QTL MAPPING FOR IMPROVEMENT OF DOWNY MILDEW [*Sclerospora graminicola* (Sacc.) J. Schroet.] RESISTANCE (DMR) IN PEARL MILLET (*Pennisetum glaucum* (L.) R. Br.) HYBRID PARENTAL LINE ICMB 89111

BY

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Thesis submitted to the Chaudhary Charan Singh
Haryana Agricultural University in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

IN

PLANT BREEDING

College of Agriculture
Chaudhary Charan Singh Haryana Agricultural University
Hisar-125 004 **INDIA**

2004

DEDICATED

TO

MY REVERED PARENTS

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MY LOVING WIFE

CERTIFICATE I

This is to certify that this thesis entitled, **"QTL mapping for improvement of downy mildew [*Sclerospora graminicola* (Sacc.) J. Schroet.] resistance (DMR) in pearl millet [*Pennisetum glaucum* (L.) R. Br.] hybrid parental line ICMB 89111"** submitted for the degree of **Doctor of Philosophy** in the subject of **Plant Breeding** of the Chaudhary Charan Singh Haryana Agricultural University, is a bonafide research work carried out by **Surinder Kumar Gulia** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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ACKNOWLEDGEMENT

It gives me immense pleasure to express a great sense of gratitude to the Chairman of my Advisory committee, Dr. R.S. Sangwan, Section Head, Oilseeds, Department of Plant Breeding, CCS HAU, Hisar, for his kind encouragement, guidance and unceasing support during my study at HAU and during the course of achieving the final shape of this thesis.

Zealously and self-effacingly, I eulogize the genuine cooperation and impetus offered to me by Co-chairman of my Advisory committee, Dr. C.T. Hash, Principal Scientist, Molecular Breeding, ICRISAT, Patancheru and, for providing excellent research facilities, meticulous guidance and constructive criticism throughout the period of this investigation and preparation of the manuscript.

I am also very thankful to other members of my advisory committee, Dr. R.K. Behl, Professor and Head-Teaching section, Plant Breeding, Dr. M.S. Kuhad, Professor, Department of Plant Physiology, Dr. B.K.. Hooda, Senior Scientist, Department of Mathematics & Statistics, and Dr. O.P. Luthra, Professor, Genetics for constructive suggestions and guidance for completion of this endeavor.

I wish to express my sincere and wholehearted gratitude to Dr. I.S. Khairwal, Professor and Co-ordinater, All India Co-ordinated Pearl Millet Improvement Program (AICPMIP) for his continuous encouragement and great support to carry out my doctoral research at ICRISAT.

I am sincerely thankful to Dr. R..S. Waldia, Professor & Head, Dr. B.P.S. Lather, Professor and ex-Head, Department of Plant Breeding,CCS HAU Hisar for their invaluable encouragement and able direction throughout the course of study. I would also like to thank the Dean PGS, CCS HAU for his kind authorization and full support for carrying out my research work at ICRISAT.

I would like to express my heartfelt and profound gratitude to Dr. J.H. Crouch, Head, Applied Genomics Laboratory, Dr. R.P. Thakur, Senior Scientist (Pathology), Dr. K.N. Rai, Principal Scientist (Pearl millet Breeding), Dr. Hutokshi Buhariwala and *Dr. F. Roelf for* their *kind help which enabled me to accomplish my doctoral research with ease.*

I express my sincere gratitude to Dr. C.R. Bainiwal, Head Bajra section, Dr. Krishan Shehrawat, CCS HAU, Hisar, Dr. Yaspal Yadav, Sr. Scientist, RRS, CCS HAU, Bawal, and Dr. Sriknat, Sr. Scientist, RRS RAU, Durgapura, for providing all necessary facilities and technical assistance in carrying out multilocational trials at their research stations.

I am also grateful to Dr. Rattan Yadav, Molecular Geneticist, (IGER, Aberystwyth, UK), Dr. F.R. Bidinger, Principal Scientist (Cereal Physiology), Dr. Maria Kolesnikova-Allen, former Special Project Scientist, PMB, and Dr. H.C. Sharma, Principal Scientist, Entomology, ICRISAT for being supportive and encouraging throughout this research project, and for giving me time for scientific discussions.

With earnest regards and immense pleasure, I wish to acknowledge and express my thanks to Dr. S. Chandra (Senior Statistician), Ms. Rupa Devi and Mr. Prashant Kumar (Scientific

Officers, Statistics unit), ICRISAT, for their valuable advice and help rendered during analyses.

Assistance rendered by the AGL technical and scientific staff especially Mr. Narsi Reddy, Mr. Brian Moss, Ms. Seetha Kanan, and technical staff-Plant Pathology, ICRISAT, who helped me in completing my lab work efficiently, expediently and smoothly, is also acknowledged.

I express heartfelt words of appreciation to all ICRISAT PMB staff especially Mr. Bhaskar Raj, Mr. Ganapathi, Mr. Om Parkash, Mr. Bashir, Mr. Murali, Mr. Ahmed, Mr Jaihind for their kind cooperation and help rendered. I thank Ms. S. Devi and Mr. S.B. Stanley, Senior Secretaries (PMB) and former secretary Mr. K. Prabhakar for their kind and prompt cooperation.

Special thanks are extended to Library staff, Learning Systems Unit staff especially Mr. Prasad Rao and Mr. Vidya Sagar (Photographer) for their excellent assistance during my research work at ICRISAT. I also thank Housing and Food services staff for making my stay at ICRISAT comfortable and enjoyable.

It was with the highest blessings and good wishes of my parents, my brothers, sister-in-laws, sisters and brother-in-law, and affection of my nieces and nephews that I could attain academic heights by undergoing doctoral studies successfully. I shall be failing in my duty if I do not express my deepest and heartfelt thanks to my caring and loving wife for her sacrifices, constant and all round support at every moment of course of this study.

I express my cordial thanks to all my senior friends Ranjana, Raghu, Lava, Gauri, Rizvi, Senthil, Arun, and colleagues Devvart, Santosh, Mehtre, Mukesh, Mohan, Pradeep, Nepolean, Satish, Velu, Ranvir, Ramesh, Amit, and Gaurav for giving a nice company and full assistance during my stay, both at ICRISAT and CCS HAU Hisar. My sincere and special thanks go to Pranjan Barman, Anil Ahlawat and Virender Mor for their untiring help, whole-hearted cooperation and moral support all along the course of this study.

I take this opportunity to thank Dr. Balaji, Head Learning Systems Unit, Dr. C.L.L. Gowda, Program Leader, Global Theme-Crop Improvement, and ex-head, Learning Systems Unit, ICRISAT for their kind cooperation to work in collaboration with PMB division of ICRISAT on Memorandum of Understanding.

I gratefully acknowledge Department For International Development (DFID), Plant Sciences, UK, for providing funds and Research Scholarship and excellent facilities by ICRISAT to carry out my doctoral research.

30-11-04

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HISAR
November, 2004

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ABBREVIATIONS

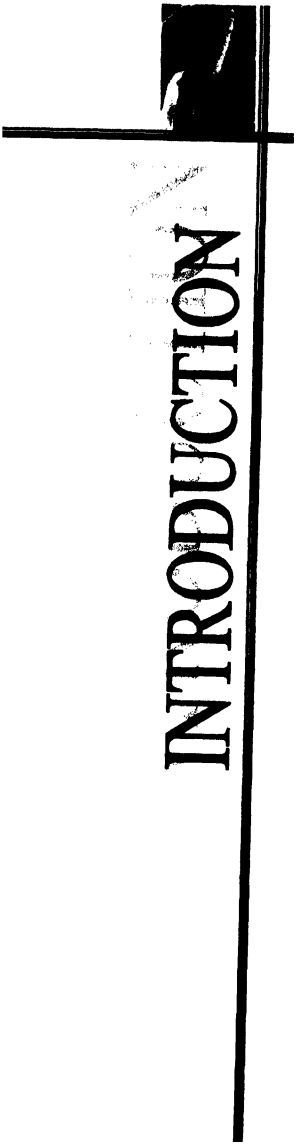
°C	: degree celsius
^{32}P	: Radioactive material (used for labeling RFLP probe)
µg	: microgram
µL	: microlitre
A	: Homozygote for the allele a from parental strain P_1 at this locus
AFLP	: Amplified fragment length polymorphism
AGL	: Applied genomic laboratory
AGN/P	: Average grain number per panicle
ANOVA	: Analysis of variance
APS	: Ammonium per sulphate
dATP	: Deoxyadenosine triphosphatase
B	: Homozygote for the allele b from parental strain P_2 at this locus
BAC	: Bacterial artificial chromosome
BC	: Back cross
bp	: base pair
BSA	: Bovine serum albumin
C	: Not a homozygote for allele a (i.e. either B or H)
CIM	: Composite interval mapping
cM	: centi Morgan
cm	: Centimeter(s)
CMS	: Cytoplasmic male sterility
CTAB	: Cetyl trimethyl ammonium bromide
dCTP	: Deoxycytidine triphosphatase
CV	: Coefficient of variation
D	: Not a homozygote for allele b (i.e. either A or H)
df	: Degree of freedom
dH_2O	: Distilled water
DNA	: Deoxyribo nucleic acid
dNTP	: Deoxy nucleotide tri-phosphate
*Dra*I	: *Deinococcus radiodurans*
DSY	: Dry straw yield
EB	: Ethidium bromide
*Eco*RI	: *Escherichia coli* RY13
EDTA	: Ethylene diamine tetra acetic acid
ENT	: Effective number of tillers
EPS	: Effective plant stand
FSY	: Fresh straw yield
g	: gram(s)
G × E	: Genotype × environment
gDNA	: genomic DNA
GTE	: Glucose tris-HCL EDTA
dGTP	: Deoxyguanosine triphosphatase
GY	: Grain yield
H	: Heterozygote carrying alleles from both P_1 and P_2 parental strains i.e. genotyping comparable to the F_1

h^2 (ns, bs)	: Heritability (narrow sense, broad sense)
HCL	: Hydrochloric acid
HI	: Harvest Index
*Hind*III	: *Haemophilus influenzae* Rd
hsd	: Honestly significant difference
IAA	: Iso-amyl Alcohol
Kb	: Kilobase
LB	: Luria-bertani media
LG	: Linkage group
LOD	: Log_{10} of the likelihood ratio
M	: Molar
M ha	: Million hectares
MAB	: Marker-assisted breeding/backcrossing
MAS	: Marker-assisted selection
Mb	: Million bases
Min	: Minute(s)
mL	: millilitre
mM	: milliMolar
MSS	: Mean sums of squares
Na_2SO_3	: Sodium sulphate
NaCl	: Sodium chloride
NaOH	: Sodium hydroxide
ng	: nanogram
No.	: Number(s)
OD	: Optical density
p	: Probability
PAGE	: Poly acrylamide gel electrophoresis
PCR	: Polymerase chain reaction
PG/D	: Panicle girth/diameter
PH	: Plant height
PL	: Panicle length
PM	: Panicle mass
pmol	: picomole
*Pst*I	: *Providencia stuartii*
QTL	: Quantitative trait loci
RAPD	: Random amplified polymorphic dna
RE	: restriction enzyme(s)/ endonuclease(s); relative efficiency
REML	: Residual maximum likelihood
RFLP	: Restriction fragment length polymorphism
RIL	: Recombinant inbred line
RNA	: Ribonucleic acid
RNase	: Ribonuclease
rpm	: Revolution per minute
SCAR	: Sequence characterized amplified region
SDS	: Sodium dodecylsulphate
SDW	: Sterile distilled water
SE	: Standard error
Sec	: Second
SIM	: Simple interval mapping

SMC	: Straw moisture content
SNPs	: Single nucleotide polymorphism
SSR	: Single/simple sequence repeat (microsatellites)
$T_{10}E_1$	: 0.5 M Tris HCl pH 8.0 and 0.5 M EDTA pH 8.0
TAE	: Tris acetic EDTA
TAGBY	: Total above ground biomass yield
Taq	: *Thermos aquatus*
TBE	: Tris Borate EDTA
TE	: Tris- EDTA
TEMED	: N,N,N,N-Tetramethylethylenediamine
TF	: Time to 50% flowering
TSM	: 1000-Seed mass
dTTP	: deoxythymidine triphosphatase
UWB	: University of Wales, Bangor, UK
UV	: Ultraviolet
V	: Volt
v/v	: Volume/volume
W	: Watts
YAC	: Yeast artificial chromosomes
λ-DNA	: *lambda* DNA/used as marker
χ^2	: Chi square
-	: Missing data for the individual (A, B, H) locus

CHAPTER 1

CHAPTER 1



INTRODUCTION

1. INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.], a monocot species belonging to the *Poaceae* family, is a staple food grain crop for about 90 million people living in the semi-arid tropical regions of Africa and the Indian sub-continent. Grain yields are generally low (around 500-600 kg/ha). It is better adapted than other cereals to marginal lands of low fertility and low rainfall that receive application of little or no inputs. It is a multi-purpose cereal grown for grain, stover and green fodder on more than 26 million hectares, primarily in arid and semi-arid regions of India and Africa (FAO, 2000). Pearl millet ranks sixth among cereals in terms of global area. In India, it ranks fourth among cereals, both in area under cultivation (9.55 m ha) and production (8.36 m tones) and sixth in productivity (8.8 q/ha). In Haryana, pearl millet occupies fifth, fourth and sixth positions for area, production and productivity, respectively (Statistical Abstract, India, 2002).

Pearl millet is an excellent forage and, and because of its low hydrocyanic acid content, is the best annual grazing crop for the southern USA (Burton, 1995) and an important summer forage crop in Australia and South America as well (Hanna, 1996). The energy density of pearl millet grain is relatively high, arising from its higher oil content relative to maize, wheat or sorghum (Hill and Hanna, 1990). Pearl millet grain contains 27 to 32% more protein, higher concentrations of essential amino acids, twice the extract, and higher gross energy than maize (Ejeta *et al.*, 1987).

It is a good experimental plant for genetical studies because of its low diploid chromosome number (2n=14) with a moderately high DNA content of IC = 2.36 pg (Martel *et al.*, 1997), its short duration (60-90 days), and its protogynous flowering that facilitates control of pollination. It has also been recognized as well suited for molecular studies.

Downy mildew, also known as green ear disease, is one of the most widely spread and destructive diseases of pearl millet, potentially resulting in devastating yield losses in India and western Africa. It is caused by systemic infection by the obligate biotrophic pseudo-fungus *Sclerospora graminicola* (Sacc.) J. Schroet., first reported on pearl millet in India by Butler (1907). The pearl millet downy mildew pathogen reproduces asexually

by means of sporangia that germinate to release motile zoospores and sexually to produce soil-borne oospores. Downy mildew disease of pearl millet develops after colonization by the pathogen of undifferentiated host tissue, which results in symptoms resembling those of a systemic virus disease, with growth disturbance and chlorosis. Pearl millet plants infected at an early growth stage will produce no grain at all, instead transferring in to leafy structures (green ear) in panicle. Later infection can severely affect basal and nodal tillers, although the main shoot may have escaped disease. Pearl millet originated in West Africa (Purseglove, 1976) and the pearl millet downy mildew pathogen, which is highly host specific, is likely to have co-evolved with pearl millet in that region (Rachie and Majumdar, 1980). Following the release and widespread adoption of genetically uniform pearl millet single-cross hybrid in India in the late 1960s (Dave, 1987), downy mildew became an economically important disease and the first major epiphytotic occurred in the early 1970s (Singh and Govind Singh, 1987; Hash, 1997). Since then, downy mildew has been a major production constraint and a major focus of pearl millet improvement research both by ICRISAT and the Indian National Program (Rai and Singh, 1987; Shetty, 1987; Singh *et al.*, 1987; Singh *et al.*, 1993a; Singh, 1995; Hash *et al.*, 1997, 1999). As the host is a crop of poor and marginal areas, the use of resistant cultivars is the most appropriate control method for pearl millet downy mildew. Breeding for resistance to diseases of current and potential economic importance contributes to increase productivity and stability of pearl millet grain, stover and forage yields. Disease resistance is a major concern in pearl millet improvement programs, and has been the subject of several reviews (Louvel, 1982; Williams and Andrews, 1983; Williams, 1983, 1984; Andrews *et al.*, 1985; Rai and Anand Kumar, 1994; Talukdar *et al.*, 1994; Hash *et al.*, 1997; Hash *et al.*, 1999). In breeding improved pearl millet cultivars, it is necessary to maintain moderate levels of resistance to many potential pathogens currently of minor importance in the breeder's target environments (Mohan *et al.*, 1978; Singh *et al.*, 1993b). This helps ensure that these potential constraints do not become actual problems later on.

For any resistance-breeding program, knowledge of the inheritance of host plant resistance against available pathogen populations is a prerequisite for an effective resistance-based disease management strategy. Previous studies have reported that downy mildew resistance in the host plant shows continuous variation (Safeeulla, 1976; Basavaraju *et al.*, 1981b; Shinde *et al.*, 1984), or in some cases can be rather simply inherited (Singh and Talukdar, 1998). Resistance is generally dominant and variation in

segregating populations is typically continuous (Singh *et al.*, 1980). Non-additive gene action is responsible for much of the heritable variability (Deswal and Govilla, 1994) with complementary epistasis (Joshi and Ugale, 2002). The allogamous and highly variable nature of both the host and the pathogen (Thakur *et al.*, 1992) is a hindrance to breeding for host plant resistance to this disease. Because of the complexity of this host-pathogen system, the mechanisms and inheritance of host plant resistance to pearl millet downy mildew continue to be poorly understood.

ICRISAT has developed highly effective field (Williams *et al.*, 1981) and green house (Singh and Gopinath, 1985; Singh *et al.*, 1993a; Weltzien and King, 1995) screening techniques that can easily differentiate between resistant and susceptible progenies.

Genotypes by environment (G × E) interactions are almost unanimously considered to be among the major factors limiting response to selection and, in general, the efficiency of plant breeding programs. G × E interactions become important when the rank of breeding lines changes to a large extent in different environments. This change in rank has been defined as crossover G × E interaction (Baker, 1988). G × E interactions are reported to be statistically significant (but not always important) for most agronomic characters in pearl millet (Ali *et al.*, 2001). For example, QTL × environment interactions for downy mildew were significant at each QTL due to difference in the magnitude rather than direction, of the QTL effects (Jones *et al.*, 2002).

A very good understanding of, and ability to manipulate, oligogenic and quantitative traits is offered to plant breeders by recent advances in genetic marker technology (Young, 1999). A major advantage of using molecular markers for the introgression of resistance genes into cultivars is a gain in time (Tanksley *et al.*, 1989; Melchinger, 1990) by guiding and expediting conventional plant breeding program by reducing number of breeding cycles. The second major advantage is that it facilitates effective selection even when phenotypic selection is likely to be ineffective (e.g. for drought tolerance during rainy season or for resistance in absence of a target pest or pathogen). DNA sequence that match specific chromosomal loci can be used to detect restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), microsatellite polymorphisms (SSRs and others), amplified fragment length polymorphisms (AFLP) or other molecular genetic marker loci (Jones *et al.*, 1997; Mohan

et al., 1997; Prioul *et al.*, 1997, Qi *et al.*, 2001 and Allouis *et al.*, 2001). The development and availability of abundant, naturally occurring, molecular genetic markers (RFLP, RAPD, SSRs, isozymes, etc.) during the last two decades has generated renewed interest in counting, locating and measuring the effects of genes (polygenes or QTLs) controlling quantitative traits. When there is a marker map and a segregating population for a character of interest, it is often possible to obtain information about the number, effects and positions of the QTLs affecting the trait (Paterson *et al.*, 1988). Microsatellites remain the markers of choice for practical breeding applications with several advantages over RFLP, RAPD and AFLP markers. In addition, microsatellites exhibit co-dominant inheritance and their detection is readily automated (Hernandez *et al.*, 2002). Marker-assisted selection could be more efficient than purely phenotypic selection in quite large populations and for traits showing relatively low heritabilities (Hospital *et al.*, 1997; Moreau *et al.*, 1998). Molecular marker technologies can be effectively used to pyramid several resistance genes into a single host genotype, using marker-assisted selection-based backcrossing. The product of such marker-assisted backcrossing can be used in more effective resistance gene deployment strategies (Witcombe and Hash, 2000; Hash and Witcombe, 2002). Pyramiding of the resistance genes in a breeding program could be very well possible using molecular-marker-based genotyping, even when the phenotypes result from epistatic interaction of alleles at two or more loci.

The building up of a saturated molecular map using molecular markers like restriction fragment length polymorphisms (RFLPs) and microsatellites (SSR) makes it possible to dissect Mendelian factors underlying a complex trait such as disease resistance, and consequently enhance the effectiveness and accelerate the rate of breeding programs to improve pure line varieties of self-pollinated crops and hybrid parental lines of cross-pollinated crops. Linkage drag and confounding effects of environmental variation associated with conventional breeding can also be reduced. With QTL mapping, the role of specific resistance loci can be described, race-specificity of partial resistance genes can be assessed, and interactions between resistance genes, plant development, and environment can be analyzed. As the molecular-marker-based genetic linkage map for pearl millet has been constructed (Liu *et al.*, 1994) and extended (Devos *et al.*, 2000; Qi *et al.*, 2004), QTL analysis is now possible even for this relatively little-studied crop. An added advantage of mapping resistance genes using these molecular markers is that homogeneous lines of the pathogen need not be isolated, because resistance QTLs

effective against all individual pathogenic components that may exist within the pathogen population can be detected through inoculation with a representative sample of the whole pathogen population. Keeping in view the aspects mentioned above, the present study was carried out with the following objectives:

1. To construct a skeleton linkage map for a pearl millet mapping population based on cross ICMB 89111 × ICMP 423.
2. To study the inheritance of downy mildew resistance based on screening of the segregating mapping population against diverse Indian and African isolates of *Sclerospora graminicola.*
3. To identify and map QTLs controlling downy mildew resistance effective against diverse pathogen populations of Indian and African origin.
4. Multilocational assessment of the agronomic performance and downy mildew reaction of versions of hybrid HHB 94 based on sub-selections of ICMB 89111 differing in their downy mildew incidence reactions.

CHAPTER 2

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. PEARL MILLET AND ITS IMPORTANCE

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is grown principally for grain in the hot arid and semi-arid areas of Africa and the Indian subcontinent. It is sown on approximately 14 million ha in Africa and 9 million ha in India, annually producing 10.5 and 6.5 million tons of grain, respectively. In terms of global production, pearl millet is the sixth most important cereal crop after wheat, rice, maize, barley, and sorghum (FAO and ICRISAT, 1996). Increasing the productivity of pearl millet to keep pace with the growing food demand of human and feed demand of livestock populations in the semi-arid tropics is a gigantic task requiring concerted efforts from national and international research and development organizations including both public-sector and private-sector agencies (Rai and Anand Kumar, 1994). More than 70 single-cross F_1 pearl millet hybrids released so far, occupy more than 60 percent of the major pearl millet areas of Gujarat, Maharashtra, Haryana, Uttar Pradesh, Rajasthan and Tamil Nadu, and have significantly contributed towards enhanced productivity (875 kg/ha) and production (8035 million tones) despite a gradual decline in total cultivated area in India (Bhatnagar, 2003).

2.1.1. As a nutritive food grain

Pearl millet grain has a high nutritional value and typically has higher protein content than maize or sorghum (Maiti and Bidinger, 1981). The amino acid profile of pearl millet grain is better than that of normal sorghum or normal maize and is comparable to those of the small grains wheat, barley and rice (Ejeta *et al.*, 1987) with a less disparate leucine/isoleucine ratio (Hoseney *et al.*, 1987; Rooney and McDonough, 1987). The content of lysine in protein reported in pearl millet grain ranges from 1.9 to 3.9 g per 100 g protein (Ejeta *et al.*, 1987). Pearl millet grain appears to be generally free of any major anti-nutritional factors, such as the condensed tannins in sorghum grain having a pigmented testa, which reduces protein availability. As with others cereals, phytic and nicotinic acid contained in pearl millet grain are mainly found in the germ (Simwemba *et al.*, 1984; McDonough, 1986).

In some parts of Africa there is a strong positive correlation between goiter incidence and per capita pearl millet production (Klopfenstein *et al.*, 1983b). Epidemiological studies have suggested that pearl millet might be at least partially responsible for the high goiter incidence in the Durfur area of Sudan (Klopfenstein *et al.*, 1983a). Rats fed a pearl millet diet tend to develop symptoms similar to those of colloid goiter in humans. Thyroid colloid follicles were some what enlarged, but serum thyroid hormone concentration was normal in rats that were fed with pearl millet weaning food (Klopfenstein *et al.*, 1985). Autoclaving the grain appears to alleviate the symptoms.

2.1.2. As a feed grain and fodder crop

Hanna *et al.* (1991) suggested that pearl millet grain has a good potential to be used as a high quality feed grain like corn and sorghum in rations of chickens, beef cattle and swine. The energy density of pearl millet grain is relatively high, arising from its higher oil content relative to maize, wheat or sorghum (Hill and Hanna, 1990). It contains 27 to 32% more protein than maize, higher concentration of essential amino acids, twice the extract (fat) and higher gross energy than maize (Ejeta *et al.*, 1987).

Studies conducted by several workers (French, 1948; Singh and Barsaul, 1976; Sharma *et al.*, 1979; Stringhini and Franca, 1999) show that pearl millet grain compares favorably with maize in poultry diets. Lloyd (1964) observed that broilers fed on pearl millet rations were heavier and had better-fed conversion than those fed on maize rations. Fancher *et al.* (1987) reported that the metabolizable energy (MA_n) content of ground pearl millet grain varied from 2.9 to 3.2 kcal g^{-1} dry matter. Sullivan *et al.* (1990) observed that pearl millet in comparison with sorghum and maize offers an excellent alternative as feed grain for cattle and broilers, having higher feed conversion rates.

Costa (1992) and Gates *et al.* (1999) reported that pearl millet has demonstrated great potential as a forage crop, where it can be used as pasture or to make silage and hay that is free of prussic acid glycosides. Pearl millet produces high yields of good quality forage when properly managed. Earliness is a major advantage of pearl millet when harvested for forage. However, its high quality is also important with dry matter crude protein levels reaching 18-20% between the boot leaf and the milky grain growth (Tabosa *et al.*, 1999).

Collins *et al.* (1997) noted commercial layers given feed containing pearl millet grain presented lower ratios between the omega-6 and omega-3 fatty acids, endowing the eggs with a fatty acid profile more favorable to human health. Gelaye *et al.* (1997) found that pearl millet grain increased the neutral detergent fiber content in the rations, with increases of lignin and cellulose concentrations in the diets, but the levels of calcium, phosphorus and crude protein were also higher than those of maize.

Pearl millet pasture grazed rotationally by dairy cows provides total digestible nutrients (TDN) in the range of 1400-2300 kg ha^{-1}, a quantity generally superior to that of Sudan grass and sorghum (Faires *et al.*, 1941; Roark *et al.*, 1952; Marshall *et al.*, 1953). Miles *et al.* (1956) have shown that Tift Sudan consistently produces more dry matter, milk and TDN than pearl millet but that pearl millet consistently provides higher quality pasture than permanent pastures. Pearl millet is an excellent forage and is the best annual grazing crop in southern USA (Burton, 1995) and an important summer forage crop in Australia and South America as well (Hanna, 1996).

2.1.3. As a crop for harsh production environments

Pearl millet is a hot climate plant that is xerophilous and has efficient drought escape and tolerance mechanisms. Pearl millet growing covers 26 million hectares in sub-Saharan Africa and South Asia. Low input farming is the main activity for the approximately 400 million people living in these regions. It is also grown on millions of hectares as a forage and mulch component minimum tillage soybean production systems in USA and Brazil, respectively. It is almost the only cereal crop that can be grown in parts of tropical and subtropical Asia and Africa with annual rainfall under 400 mm (parts of countries such as India, Pakistan, Yemen, Mauritania, Mali, Burkina Faso, Senegal, Chad, Niger, Nigeria, Sudan and others) (Scalea, 1999). Because of its sharp crop life cycle, rapid grain filling, and exceptional ability to tolerate drought, pearl millet extends warm rainy season food grain production into regions too arid for sorghum and maize (Burton, 1983). It tolerates drought, low soil fertility, and low soil pH, while responding well to water and good management (Anand Kumar, 1989).

Pearl millet performs better than any other cereal in sandy soils and under poor fertility conditions and has the greatest drought tolerance (Maciel and Tabosa, 1982). Its better adaptation to low-fertility soils is based on its superior ability to extract nutrients

due to a deep fibrous root system that ultimately endows it with superior shoot matter production resulting in green matter yields of 20-70 tons ha^{-1} (Scalea, 1999).

2.1.4. As a component of sustainable agriculture on more intensively cultivated lands

Saturnino and Landers (1997) advocated zero-tillage seeding of pearl millet following the soybean crop, and stressed that compared with conventional tillage this technology provides greater soil protection, results in more intensive land use, ensures better weed control, and better supports crop-livestock production systems.

The recent rapid expansion of pearl millet in Brazil (Bonamigo, 1999) is based on increased adoption of minimum-tillage planting systems. The main uses of pearl millet in Brazil are as a mulch component of minimum-tillage technology, grain production for monogastric livestock feed, biomass production for hay or direct grazing, and crop rotation following soybeans (Val, 1994). No-till millet is a widely adopted alternative due to a series of crop characteristics, particularly its ample output of biomass with high C/N ratio and good drought tolerance (Spehar, 1999). Pearl millet was adopted by the farmers attracted by the advantages of more efficient sowing and lower production costs through no-till planting (Spehar and Landers, 1997). Low desiccation costs due to greater glyphosate availability greatly facilitated pearl millet management in this system.

2.1.5. As a model crop for genetic studies

Pearl millet is potentially an ideal species for genetic studies (Jauhar and Hanna, 1998). It has a small diploid genome with a haploid DNA content of 2.36 to 2.50 pg (Bennett, 1976; Martel *et al.,* 1997), with a small number (seven) of large chromosome pairs with two distinctive nuclear organizers. Its germplasm (both landrace and elite) possesses abundant phenotypic variation (Brunken, 1977). It has a number of wild relatives with haploid chromosome complements (n) of 5, 8 and 9 in addition to a large group with n=7 with which it can be intercrossed (Jauhar, 1981). Pearl millet is also of interest as a biological model of studying domestication and crop/wild complex evolution (Poncet *et al.*, 1998, 2000, 2002).

2.2. PEARL MILLET DOWNY MILDEW AND ITS IMPORTANCE

2.2.1. Pearl millet downy mildew history

Pearl millet downy mildew is caused by *S. graminicola*, a member of the class Oomycetes, order Peronosporales, and family Peronosporaceae. The first report of downy mildew infecting a millet was made by Saccardo (1876). He designated the species as *Protomyces graminicola* based solely on oospore characteristics. Schröter (1879) discovered the imperfect state of the fungus and designated the pathogen as *Peronospora graminicola* (Sacc.) Schroet. He was the first authority to consider a subcategory of *Sclerospora* within the genus *Peronospora* in which to classify the downy mildew fungus infecting pearl millet. *Sclerospora* was subsequently elevated to the status of genus by de Bary (1881), thereby changing the specific name of pearl millet downy mildew to *Sclerospora graminicola* (Sacc.) Schroet. Downy mildew was first reported in India on pearl millet by Butler (1907) and other hosts by Bhat (1973). This disease is of great economic importance in India but also causes pearl millet yield losses in many countries in Africa, including Burkina Faso, Chad, Eritrea, Ghana, Mali, Mozambique, Niger, Nigeria, Senegal, Sudan, Togo, Tanzania, and Zambia. This pathogen has been reported in more than 20 countries around the world (Singh *et al.*, 1993a).

Pearl millet downy mildew (caused by the oomycetic pseudo-fungus, *Sclerospora graminicola* (Sacc.) J. Schrot.) is a highly destructive and widespread crop disease in Africa and Asia. Over the past 25 years, pearl millet production area in India has come down for many reasons. One of the major causes of this reduction in pearl millet production area in India had been downy mildew. Downy mildew is the most devastating disease of pearl millet in India and a major epidemic occurred there in the early 1970s, with resurgences in subsequent years closely following the release and widespread adoption of several closely related, genetically uniform pearl millet single-cross hybrids (Dave, 1987; Singh *et al.*, 1987; Hash, 1997).

2.2.2. Pearl millet downy mildew resistance inheritance studies

Since very shortly after the onset of the hybrid era for pearl millet in India, downy mildew resistance has been a major research focus by scientists of both ICRISAT and the Indian national program involved in improvement of this crop (Singh *et al.*, 1993a; Hash, 1997; Hash *et al.*, 1997, 1999; Hash and Witcombe, 2002).

Early reports from Appadurai *et al.* (1975) indicated that a single gene for downy mildew resistance was apparently dominant over that for susceptibility, which was supported by a non-significant χ^2 goodness of fit test of F_2 segregation data to a 3:1 ratio.

Gill *et al.* (1978) reported two dominant duplicate factors conferring resistance to downy mildew and proposed the gene symbols DM_1DM_2, DM_1dm_2, and dm_1DM_2 for resistant and dm_1dm_2 for susceptible genotypes. Dass *et al.* (1984), Thakur *et al.* (1992), and Singh (1995) reported resistance to be dominant over susceptibility and probably controlled by one or a few genes. Except in one case where resistance was reported to be recessive (Singh *et al.*, 1978) resistance is generally observed to be dominant and variation in segregating populations is continuous (Singh *et al.*, 1993a).

Quantitative inheritance of resistance to downy mildew was reported by Singh *et al.* (1978), who observed significant additive and non-additive genetic variance. Basavaraju (1978) and Basavaraju *et al.* (1980) concluded that resistance to downy mildew is not simply inherited, but is due to a series of non-allelic interactions. Singh *et al.* (1980) and Dass *et al.* (1984) have observed continuous variation for segregation in pearl millet downy mildew disease resistance studies. Many authors (*e.g.,* Tyagi and Iqbal Singh, 1989; Deswal and Govila, 1994; Kataria *et al.*, 1994) have concluded that non-additive gene action is responsible for much of the heritable variability for host plant reaction of downy mildew, agreeing with simpler studies that show resistance to be dominant or partially dominant.

Deswal and Govila (1994) reported inheritance of pearl millet downy mildew resistance as digenic based on generation mean analysis of crosses involving resistant and susceptible parents. The inheritance of resistance was complementary at Delhi (9:7) and duplicate at Villupuram, Tamil Nadu (15:1). In few cases where clear Mendelian segregation have been observed, Hash *et al.* (2003) also reported one, two or even three dominant genes governing the resistance to downy mildew in pearl millet The results from Kataria *et al.* (1994) supported a more complex pattern of inheritance of resistance to downy mildew and indicated simple selection would not be effective for incorporation of resistance to downy mildew disease.

Weltzien and King (1995) subjected one population of pearl millet highly susceptible to downy mildew to two cycles of recurrent selection for downy mildew

resistance and demonstrated that even in a susceptible population, recurrent selection effectively increased the level of resistance to this disease. However, progress in the second cycle of selection was much less than that in the first suggesting fixation had occurred after the first selection cycle at the loci contributing most to disease reaction in this population.

Gill *et al.* (1975) studied the reaction of some F_1 hybrids of crosses involving resistant and susceptible parents as well as some crosses in the reverse directions. The F_1 data indicated that the inheritance pattern of resistance was rather complex and could involve genic interactions.

Singh *et al.* (1978), using a sick plot screening protocol, observed resistant and susceptible reactions in the F_1 and F_2 generations of crosses between resistant and susceptible pearl millet inbreds. In the F_2 generations the frequency of resistant plants was higher in R × R crosses than in R × S and S × S crosses, suggesting polygenic inheritance.

The inheritance of resistance to downy mildew in pearl millet was studied by Besavaraju *et al.* (1981a) using 16 parental lines and their F_1, F_2, BC_1 and BC_2 progenies under artificial epiphytotic conditions. The χ^2 values for different ratios and heterogeneity components showed that resistance to downy mildew in pearl millet was not simple but exhibited quantitative inheritance.

A study by Shinde *et al.* (1984) revealed the contribution of both additive and non-additive gene effects in the inheritance of pearl millet host-plant resistance to downy mildew. Duplicate effects were observed in most of crosses studied. Singh and Talukdar (1998) studied F_1, F_2 and BC progenies of the cross IP 18292 × Tift 23DB for inheritance of downy mildew resistance. A single dominant gene from IP 18292 controlled resistance and they suggested simple and straightforward selection techniques to transfer this to economically important backgrounds.

Although in the above studies pearl millet downy mildew resistance was generally found to be dominant over susceptibility and controlled by one or more dominant genes with some modifiers, a complete picture of its inheritance is not yet available. The use of regional variability present in pathogen populations of *S. graminicola* and difficulties in maintaining high and uniform disease pressure in downy mildew disease screening studies have led to the conflicting conclusions from earlier studies (Jones *et al.*, 1995).

However, with the availability of more precise inoculation techniques (Singh and Gopinath, 1985; Jones *et al.*, 2001) highly homozygous resistant and susceptible parental lines, and effective molecular mapping procedure, more precise information on the genetics of resistance will soon become available (Singh, 1995; Hash *et al.*, 1997, 1999; Hash and Witcombe 2002).

Limited information on downy mildew resistance QTLs detected using molecular marker techniques has been published (Jones *et al.*, 1995; Hash *et al.*, 1995; Hash and Bramel-Cox, 2000; Breese *et al.*, 2002; Jones *et al.*, 2002). However, it is clear that this technique facilitates manipulation, including pyramiding, of resistance genes in genetic backgrounds of elite inbred hybrid parental lines (Hash *et al.*, 1997; Witcombe and Hash, 2000; Sharma, 2001; Hash and Witcombe, 2002). The literature on inheritance of downy mildew resistance has been adequately discussed in several fairly recent reviews (Koduru and Krishna Rao, 1983; Hash *et al.*, 1997, 1999). Maiti and Singh (2004) also reviewed all biotic factors, especially downy mildew disease, affecting pearl millet growth and productivity.

Significant variability in downy mildew incidence due to genetic differences among A-lines, due to pathotypes, and due to line × pathotype interactions were observed by Thakur *et al.* (2001). They further reported that among the sources of variation, the largest proportion of variability for downy mildew incidence was accounted for by lines, followed by line × pathotypes interaction and pathotypes, both in field and green house tests.

Joshi and Ugale (2002) reported digenic Mendelian ratios of 15:1 in cross 1 at environment 1 and 13:3 at environment 2 and hence concluded that the nature of inheritance of downy mildew for this cross was digenic. Further, in cross II, they reported tetragenic ratios (229:27) and in cross III, trigenic ratios (55:9) and suggested that resistance is not simply inherited but controlled by several loci. They also reported preponderance of duplicate dominant and non-allellic interactions, especially complementary epistasis.

2.2.3. Pearl millet downy mildew - screening

The life cycle of *Sclerospora graminicola* (Sacc.) J. Schröt. is comprised of both sexual and asexual phases. The sexual stage produces oospores, which are soil or seed borne,

provide the primary source of inoculum each season (Shetty, 1987). The asexual sporangia are produced at night under conditions of moderate temperatures and high relative humidity. Maximum sporangia production occurs at 20°C. No sporulation is recorded at relative humidity levels below 70%. Sporangia germinate to produce motile zoospores (which identify the causal organisms of pearl millet downy as a member of the Protistae, and not of the Fungi) and generally do not remain viable for very long after daybreak. Sexual oospores are thick-walled, spherical, brownish yellow, and 22 to 35 μm in diameter. Oospores form following sexual recombination in colonized tissue and can survive from 8 months to 13 years under laboratory conditions (Wilson, 1999). Early attempts to screen for sources of resistance to pearl millet downy mildew depended on "sick plots" *i.e.*, plots into which infected, oospores-bearing pearl millet plants had been ploughed for several years (Nene and Singh, 1976). The test materials were sown in these plots and infection was initiated by the oospores in the soil. Large-scale field screening techniques have now been developed based on pre-sown infector rows that provide sporangial inoculum (Williams *et al.*, 1981). This technique involves the sowing of infector rows (every fifth or ninth row) with a mixture of susceptible genotypes three weeks before sowing the test materials.

Singh and Gopinath (1985) described a laboratory downy mildew screening technique using a micro-syringe that is more effective than field screening in producing downy mildew infection in susceptible genotypes. The procedure resembles natural infection but provides greater inoculum uniformity and does not affect normal host activity. A modified greenhouse method for assessing resistance to downy mildew described by Weltzien and King (1995) is more rapid and is suitable for use throughout the year, independent of season. In this method, instead of inoculating plants individually, seedlings at the coleoptile to one-leaf stage were spray-inoculated with an aqueous suspension of freshly prepared sporangia (about 10^5 sporangia mL^{-1}).

Singh *et al.* (1997) explained all screening techniques available for this disease including dip inoculation, spray inoculation, drop inoculation, injection inoculation, setting tower inoculation and field screening infector-row techniques. Jones *et al.* (2001) discussed effective ways to maintain infection potential of inoculum by spraying a chilled suspension of sporangia. Spraying seedlings with a suspension of sporangia that had been chilled before zoospore release gave uniform and adequately high disease pressure over

many hours. Thus there has been tremendous improvement over the past 30 years in the screening methods available to detect the genetic differences in host plant resistance to pearl millet downy mildew (Singh *et al.*, 1997; Hash, 1997; Hash *et al.*, 1997, 1999; Hash and Witcombe, 2002).

2.2.4. Pearl millet downy mildew - pathogen variability

There is a clear evidence for the existence of physiological specialization within *S. graminicola* (ICRISAT, 1989; Thakur and Rao, 1997). Ahmad *et al.* (1978) and Shetty *et al.* (1980) reported existence of pathogenic races within *S. graminicola* on the basis of differences in size of the asexual structures, number of nuclei, seed-borne nature and soluble proteins in the pathogen. Shetty and Ahmad (1981) reported two races from Gulberga and Mysore (in the Indian state of Karnataka) based on the differences in size and shape of sporangiophores, sporangia, and zoospores and the number of nuclei in sporangia. Pearl millet downy mildew pathogen variability was discussed by Ball (1983), who reported that the host and pathogen genotypes determine observed variation. West African isolates of S. *graminicola* were generally more pathogenic than Indian isolates.

Thakur *et al.* (1992) reported both the pathogen and host are allogamous and highly variable, and the resulting heterogeneity has consequently hampered most studies on the inheritance of resistance to pearl millet downy mildew.

Ball and Pike (1983) showed that host cultivars responded differentially to different sources of inoculum. Ball and Pike (1984) discussed intercontinental variation of *S. graminicola.* The West African hosts were potentially more susceptible to Indian than to West African pathogen isolates; conversely some Indian hosts were more vulnerable to West African than to Indian isolates (Ball *et al.*, 1986). They further reported that isolates from Africa and India vary in pathogenicity and have been shown to be sexually compatible.

Large variation in pathogenicity has also been reported among isolates of *Scelrospora graminicola* by Thakur *et al.* (1998; 1999; 2002) and by Sivaramakrishnan *et al.* (2003). Sastry *et al.* (1995) reported the use of DNA fingerprinting with simple sequence repeats (SSRs) to detect genetic diversity among pathotypes of *S. graminicola.* They also reported that $(GATA)_4$ identified most of the polymorphism among the host pathotype-specific isolates of downy mildew disease in pearl millet. In a study of host-

pathogen specificity, Sastry *et al.* (2001) demonstrated a change in virulence of a *S. graminicola* pathotype on a pearl millet line 700651 with a corresponding change in virulence in RAPD and DNA finger printing profiles in two extreme asexual generations of the pathotype.

A number of downy mildew resistant pearl millet inbred lines have been developed by pedigree and backcrossing programs, but the effectiveness of resistance in these has intended to be short lived because of the high level of genetic variability in the pathogen populations (Thakur *et al.*, 1997). At least six distinct pathogen populations have now been identified in India. Isolates of these six distinct pathogen populations are being maintained at ICRISAT- Patancheru (Thakur and Rao, 1993). Assessing regional variability between populations of the pearl millet downy mildew pathogen initially required expensive, time consuming, multilocational trials to study the patterns of differential effectiveness of host-plant resistance (Jones *et al.*, 1995). However, greenhouse screening at a single location against differential lines selected on the bases of their response to asexually-maintained pathogen populations known to differ in virulence can now reduce the time required for such studies as well as improve upon the heritability of data obtained.

Singru *et al.* (2003) detected genomic variation among 19 fungal isolates from different cultivars of pearl millet grown in various regions of India using fourteen AFLP primer combinations, which produced 184 polymorphic bands. An unweighted pair-group method of averages cluster analysis represented by dendrogram and principal coordinate analysis separated the mildew collections into four distinct groups. Isolates having characteristic opposite mating abilities, geographic relatedness, virulence, common host cultivars, and changes through asexual generations reflected heterogeneity of the pathogen.

Based on recent virulence analysis of 15 *S. graminicola* isolates from various parts of India, inoculated on putative host differentials, Sivaramakrishnan *et al.* (2003) classified them in to major five pathotypes groups distributed in hybrid-intensive states of Rajasthan, Maharashtra, Gujarat, Madhya Pradesh, Karnataka, and Andhra Pradesh.

2.3. AGRONOMIC PERFORMANCE IN MULTILOCATIONAL TRIAL

2.3.1. Genotype × Environment interactions

Genotype by environment (G × E) interactions is almost unanimously considered to be among the major factors limiting response to selection and, in general, the efficiency of breeding programs. G × E interactions become important when the rank of breeding lines changes in different environments. This change in rank has been defined as crossover G × E interaction (Baker, 1988).

Yadav *et al.* (2003) studied the genetic architecture of grain yield and its component traits among a set of testcross hybrids produced on male-sterile line 843A using as pollinators F_3-self-bulk progenies derived from individual RFLP-skeleton mapping F_2 individuals from the cross H 77/833-2 × PRLT 2/89-33 in wide range of different terminal drought stress and fully-irrigated control environments. They reported significant genotype × moisture regime (G × E) interactions for grain yield and its various component traits, indicating that the testcrosses did react differently to stress even where their mean yields across a particular stress treatment did not differ. Some of QTLs for drought tolerance of grain yield that mapped on linkage group 2 were common across the stress environments, where as others were specific to only particular stress environments.

Jones *et al.* (2002) screened seedlings of F_2 -derived F_4 self bulks from the cross 7042(S) × P7-3 under field as well as more controlled greenhouse downy mildew screening conditions in India against Indian pathogen isolates and in greenhouse conditions in the UK against African pathogen isolates. They consistently observed pair-wise marker interactions as dominant × additive and dominant × dominant between M298 on LG3 and M390 on LG5 in each screen and across–screen predicted means and detected two additional QTLs across screens by examining pair-wise marker interactions. But in India/Field-1, an additional significant interaction of smaller effect as additive × additive was also observed between same markers. They further found significant QTL × screening environment interactions at each QTL due to differences in the magnitude (rather than direction) of the QTL effects. The differences in magnitude appeared to be consequences of the degree or normality of the disease incidence distribution in the screens being compared, rather than any differences between screening methods.

Ali *et al.* (2001) have reported the significance of G × E interactions for five agronomic characters in a population diallel of pearl millet (all observed characters except productive tillers, biomass and growth index), indicating that relative performance of genotypes differed with the test environment. Although statistically significant for five characters, these G × E interactions were large enough to be of practical importance in case of grain yield and plant height. Coefficients of variation (CV) were <13% for individual environments for most characters studied, except for productive tillers and panicle length, for which the CV was >18%.

Chikurte *et al.* (2003) tested seventeen genotypes of pearl millet under four environments for twelve characters and indicated that mean differences due to genotypes were statistically significant when tested against G × E interactions (GEI) and pooled deviation. Environmental variances were significant for all characters suggesting the presence of variation among genotypes due environments. They further partitioned GEI into linear and non-linear effects and reported the major portion of the GEI as linear in nature for all characters except time to 50% flowering, plant height, and number of total and effective tillers, indicating the possibility of prediction over environments for eight of the twelve observed characters. Significance of pooled deviations suggested the importance of the non-linear components in all characters; therefore both linear and non-linear components of GEI appear to be important in pearl millet in India (Tyagi *et al.*, 1979; Dass *et al.* 1985; Dahiya *et al.*, 1987; Bhaviskar, 1990; and Anarase *et al.*, 2000).

Using simple analysis of variance, Gupta *et al.* (1975) have observed occurrence of environmental (seasonal) and genotypic × environmental interactions in pearl millet for fodder yield and its component traits. The presence of significant G × E interactions has been further reported by Mangat (1992) and Wilson *et al.* (1993). However, such studies simply give preliminary information about the phenotypic stability of genotypes.

Khairwal and Singh (1999) have suggested that such types of multi-location/phenotypic studies help the breeders in two ways: (1) to identify genotypes that perform better over a range of environments, and those that perform best under specific environmental conditions, and (2) to minimize the bias caused by genotype × environmental interactions in the estimates of different components of genetic variation. They further reported the use of regression procedures in most of pearl millet population

studies, which allow partitioning of G × E interactions into linear (predictable) and non-linear (unpredictable) components.

Bramel-Cox (1996) and Evan (1993) emphasized the need to balance high yield potential, wide adaptability with reliable performance in specific conditions in breeding programs. For this breeding programs need to better characterize the target region, develop better strategy to allocate resources to the test environments, develop the optimal population type to buffer against diverse environment constraints, define the optimal selection criteria to enhance mean performance and reduce environmental sensitivity, improve trait identification for selection and increase the use of genetic diversity within a crop species for specific adaptation to various stresses.

2.3.2. Mean performance of yield and its component traits

Whilst studying yield and its component in pearl millet in Botswana by Karikari and Mosekiemang (2002) reported that as the population increased, the development of tillers terminated earlier in growth of the plant resulting in a reduced tiller survival rate therefore reduced productive tillers per plant. Consequently, grain yield per plant declined owing to head number and also to lower seed numbers per head. Kassam (1976) reported that tillers contributed about 25% of the total grain yield. Carberry *et al.* (1985) and Crawfurd and Bidinger (1989) reported that primary yield component of millet affected by population was tiller numbers per plant. Karikari and Ngwako (1999) have advocated maintaining three tillers to have grain yield advantages.

Rai *et al.* (2000) reported that grain yield of all nine F_1 seed parents averaged over 11 environments, was significantly greater than the respective higher yielding (*i.e.* better) inbred seed parent. The average heterosis of the F_1 seed hybrid parents over the better inbred parental line (*i.e.* better parent heterosis) varied from 27% for F_1SP2 to 107 % for F_1SP8. They further revealed highly significant differences among genotypes as well as highly G × E interaction ($p< 0.001$). These two sources and error terms accounted for 43, 17 and 11% of the total variability, respectively. In previous studies in sorghum, the comparison of F_1's and A-line has shown that high yielding A-line do not necessarily produce high yielding F_1 (Hookstra and Ross, 1982; Gorz *et al.*, 1984).

2.3.3. Effect of cytoplasm on grain yield

Genetic diversification of hybrid seed parental lines in pearl millet is now achieved by using more than one CMS system and several nuclear genotype combinations within each system. Several CMS systems other than A_1 are currently available in pearl millet and a few of them are being used in pearl millet in CMS line development (K N Rai, 2004, pers. comm.).

In a study on cytoplasmic male sterile and male fertile lines for agronomic and morphological characters of sorghum, Lenz and Atkins (1981) reported higher grain yield, seed per head, head per plant, leaf area and leaves per plant in normal cytoplasmic male fertile plants compared to different male sterility inducing cytoplasms while 100 seed weight and flowering days were higher in male sterility inducing cytoplasms. But none of the male sterility inducing cytoplasm (possessed by the line KS 34 through KS 39) differed from *Milo* sterility inducing cytoplasm for their effects on grain yield and its primary components. Further more, Ross and Kofoid (1979) reported that neither of the cytoplasm nor the nuclear factors of the Kansas lines (KS 34 to KS 39) differed significantly from those of CK (Combine Kafir 60) for agronomic performance like-days to flowering, plant height, grain yield, panicles per plant, seed weight and numbers of seed per panicle.

The nuclear line in isonuclear different cytoplasmic lines [(*Triticum timopheevii* (A/R) and *T. aestivum* (B/R) cytoplasm] showed detrimental effect on the grain weight in hybrids (Zhonggi and Youchun, 1994). The seed set on most of A-lines was shriveled but on F_1 hybrids it was not shriveled. Seed setting in wheat sterile line have been reported to be 30% lower than that of fertile lines and average grain yield of the hybrids was similar and 17 % higher than that of better parent in normal and sterile cytoplasm, respectively (Goral and Spiss, 1997).

Sushil Kumar *et al.* (1996) evaluated various lines in pearl millet, having different sources of cytoplasm which showed substantial differences for one or more of the characters, namely length of the sheath, length of leaf blade, leaf breadth, height at maturity, days to 50% flowering, days to maturity, peduncles length, ear girth, ear length, ear weight, tillers per plant and grain per plant. In general the A_1 cytoplasm source was

the best combiner for earliness and ear weight, the violaceum source was for ear length, A_3 source for ear girth and the A_2 source for tiller number and grain yield.

2.4. MOLECULAR MARKERS AND THEIR IMPORTANCE

There is such an enormous amount of diversity in the DNA of higher plants that no two organisms are likely to be identical in their DNA sequences. Variations have been detected in restricted (*i.e.,* enzymaticly digested) genomic DNA of plants and these restriction fragment length polymorphisms (RFLPs) have paved way for the development of molecular markers (Winter and Kahl, 1995). Genetic engineering and biotechnology hold great potential for application in plant breeding as they promise to reduce the time taken to produce crop varieties with desirable characters. With the use of molecular techniques, it would now be possible to hasten the transfer of desirable genes among varieties and to introduce novel genes from related species (Mohan *et al.,* 1997). Molecular markers detect unambiguous, single-site genetic differences that can easily be scored and mapped in most segregating populations. It is not difficult in populations of most crop species to identify and map 10-50 segregating molecular markers per chromosome pair (Kearsey, 1998). DNA markers (Appendix 5) can increase efficiency in breeding programs in a number of ways:

I. The ability to screen in the seedling stage for traits that are expressed late in the life of the plant.

II. The ability to more efficiently screen for traits that are extremely difficult, expensive, or time consuming to score phenotypicly. Since DNA-based markers themselves have no known effects on the phenotype of the plant, they are ideal for studying quantitative traits (Stuber *et al.*, 1992).

III. The ability to distinguish between the homozygous and heterozygous conditions of many loci in a single generation without progeny testing.

IV. The ability to perform simultaneously, marker-aided selection to screen for a character or complex of characters that could not previously be included in the program because of cost or difficulty of conventional methods based on phenotypic screens.

Molecular markers can accelerate the generation of new varieties and allow association of phenotypic characters with the genomic loci responsible for them. However, the real advantage of using molecular markers is to permit backcross transfer and pyramiding of desirable alleles in a directed manner that would not be practical with conventional phenotypic selection procedures.

Polygenic characters that were previously very difficult to analyze using traditional plant breeding methods can now be readily studied and it is now relatively easy to establish genetic relationships between even sexually incompatible crop species (Mohan *et al.*, 1997). The ability to map genes contributing towards variation in complex traits with enough accuracy to be useful for plant breeding applications has been made possible through the development of comprehensive molecular markers-based genetic linkage maps (Jones *et al.*, 1997).

DNA fingerprinting of the cereals has a very long scientific history. When DNA profiling technology first came into use, restriction fragment length polymorphism (RFLP) markers were considered state-of-an-art. RFLP technology was followed by random amplification of polymorphic DNA (RAPD) method and later by the amplified fragment length polymorphism (AFLP) technique. Most recently microsatellite markers or simple sequence repeats (SSR) have become the preferred marker technology for many plant breeding applications. Advantages of SSR markers are:

- The method is relatively simple and can be automated;
- Most of the markers detect a single locus and show Mendelian inheritance;
- SSR markers are highly informative and reproducible;
- A large number of public SSR primers are available in most major crop species;
- Cost effective per genotype and primer, and avoid use of radioactive material.

2.4.1. Importance of microsatellite (SSR) and its application

Microsatellites, alternatively known as simple sequence repeats (SSRs), short tandem repeats (STRs), simple sequence length polymorphisms (SSLPs), or variable tandem

repeats (VTRs), are tandem repeats of sequence units generally less than 5 bp in length, *e.g.* (TG)n or (AAT)n (Bruford and Wayne, 1993). SSRs have received considerable attention and are probably the current marker system of choice for marker-based genetic analysis and marker-assisted plant breeding (Akkaya *et al.*, 1992; Chin *et al.*, 1996). These markers appear to be hypervariable, in addition to which their co-dominance and reproducibility make them ideal for genome mapping, as well as for population genetic studies (Dayanandan *et al.*, 1998). Inter-SSRs are a variant of the RAPD technique, although the higher annealing temperature probably means that they are more rigorous than RAPDs. Chloroplast microsatellites (cpSSRs), are similar to nuclear microsatellites but the repeat is usually only 1 bp, *i.e.* (T)n (Provan *et al.*, 1999).

The repeat regions are generally composed of di-, tri-, tetra- and sometimes-greater length perfectly repeated, nucleotide sequences (Tautz and Ranz, 1984) that exhibit a high degree of polymorphism (Weber and May, 1989).

Microsatellite variation results from differences in the number of repeat units. These differences are thought to be caused by errors in DNA replication (Moxon and Wills, 1999; Jarne and Lagoda, 1996; Edwards *et al.*, 1992); the DNA polymerase "slips" when copying the repeat region, changing the number of repeats (Jarne and Lagoda, 1996). Larger changes in repeat number are though to be the result of processes such as unequal crossing over (Strand *et al.*, 1993). Such differences are detected on polyacrylamide gels, where repeat lengths migrate different distances according to their sizes or by capillary electrophoresis, where smaller repeat lengths migrate through the column in less time than do larger ones.

Simple sequence repeats are abundant in eukaryotic genomes. They provide a codominant, and usually highly polymorphic marker system (Bryan *et al.*, 1997; Tautz and Ranz, 1984). In plant genomes, the overall frequency of microsatellite repeats appears to be generally lower than animal genomes (Morgante and Olivieri, 1993; Wu and Tanksley, 1993). In general, plants have about 10 times less SSRs than humans (Mohan *et al.*, 1997). The incidence of closely spaced repeats AC or TC is very common, but in plants AT is more common followed by AG or TC.

Microsatellites, which detect variation at individual loci, have been thought of as the "new allozymes". Consequently much of their use has been in studies where

allozymes have previously been used, *e.g.* diversity studies (*e.g.* Rossetto *et al.*, 1999), gene flow and mating systems (Chase *et al.*, 1996), and paternity analysis (Streiff *et al.*, 1999). Rossetto *et al.* (1999) studied the partitioning of variation within and between populations of *Melaleuca alternifolia* (Myrtaceae) to facilitate the identification of genetic resources and assist in the conservation of genetic diversity. Chase *et al.* (1996) studied the gene flow and mating patterns of *Pithecellobium elegans* (Leguminosae) in a forest fragment in Costa Rica, whilst Aldrich *et al.* (1998) analysed the genetic structure and diversity of fragmented populations of *Symphonia globulifera* (Clusiaceae). However, there are few phylogenetic studies that use microsatellite markers, perhaps because few microsatellite markers are transferable across species (Sorrells *et al.*, 2003). Many microsatellite studies appear to be expansions of groups that have been studied using biochemical or molecular markers. Rossetto *et al.* (1999) study on genetic structure in *Melaleuca alternifolia* is an expansion of allozyme studies by Butcher *et al.* (1992), albeit Rossetto *et al.* (1999) used a greater number of individuals and populations.

Unique sequences that flank the tandem repeats can be used as highly polymorphic probes or for making PCR primers. There are well-established methods of finding microsatellites by screening phage libraries with oligonucleotide probes. But a quicker, if limited, approach is to examine sequence data banks for their presence (Burr, 2001). SSR-based primers representing tri-, tetra- and penta-nucleotide repeats have been used successfully to generate distinct banding patterns that are resolvable on low-resolution agarose gels using ethidium bromide staining (Gupta *et al.*, 1994; Weising *et al.*, 1995), on high-resolution polyacrylamide gels by silver staining (Buscot *et al.*, 1996), through primer radioactive labeling followed by autoradiography (Gupta *et al.*, 1994), or through primer labeling with fluorescent dyes and automated high-resolution visualization of PCR products separated by PAGE or capillary electrophoresis (Steve Kresovich *et al.*, 1994). As would be predicted, better product size discrimination is obtained with polyacrylamide-based gel analysis although agarose gel is sufficient for many applications (Vogel and Scolnik, 1997). Further, automated high-resolution visualization of dye-labeled PCR products allows effective size discrimination of 1bp.

In any case, SSRs are generally among the most reliable and highly reproducible of molecular makers. Indeed SSRs are now widely recognized as the foundation for many framework linkage maps. SSRs have played a critical role even in merging disparate

linkage maps (Bell and Ecker, 1994; Akkaya *et al.*, 1995) since they define specific locations in the genome unambiguously (Young, 2001). These markers can require considerable investment to generate but are then inexpensive to use in mapping and MAS. The large start up costs for this technique should be justifiable for crops where large-scale mapping and MAS are a practical necessity (Hash and Bramel-Cox, 2000).

Post-PCR multiplexing involves the simultaneous separation of PCR amplification products of several SSR loci in a single gel lane (Masi *et al.*, 2003). Simplex PCR conditions were optimized for each primer pair by first testing different cycling conditions and then varying (1) the amount of DNA template, (2) the concentration of primers, and (3) the concentration of $MgCl_2$, and (4) the amount of *Taq* DNA polymerase. To optimize cycling conditions, three PCR Programs (A, B and C) were tested with different times for annealing and extension, and for variation in both temperatures and number of cycles.

Reddy *et al.* (2002), in their review, have reported an Inter Simple Sequence Repeat (ISSR)-PCR technique that involves the use of microsatellite sequences as primers in polymerase chain reactions to generate multilocus markers. It is a simple and quick method that combines most of the advantages of SSRs and amplified fragment length polymorphism to the universality of random amplified polymorphic DNA (RAPD). ISSR markers are highly polymorphic and are potentially useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology.

2.4.2. Importance of RFLP and its application

Among the various DNA-based molecular markers, RFLPs were the first to be used in human genome mapping (Botstein *et al.*, 1980) and later they were adopted for plant genome mapping (Helentjaris *et al.*, 1986a; Bernatzky and Tanksley, 1986; Helentjaris, 1987; Paterson *et al.*, 1988; McCouch *et al.*, 1988; Weber and Helentjaris, 1989). RFLP is the most reliable DNA polymorphism that can be used for accurate scoring of genotypes across different species that are sexually incompatible. It has provided a relatively rapid (for its time) means of producing genetic maps of densely spaced marker loci in numerous crop species (Ellis, 1986; Helentjaris *et al.*, 1986a; Landry *et al.*, 1987; Burr *et al.*, 1988; Mohan *et al.*, 1997). The four primary advantages of RFLP markers over morphological markers are co-dominance, frequent polymorphism, absence or limited

influence of the environment, and absence of pleiotropic effects (Botstein *et al.*, 1980; Beckmann and Soller, 1983).

RFLP analysis employs cloned DNA sequences to probe specific regions of the genome for variations that are seen as changes in the length of DNA fragments produced by digestion with restriction endonucleases (Landry *et al.*, 1987).

Prior to the availability of SSR markers, two types of DNA markers have been most commonly used for most crop plant molecular marker-based linkage map developed and subsequent QTL mapping: RFLP markers (Botstein *et al.*, 1980) and RAPD markers (Williams *et al.*, 1990). Both detect DNA polymorphism and monitor the segregation of a DNA sequence among progeny of a genetic cross permitting construction of a genetic linkage map. However, co-dominant RFLP markers are more robust and repeatable than RAPD markers, which are inherited in a dominant manner.

RFLP and RAPD marker differences between plants are inherited in the same fashion as conventional Mendelian genes, thus genetic linkage maps of these molecular markers can be constructed using conventional methods. Such RFLP-based linkage maps indicate the location of specific restriction sites of chromosomal DNA relative to one another.

RFLP and morphological markers have been used in practical plant breeding programs to map quantitative trait loci (QTLs) (Tanksley *et al.*, 1982; Edwards *et al.*, 1987; Stuber *et al.*, 1987; Weller *et al.*, 1988; Mohan *et al.*, 1997) and to monitor response to recurrent selection (Stuber *et al.*, 1980, 1982). RFLP markers facilitate the selection of progenies with desirable genotypes in a relatively short span of time. However RFLP analysis is labour intensive and time consuming compared to analysis of DNA markers based on the polymerase chain reaction (Mohan *et al.*, 1997).

Costs of applying RFLPs to genetic improvement were assessed by Beckmann and Soller (1983) in terms of individuals and numbers of polymorphisms per individual that are scored for various applications including varietal identification, identification and mapping of quantitative trait loci and their marker-assisted introgression from source strain to commercial variety. Hash (1991), Gale and Witcombe (1992), Hash *et al.* (1997, 1999) and Hash and Bramel-Cox (2000) emphasized the opportunities for potential use of RFLPs in plant breeding with particular reference to downy mildew resistance in pearl

millet. A number of papers suggest that the use of RFLPs and other DNA-based markers offers a clear advantage in breeding for important qualitative and quantitative traits (Edwards *et al.*, 1987; Melchinger, 1990; Paterson *et al.*, 1991b; Arunachalam and Chandrashekaran, 1993; Mohan *et al.*, 1997; Young, 1999).

2.5. DEVELOPING A MAPPING POPULATION

The most critical decisions in constructing linkage maps with DNA markers are those made in developing the mapping population. In making these decisions, several factors must be kept in mind, the most important of which is the goal of the mapping project. Young (1994) reviewed the most important factors for a mapping project the success or failure of which is mainly dependent on which parents are chosen for crossing, the size of the population, how the cross is advanced, and which generations are used for DNA and phenotypic analysis. Hash and Witcombe (1994) described the procedures being used for development and multiplication of pearl millet mapping populations, the parentage of mapping populations then available and the traits for which they might be used for QTL mapping. Linkage maps of crop species are often constructed with segregating populations *i.e.* F_2 populations or backcrosses (Sunil, 1999)

2.5.1. DNA polymorphisms among parents

Sufficient detectable DNA sequence polymorphism between parents must be present. This cannot be over-emphasized, for in the absence of detectable DNA polymorphism, segregation analysis and linkage mapping are virtually useless. However, in many allogamous species, any cross that does not involve related individuals will provide sufficient polymorphism for mapping (Helentjaris, 1987). Miller and Tanksley (1990) reported that in naturally inbreeding species the levels of DNA sequence variations are generally low and finding suitable DNA polymorphism can be more challenging. The requirement for sufficient DNA sequence polymorphism may preclude the use of DNA markers in some narrow-based crosses (Young, 1994).

More recently developed technologies, like electrophoresis systems capable of separating DNA molecules with only a single base pair change (Riedel *et al.*, 1990), provide better methods for uncovering polymorphisms within narrow-based crosses, Probes based on minisatellites (Dallas, 1988) or simple repeated tetra-nucleotide motifs (Weising *et al.*, 1989) can uncover polymorphisms between closely related individuals.

Because these are so variable at the DNA sequence level, such sequences are likely to eventually provide markers useful for mapping in narrow-based crosses (Hüttel *et al.*, 1999; Winter *et al.*, 1999; Choumane *et al.*, 2000).

2.5.2. Choice of segregating population

Once suitable parents have been identified, the type of genetic population to be used for linkage mapping must be considered. Several different kinds of genetic populations are suitable. The simplest are the F_2 population derived from a true F_1 hybrid, and their backcross populations. For most plant species, populations such as these are easy to construct, although sterility in the F_1 hybrid can limit some combinations of parents, particularly in wide crosses. The major drawback to F_2 and backcross populations is that they are ephemeral, that is seed derived from selfing these individuals will not breed true. It is difficult or impossible to measure characters as part of QTL mapping in several locations or over several years with F_2 or backcross populations (Young, 1994). Soller and Beckmann (1990) describe advanced generation progeny-based phenotyping of F_2-genotyped individuals. Based on this, Hash and Witcombe (1994) described a method for developing and maintaining a pearl millet mapping population based on F_2 plants derived by selfing a single F_1 plant that will provide an "immortal" mapping population available for several seasons. The uses of inbred populations comprised of recombinant inbred lines (RILs) derived from individual F_2 plants are an excellent strategy to provide more permanent mapping resources (Burr *et al.*, 1988; Burr and Burr, 1991). Similar types of inbred populations, such as doubled haploids, can also be used for linkage mapping with many of the same advantage of RILs (Heun *et al.*, 1991). A doubled haploid population is only a form of RIL population differing from conventional RIL populations in the procedure used to produce it.

2.6. LINKAGE MAPPING

Linkage mapping is putting marker loci (and QTLs) in order, indicating the relative distances among them, and assigning them to their linkage groups on the basis of their recombination values from all pair-wise and three-point combinations. The first linkage map of the human genome based on molecular markers (Botstein *et al.*, 1980) fuelled the development of molecular marker-based genome maps in other organisms.

2.6.1. The basis of linkage mapping

The theory of linkage mapping is same for DNA markers as in classical genetic mapping; however, several new considerations must be kept in mind. This is primarily a result of the fact that potentially unlimited numbers of DNA markers can be analyzed in a single mapping population. DNA-based maps can be related to existing cytogenetic maps through the use of aneuploid or substitution lines (Helentjaris *et al.*, 1986b; Sharp *et al.*, 1989; Young *et al.*, 1987) or *in situ* hybridization (ISH) (Zhang *et al.*, 2000).

Since DNA marker technology was first applied to plants, there has been an explosion in the development and application of genetic linkage maps (Mohan *et al.*, 1997). Using these new DNA based markers, scientists have constructed maps in species where only poorly populated classical maps existed before (Bonierbale *et al.*, 1988; Gebhardt *et al.*, 1991; Liu *et al.*, 1994), located genes governing quantitative characters, often in great detail, and gone on to attempt (sometimes successfully) gene cloning based on genetic map position. Detailed genetic linkage maps are also fundamental tools for studies on selection, identification and organization of plant genomes (Beckmann and Soller, 1986; Landry and Michelmore, 1987; Tanksley, 1993).

2.6.2. Success achieved so far

2.6.2.1. In crops other than pearl millet

Using RFLPs as genetic markers, Helentjaris *et al.*, (1986a) constructed linkage maps for maize and tomato. The first true RFLP-based genetic linkage map in a crop plant (tomato) was constructed in 1986 with only 44 F_2 plants and 57 marker loci (Bernatzky and Tanksley, 1986). Since then, DNA marker-based genetic linkage maps for many plants species have been constructed (Helentjaris, 1987; McCouch *et al.*, 1988; Heun *et al.*, 1991; Tanksley, 1993; Mohan *et al.*, 1997).

Landry *et al.* (1987) constructed a detailed map of lettuce, using 53 genetic markers. These included 41 RFLP loci, 5 downy mildew resistance genes, 4 isozyme loci and 3 morphological markers covering 404 cM.

McCouch *et al.* (1988) reported the construction of an RFLP-based genetic linkage map of rice. The map was comprised of 135 loci corresponding to clones selected from a *Pst*I genomic library covering 1389 cM of the rice genome. Causse *et al.* (1994)

developed a rice genetic map using ca. 800 RFLPs that expanded the length of the rice linkage map to 1491 cM. Chao *et al.* (1989) attempted RFLP mapping in wheat (*Triticum aestivum*) using 18 cDNA clones: 14 anonymous and 4 known functions. The loci identified by these probes were mapped on one or more of wheat homeologus group 7 chromosomes. Graner *et al.* (1991) analyzed two populations to construct an RFLP-based genetic linkage map of barley using 250 genomic and cDNA markers. Maps of chromosomes 3A, 3B and 3D of wheat and 3R of rye were developed by Devos *et al.* (1992) using 22 DNA probes and 2 enzyme marker systems.

2.6.2.2. In pearl millet

The first detailed molecular marker-based genetic linkage map of pearl millet was published in 1994, and was comprised primarily of RFLP markers (Liu *et al.*, 1994). They placed 181 loci on a linkage map by studying segregation in two F_2 populations. Two crosses (LGD 1-B-10 × ICMP 85410 and Tift $23D_2B_1$ × IP 18292) were employed. The total length of this map, which comprised the seven expected linkage groups, was only 303 cM and the average distance between loci was about 2 cM. The individual linkage groups (LG) varied in length from 90 cM for LG1 to only 30 cM for LG6 (Devos *et al.*, 1995).

Genetic linkage maps in pearl millet have been constructed and quantitative trait loci (QTLs) have been identified and mapped for downy mildew resistance (Jones *et al.*, 1995, 2002; Breese *et al.*, 2002), rust and blast resistance (Morgan *et al.*, 1998) drought tolerance and grain yield (Yadav *et al.*, 2002; 2003; 2004) and for characters involved in domestication (Poncet *et al.* 2000; 2002). An integrated genetic linkage consensus map for this crop has recently been accepted for publication (Qi *et al.*, 2004).

The integration of markers previously mapped in other grass species has provided the anchor points to align the pearl millet linkage groups to other cereal genetic maps, including the cereal model, rice. Although the pearl millet genome appears to be relatively highly rearranged relative to rice, regions of colinearity between the two species can clearly be identified (Devos *et al.*, 2000). These now form a framework for exploitation of the rice genomic sequence as a source of new markers and candidate genes underlying traits in pearl millet.

The pearl millet genetic linkage map originally reported by Liu *et al.* (1994), is unusual among grass genomes in that it is particularly short, but this difference is expected to reduce with time. Subsequent studies have extended the length of the pearl millet genetic linkage map to circa 700 Haldane cM, but to date no significant linkage has been detected between the marker loci in these seven linkage groups and telomeric sequences that are expected to cap the ends of each (Katrien M. Devos, pers. comm.). This suggests that the pearl millet genetic linkage map will eventually extend to at least 1400 cM (Haldane).

2.6.3. Computer software packages for genetic linkage mapping

Advances in computer technology have been essential to progress in DNA marker-based genetic linkage maps. The theory behind linkage mapping with DNA markers is identical to mapping with classical genetic markers, but the complexity of the problem has dramatically increased because of the larger numbers of markers that can and must be used. This increase in numbers of segregating loci (and the number of progenies in which they are segregating) relative to studies of classical genetic markers has necessitated the development of complex computer algorithms and software packages specifically for this purpose.

Construction of a genetic linkage map from a DNA marker data set requires computer software packages capable of running κ^2 contingency table analysis. The program LINKAGE-1 (Suiter *et al.*, 1983) carries out this type of analysis automatically and also compares the observed allelic distributions to expected distributions. In a different strategy for optimizing the use of DNA marker information, the computer program "Hyper Gene" converts genotypic data into a "graphical genotype" (Young and Tanksley, 1989a, b). In this complete genome of an individual from the mapping population is displayed.

MAPMAKER/EXP is a linkage analysis software package for constructing primary linkage maps of markers segregating in experimental crosses. It performs full multipoint linkage analysis for dominant, recessive and co-dominant (*e.g.* RFLP-like) markers in BC_1 backcrosses, F_2 and F_3 (self) intercrosses and recombinant inbred lines (Lander *et al.*, 1987; Lincoln *et al.*, 1992a, b).

The software package Join Map (Stam, 1993; Stam and Van Ooijen, 1995) can be used to analyse all types of mapping populations and can combine maps of different mapping populations provided there are common markers. Another software for linkage mapping is Gmendel from Oregon State University, USA (Holloway and Knapp, 1994). The package MapMaker, with different versions such as QTS, QTXP and QTX-Classic for Macintosh- and IBM-compatible computers (Manly, 1993; Manly and Olsen, 1999), can be used to analyses the results of genetic mapping experiments using backcrosses or recombinant inbred lines.

2.7. MAPPING QUANTITATIVE TRAIT LOCI (QTLs)

2.7.1. The basis of QTL

A "QTL", the acronym for Quantitative Trait Locus, is one of the genes or gene blocks that underlie quantitative traits (Gelderman, 1975). Before the discovery of molecular markers, QTLs were referred to as polygenes (Mather, 1949). QTL analysis is predicated on associations between phenotypic values for the quantitative trait and the marker alleles segregating in the mapping population. It has two essential stages: the mapping of markers and the association of the trait phenotype values with the marker genotypes. The basic theory underlying marker mapping has been available since 1920.

Sax (1923) first reported association of simply inherited genetic markers with a quantitative trait in plants when he observed segregation for seed size associated with segregation for a seed coat colour marker in beans (*Phaseolus vulgaris* L.). Rasmusson (1935) demonstrated linkage of flowering time (a quantitative trait) in peas (*Pisum sativum* L.) with a simply inherited gene for flower color. Everson and Schaller (1955) found morphological markers that flanked a chromosomal region affecting yield in barley (*Hordeum vulgare* L). Hash and Blake (1981) reported variation in prolamin seed storage proteins conditions by genes at the highly polymorphic loci *Hor1* and *Hor2,* flanking the multiallelic *Mla* locus for resistance to powdery mildew, and suggested their use as selectable markers for specific resistance alleles by half-seed screening of segregating progenies.

Extensive work in *Drosophila melanogaster* (Mather and Harrison, 1949) demonstrated the effects of individual chromosomes on quantitative traits. Cavalli (1952) crossed lines of *D. melanogaster* selected for high and low abdominal bristle number, and

found evidence of linkage between polygenes. Harrison and Mather (1950) and Gibson and Thoday (1962) by selection experiments in *D. melanogaster*, were able to locate polygenes for bristle number on a particular chromosome. Thoday (1961) developed methods for detecting linkage of polygenes with marker loci. In domesticated animals, associations of quantitative traits with segregation for blood group markers have been reported (Niemann-Sorenson and Robertson, 1961). In wheat (*Triticum aestivum* L.) monosomics have been used to identify association of quantitative traits with individual chromosomes (Law, 1967). These earlier studies provided a background of theory and observation for more recent work with molecular markers (Dudley, 1993).

The first use of a reasonably complete crop linkage map based on RFLP markers was reported in tomato by Paterson *et al.* (1988). They resolved quantitative traits to discrete Mendelian factors in an inter-specific backcross of tomato, mapping at least six QTLs controlling fruit mass and four QTLs for soluble solids.

2.7.2. QTL mapping for disease resistance

With DNA markers and QTL mapping, complex forms of disease resistance and their underlying genes are now far more accessible to applied plant breeders and pathologists. Quantitative genetics is unsuited for dissecting polygenic resistance characters into discrete genetic loci or defining the roles of individual genes in disease resistance. With QTL mapping, the role of specific resistance loci can be described, race-specificity of partial resistance genes can be assessed, and interactions between resistance genes, plant development and the environment can be analyzed (Melchinger, 1990; Young, 1996).

The quantitative host-plant resistance system for rice blast caused by *Pyricularia oryzae* has been especially well characterized (Wang *et al.*, 1994). Two dominant qualitative resistance loci were identified on chromosomes 4 and 11 of rice (Yu *et al.*, 1991). Another disease system that has been studied with QTL mapping is late blight of potato caused by *Phytophthora infestans.* Leonards-Schippers *et al.* (1994) identified eleven genomic segments on nine chromosomes that were associated with host plant resistance to potato late blight.

Inheritance of disease reaction to leaf spot caused by *Cercospora zeae-maydis* in three maize F_2 populations was examined to study quantitative resistance using RFLP

markers (Bubeck *et al.*, 1993). One QTL on maize chromosome 2 was found to be significantly associated with resistance in all three populations.

A study of resistance to bacterial wilt caused by *Pseudomonas solanacerarum* in tomato was reported by Danesh *et al.* (1994) using DNA marker genotypes and disease resistance reactions for 71 F_2 individuals. Two genomic regions were significantly associated with resistance, one on chromosome 6 and another on chromosome 10. Loci contributing towards quantitative variation have been mapped in tomato for resistance against insects (Nienhuis *et al.*, 1987), in potato for resistance against cyst nematode (Kreike *et al.*, 1993), in peas for resistance against ascochyta blight (Dirlewanger *et al.*, 1994), in maize for resistance to northern corn leaf blight (Freymark *et al.*, 1993), stalk and ear rot (Pê *et al.*, 1993), and in Sorghum for resistance to green bug (Agrama *et al.*, 2002).

Manzanares-Dauleux *et al.* (2000) identified QTLs against clubroot disease of *Brassica napus* caused by *Plasmodiophora brassicae*. Inheritance of *Cercospora* leaf spot resistance in sugar beat was studied by Nilsson *et al.* (1999) and they identified QTLs for this trait. Four QTLs associated with *Cercospora* resistance on chromosomes III, IV, VII and IX were revealed using composite interval mapping (Setiawan *et al.*, 2000). Four QTLs were localized for leaf rust *(Puccinia hordei)* resistance in barley, which explained 96% of the segregating genetic variation in the mapping population studies (Kicherer *et al.*, 2000). Brown stem rot (*Phialophora gregata*) resistance QTLs were identified by Lewers *et al.* (1999) in a RIL mapping population of soybean using 146 RFLPs, 760 AFLPs and 4 probes for resistance gene analogs (RGAs).

2.7.3. QTL analysis: statistical methods

Jayakar (1970) suggested mathematical-statistical methods for the detection and estimation of linkage between a qualitative marker gene and a locus influencing a quantitative character. Since then, experimental designs for determination of linkage between marker loci and QTL have been widely described (Elston and Stewart, 1971; Gelderman, 1975; Hill, 1975; Soller and Beckmann, 1983, 1990; Jensen, 1989; Lander and Botstein, 1989; Knapp *et al.*, 1990).

Marker-QTL association detection can be conducted through t-tests based on single markers (Soller *et al.*, 1976) or by means of likelihood ratio tests that involve that

use of a pair of markers bracketing a QTL, a procedure termed 'Interval Mapping' (Weller, 1987; Jensen, 1989; Lander and Botstein, 1989; Knapp *et al.*, 1990), although simpler approaches are also possible (Thoday, 1961; Weller, 1987; Haley and Knott, 1992).

Lander and Botstein (1989) described a set of analytical methods that modify and extend the classical theory for mapping QTLs and that are implemented in the computer software package MAPMAKER/QTL. In this, interval mapping is applied to several population types. Each interval between adjacent pairs of markers along a chromosome is scanned and the likelihood profile of a QTL being at any particular point in each interval is determined.

Michelmore *et al.* (1991) used a modification of "conventional QTL mapping" to detect QTLs for downy mildew resistance in lettuce in a procedure they called "bulk segregant analysis", which is remarkably similar to that previously described by Burton and Wells (1981) for assessing the value of a trait in near-isogenic F_3 populations of pearl millet.

Prioul *et al.* (1997) described the genetic methods required to analyze possible associations between traits that are inherited in a quantitative manner using QTL analysis. Advantages and some limitations of QTL analysis over other methods then in use by plant physiologists to test associations between traits were also discussed.

Particularly in the case of cross-pollinating crop populations, interval mapping has been enhanced to "all marker mapping". To calculate the likelihood of a segregating QTL, the segregation information of all linked markers is employed. Each segregating marker may follow a different segregation type, with two to four alleles (Maliepaard and van Ooijen, 1994).

An alternate approach was developed for QTL analysis using regression by Knapp *et al.* (1990) and Haley and Knott (1992). It produces results very similar to interval mapping both in terms of accuracy and precision, but has the advantage of speed and simplicity of programming. This method used the coefficient of regression of the phenotype on the genotype of the different markers (Martinez and Curnow, 1992; Wu and Li, 1994). A significant regression coefficient is indicative of an association between the marker locus and gene(s) contributing to phenotypic difference.

Estimating the location and the size of effects of QTLs using flanking markers was discussed by Martinez and Curnow (1992) in the framework of a backcross using a regression model as the analytical tool. Conneally *et al.* (1985), in the field of linkage analysis, proposed the use of confidence intervals based on limits of the χ^2 distribution of the likelihood ratio test between two positions. This idea leads to a very simple construction of the confidence interval. Mangin *et al.* (1994) described a method for construction the confidence interval of the QTL location parameter, developed in the local asymptotic framework, leading to a linear model at each position of the putative QTL.

Kearsey and Hyne (1994) developed the marker regression approach. It attempts to model to all the marker means on a given chromosome simultaneously, and obtains significance tests by weighted least squares or by simulation. The method involves regressing the additive difference between the marker genotype means at a locus against the function of the recombination frequency between the locus and the putative QTL.

Two classical approaches used for QTL detection are marker-by-marker ANOVA and multiple marker methods. The principle of the ANOVA is to test whether there are significant differences between the phenotypic means of genotypes classes at a particular marker locus (Prioul *et al.,* 1997). van Ooijen (1999) presented methods that provide reasonably accurate approximations to LOD significance thresholds for QTL analysis, which were obtained by large-scale simulations. Churchill and Doerge (1994) described an empirical method, based on the concept of permutation tests, for estimating threshold values for declaring significant QTL effects.

2.7.4. QTL mapping software

Normally all QTL mapping software require input of the data for

1. the quantitative trait value(s) for each progeny
2. the genotype (molecular markers) for each progeny

There are over one hundred genetic analysis software packages available. Here is the brief list of some commonly used software packages:

MAPMAKER/QTL (*ftp://genome.wi.mit.edu/pub/mapmaker3/*) is the original QTL mapping software for Macintosh and IBM computers (Lincoln *et al.,* 1992b). It is

user-friendly, freely distributed, and runs on almost all platforms. It will analyze F_2 or backcross data using standard interval mapping procedures.

MQTL is an IBM-compatible computer program for composite interval mapping in multiple environments (van Ooijen and Maliepaard, 1996). It can also perform simple interval mapping. Currently, MQTL is restricted to the analysis of the data from homozygous progeny (doubled haploids or recombinant inbred lines). Progeny types with more than two marker classes (*e.g.* F_2) are not handled.

PLABQTL *(http://www.uni-hohenheim.de/~ipspwww/soft.html)* is a freely distributed IBM-compatible computer program for composite interval mapping and simple interval mapping of QTLs (Utz and Melchinger, 2000; Utz *et al.*, 2000). Its main purpose is to localize and characterize QTLs in mapping populations derived from a biparental cross by selfing or production of double haploids. Currently, this program is the easiest software to use for composite interval mapping.

QTL Cartographer *(http://statgen.ncsu.edu/qtlcart/cartographer.html)* is a QTL-mapping package written for UNIX, Macintosh, or Windows computer operating systems. It performs single-marker regression, interval mapping, and composite interval mapping. It permits analysis of F_2 or backcross populations. It displays map positions of QTLs using the GNUPLOT software. QTL Cartographer was developed by the group of Zeng in USA (Zeng, 1993, 1994; Basten *et al.*, 1994, 1997). It allows markers to be chosen as cofactors to reduce the background genetic noise and increase the resolutions of QTL detection. This is an effective strategy for improving the ability to detect QTLs of small effect provided that the number of progenies in the mapping population is reasonably large.

MapQTL (http://www.cpro.dlo.nl/cbw/) is a similar composite interval mapping methods package has been developed by Jansen and co-workers (Jansen, 1993; Jansen and Stam, 1994) called multiple QTL modeling (MQM).

Multimapper (Sillanpaa and Arjas, 1988), based on Bayesian modeling and inference, treats the number of quantitative trait loci as an unobserved random variable using ideas similar to composite interval mapping. This method is introduced for inbred lines and it can be applied also in situations involving frequent missing genotypes.

Qgene is a QTL mapping and marker-aided breeding package written for Macintosh computer operating systems. It has a user-friendly graphical interface and produces graphical outputs. QTL mapping is conducted by either single-marker regression or interval regression.

QTLSTAT is based on interval mapping using nonlinear regression for F_2, backcross, RIL and DH populations and outputs results in graphical form (Knapp *et al.*, 1992; Liu and Knapp, 1992).

PGRI calculates based on the functions of t-test, conditional t-test, linear regression, multiple QTL modeling, and permutation tests (Lu and Liu, 1995). It is for F_2, backcross, RIL, heterozygous F_1 and open-pollinated populations.

SAS (SAS, 1999) is a general statistical analysis software package. It can detect QTL by identifying association between marker genotype and quantitative trait by single marker analysis approaches such as ANOVA, t-test, and regression (eg. PROC ANOVA, PROC GLM or PROC REG).

2.7.5. Selective genotyping and QTL reliability estimates

Selective genotyping (Darvasi and Soller, 1992; Lander and Botstein, 1989; Lebowitz *et al.*, 1987) was suggested as a design that can reduce the number of individuals genotyped for a given power of QTL detection, by genotyping only the most informative individuals in the experimental population. Genotyping only individuals from high and low phenotypic tails of the entire sample population, the number of individuals genotyped for a given power can be decreased considerably, at the expense of an increase in the number of individuals phenotyped (Lebowitz *et al.*, 1987).

Muranty and Goffinet (1997) extended the concept of selective genotyping to multiple trait QTL mapping, showing that selection on one trait can increase the power of QTL detection for a correlated trait. Adequate power, precision and accuracy of QTL analysis can only be expected from a large well suited mapping population, using a marker set with good genome coverage and phenotypic values based on multi-environment trials (van Ooijen, 1992; Utz and Melchinger, 1994; Beavis, 1998).

Such selective genotyping is a cost-effective strategy in mapping QTLs. When the proportion of individuals selected for genotyping is low, the majority of the individuals

are not genotyped, but their phenotypic values, if available, are still included in the data analysis to correct the bias in parameter estimation using an expectation-maximization (EM) algorithm (Xu and Vogl, 2000; Vision *et al.*, 2000).

Three percent error rates in genotyping can double estimates of genetic map distance (Brzustowicz *et al.*, 1993). The quality of marker data from the segregating population is very important to the success or failure of the QTL analysis. There was a great surprise when the map length of species as maize and wheat suddenly increased with the advent of molecular markers beyond the lengths predicted from chiasma frequency (Nilsson *et al.*, 1993), but corrections of errors in marker genotyping data sets have largely done away with these artifactual surprises.

Kearsey and Farquhar (1998) reported that the available analytical methods locate QTL with poor precision unless the heritability of a particular trait is high. Also the estimates of the QTL effects, particularly the dominance effects, tend to be inflated because only large estimates are detected as being statistically significant. This is especially problematic where size of a mapping population is less than optimal (as it usually is).

Darvasi *et al.* (1993) showed that the power of detecting a QTL was virtually the same for a marker spacing of 10 cM as for an infinite number of markers and was only slightly decreased for marker spacing of 20 cM or 50 cM. However, a very important consideration is the confidence interval for the QTL position on the linkage group. Effective utilization of molecular marker technology to manipulate loci controlling quantitative traits is considered to be dependent on tight linkage between the marker(s) and the QTL (Dudley, 1993), but in fact, even loose linkages can be exploited in an applied breeding program (Sharma, 2001).

In most published QTL studies, the number of QTLs is considerably under estimated and the percentage of genetic variation explained by markers is highly erratic and often over-estimated (Lynch and Walsh, 1998). These problems can be overcome by backcross transfer of putative QTLs to near-isogenic backgrounds and/or QTL mapping in independent (and large) samples of the mapping population for verification studies of any putative QTLs detected. An additional need is to verify estimated QTL effects and

the possible epistatic interactions of QTL alleles with the genetic background of the material to be improved (Phillips, 1999; Kerns *et al.*, 1999).

Hackett (1997) described diagnostic tools based on residuals, likelihood profiles and regression coefficients for fitting QTL models. These are used to assess the agreement between linkage data and fitted normal mixture models for interval mapping.

Nearly every agronomic trait imaginable has been subjected to DNA marker mapping and QTL analyses: *e.g.*, drought tolerance (Martin *et al.*, 1989; Yadav *et al.*, 2002, 2004), seed hardness (Keim *et al.*, 1990), seed size (Fatokun *et al.*, 1992), maturity and plant height (Lin *et al.*, 1995; Yadav *et al.*, 2003), disease resistance (Jones *et al.*, 1995, 2002; reviewed by Young, 1996), oil and protein content (Diers *et al.*, 1992), soluble solids (Paterson *et al.*, 1988), and, of course, yield (Stuber *et al., 1987; Yadav et al., 2003*). Even when a well-performed mapping experiment indicates promising QTLs, there is often much more that needs to be done to make the mapping results ready for application in marker-assisted selection (MAS). Repetition over several years and several locations, repetition in genetically unrelated populations, and detailed analysis in marker-generated populations that isolate the effects of individual QTLs, are factors to increase the efficiency and reliability of use of QTLs in applied plant breeding programs (Young, 1999). However delay in their use can be as costly as using them too soon, so several alternative strategies for application of marker-assisted selection to backcross improvement of elite inbred lines have been described by Hash *et al.* (2000) and Hash (2000) to speed up adoption of this technology while minimizing cost and risk.

For marker-assisted selection (MAS) to be most effective, reliable estimates of QTL positions and effects are required. Adequate power, precision and accuracy of QTL analyses can only be expected from large, well-suited mapping populations, using a marker set with good genome coverage, and phenotypic values based on multi-environment trials (van Ooijen, 1992; Utz and Melchinger, 1994; Beavis, 1998). In verification studies with maize, Melchinger *et al.* (1998) found that 50% or less of variance attributable to markers in the calibration experiment could be recovered in an independent sample of progenies of the same initial F_2 population. Such uncertainties of QTL analyses have the potential to seriously reduce the efficiency of MAS. Verification of individual QTLs, e.g. by re-estimation in advanced generations or by evaluating near-

isogenic backcross-derived lines (NILs) contrasting for genome segments of interest (Romagosa *et al.*, 1999), is therefore imperative.

2.8. QTL FOR DOWNY MILDEW RESISTANCE IN PEARL MILLET

The first fairly detailed molecular marker map for pearl millet was constructed by Liu *et al.* (1994) so that QTL analysis is now possible. QTLs for host-plant resistance to downy mildew caused by *S. graminicola* pathogen populations from India, Nigeria, Niger, and Senegal were mapped using the susceptible × resistant cross LGD 1-B-10 × ICMP 85410 (Jones *et al.*, 1995). Host-plant resistance QTLs were detected that were effective against each of the four pathogen populations. To locate genes in mapping populations other than those for which marker-based genetic linkage maps exist, a skeleton map needs to be transferred. In pearl millet less than 40 single-copy probe-enzyme combinations will produce an RFLP-based skeleton map with an average map distance of less than 15 cM between marker loci (Liu *et al.*, 1994).

Jones *et al.* (2002) demonstrated that field screening and greenhouse pot screening of seedlings against the same pathogen population detect the same QTLs for host-plant resistance to pearl millet downy mildew using F_2-derived F_4 self bulk progenies of a mapping population derived from a cross of resistant line P7-3 and susceptible line 7042(S).

Breese *et al.* (2002) reported QTLs for downy mildew resistance from a pearl millet mapping population (originally intended to map seedling heat tolerance) produced from this cross ICMP 451 × H 77/833-2. Hash *et al.* (unpublished) worked with mapping populations from crosses PT 732B × P1449-2, 81B × ICMP 451 and 841B × 863B to locate additional QTLs for resistance to pearl millet downy mildew. QTLs for host-plant resistance effective against African and Indian pathogen populations of *Sclerospora graminicola*, the casual organism of pearl millet downy mildew, were identified in a new mapping populations based on cross W 504 × P 310-17 (Kolesnikova-Allen, 2001), and cross Tift 238D1 × IP 18293 (Azhaguvel, 2001). To date over 65 QTLs for pathogen-population-specific host plant resistance to pearl millet downy mildew have been detected (C.T. Hash, pers. Comm.)

A number of QTLs have been identified and located (Nepolean, 2002) for grain yield and its component characters using F_4 bulk testcrosses, and downy mildew disease

resistance and F_4 self bulks from an F_2–skelton mapped population derived from the cross PT 732B × P1449-2.

2.9. PEARL MILLET GENETIC LINKAGE MAP

Molecular markers also have become a powerful tool for marker-aided selection of disease resistant crop genotypes (Howarth *et al.*, 1994). Morgan *et al.* (1998) screened three segregating population for RAPDs using random decamer primers and for RFLPs and observed that rust resistance gene Rr_1 from pearl millet subspecies *P. glaucum* ssp. *monodii* was linked 8.5 cM from the RAPD OP-$G8_{350}$. The linkage of two RFLP marker loci *Xpsm*108 (15.5 cM) and *Xpsm*174 (17.3 cM), placed the Rr_1 gene on linkage group 3 of the pearl millet map and rust resistance genes from both Tift $89D_2$ and ICMP 83506 were placed on linkage group 4 by linkage to RFLP marker locus *Xpsm*716 (4.9 and 0.0 cM respectively).

Allouis *et al.* (2001) have constructed a bacterial artificial chromosome (BAC) library using nuclear DNA from pearl millet and this was used as a resource for the isolation of microsatellite markers. In future this BAC library may be used for map based cloning of downy mildew resistance genes. Using methods described by Qi *et al.* (2001), so far 42 (GT) and 8 (CT) microsatelites have been isolated from BAC clones pooled from a single 384-well microtiter plate. The primer sequence, BAC origin, SSR repeat and PIC values obtained in panel of 20 pearl millet inbred lines are given Qi *et al.* (2001) for SSR markers PSMP2201-PSMP2225 and in Allouis *et al.*, (2001) for SSR markers PSMP2224-PSMP2274. The SSR profiles of these 20 pearl millet inbred lines are available from MilletGene (http//jic-bioinfo.bbsrc.ac.uk/cereals/millet.html). More recently, Budak *et al.* (2003) have reported additional pearl millet SSR primer pairs and used these for SSR-based genetic diversity assessment of pearl millet breeding lines and germplasm accessions. Finally, Qi *et al.* (2004) report the map positions of 65 pearl millet SSR markers, including some for which primer sequences were previously reported by Qi *et al.* (2001) or Allouis *et al.* (2001).

Poncet *et al.* (2002) made a comparison of the locations and effect of QTLs controlling the morphological differences between domesticated and wild pearl millet with a focus on the organization of linkage groups (LG) 6 and LG 7. In their previous study (Poncet *et al.*, 2000), they revealed that domesticated spikelet structure is mainly

controlled by major genes located on LG 6 and LG 7. Poncet *et al.* (2000) have analyzed another cross in which the domesticated parent differs in their geographical origin, agronomic characteristics and life cycle from wild parents in the studied population. Poncet *et al.*, (2002) further compared the level of polymorphism and constructed a linkage map consisting 22 RFLP loci distributed among the seven linkage groups covering 177 cM, which corresponded to 54.6% of the original pearl millet reference map (Liu *et al.*, 1994). This is due to both a strong reduction in recombination rate in their two crosses (wild × cultivated) relative to the cross between inbreds of cultivated pearl millet used to build the reference map (Poncet *et al.*, 2000) and due to incomplete map coverage. Similar reductions in recombination rate and linkage map length in wild and cultivated crosses have been reported by Liu *et al.* (1996)

Poncet *et al.* (2002) have detected a total of 18 QTLs in an F_2 population derived from a domesticated × wild cross "Thiotande × Wild" (T × W). The location and effects of the QTLs detected in this cross were compared to those (36 QTLs) obtained in an F_2 population derived from another domestic × wild cross Souna × Mollissimum (S × M) (Poncet *et al.*, 2000). However, the T × W and S × M mapping analyses differed by map coverage levels. In the T × W cross, maximum of two QTLs were detected for each trait and the proportion of the phenotypic variance explained by each ranged from 6.1% to 66.6%. In general fewer QTLs with large effects were detected in the T × W population compared to the S × M population.

A skeleton genetic map of 562 cM (Haldane function) was constructed (Azhaguvel *et al.*, 2003) using 33 RFLP markers using a population size of 142 F_2 plants based on the cross of inbred lines IP 18293 (D_1/D_1, d_2/d_2, P/P) and Tift 238D1 (d_1/d_1, D_2/D_2, p/p). They mapped the D_1/d_1 plant height locus to pearl millet LG1, while the D_2/d_2 plant height and P/p foliage color loci were mapped to LG4. They also observed loose genetic linkage between the D_2/d_2 and P/p locus, with 42% repulsion-phase recombination corresponding to a map distance of 92 cM (Haldane).

A total of 51 alleles were detected using 16 highly polymorphic RFLP probe-enzyme combinations, nine associated with QTLs for downy mildew resistance and five associated with QTL for drought tolerance (Bhattacharjee *et al.*, 2002). Analysis of molecular allelic variation revealed high within accession variability (30.9%), but the variability between accessions was significantly higher (61.1%) than that within the

accessions. They produced a dendrogram based on a dissimilarity matrix using Ward's algorithms, which further delineated the 250 plant (25 each from 10 different landrace germplasm representative of phenotypic variation among accessions of Indian origin) plants into 10 major clusters - one per accession.

The development of 50 SSRs from pearl millet BAC clones has been described in Qi *et al.* (2001) and Allouis *et al.* (2001). The development of a further 44 SSRs from an enriched small insert library has recently reported (Qi *et al.*, 2004). Qi *et al.* (2004) also present a consensus genetic map with mapping data from four pearl millet crosses, comprising 65 SSR markers, 220 homologous and 133 heterologous RFLP markers. Recently, Budak *et al.* (2003) and Dweikat *et al.* (2004) have reported primer sequences for 18 additional pearl millet SSR markers, but the marker loci detected by these have not yet been mapped.

CHAPTER 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation was conducted during the period from May 2001 to August 2004 at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. The details of the experiments conducted in the laboratory, greenhouse and fields are given below.

3.1 MATERIALS

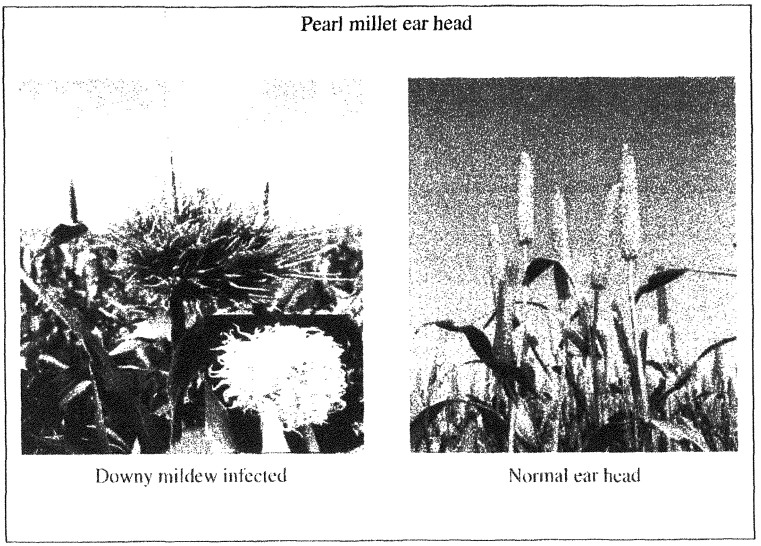
3.1.1 Mapping population parental lines

Parental lines: ICMB 89111-P6 and ICMP 423-P6 (=ICMB 90111-P6)

Table 1. Characteristics of the parental lines ICMB 89111-P6 and ICMP 423-P6 (=ICMB 90111-P6) used in the mapping population under study

Parental Line	Characteristics
ICMB 89111-P6	The more downy mildew susceptible (Figure 1) elite parent, ICMB 89111, is a d_2 dwarf maintainer of the A_1 cytoplasmic male sterility (CMS) system (Rai *et al.*, 1998). This moderately photoperiod-sensitive parent is characterized by high tillering ability and site-specific downy mildew resistance. It is the seed parent maintainer line of recently released pearl millet hybrids HHB 94 and RHB 121.
ICMP 423 –P6 (=ICMB 90111-P6)	The resistance donor parent, ICMP 423, is a genetically tall, weak restorer of male-fertility for the A_1 CMS system (Rai *et al.*, 1994). It is characterized by high tillering capacity and highly stable downy mildew resistance. It is also moderately photoperiod-sensitive. ICMP 423 is the pollinator of released hybrid MH 143 (ICMH 423) and a maintainer of the A_{egp} CMS system (this maintainer line is referred to as ICMB 90111).

Figure 1. Pearl millet ear heads with downy mildew disease infection (formation of green ear head) and a normal ear head with grain formation.



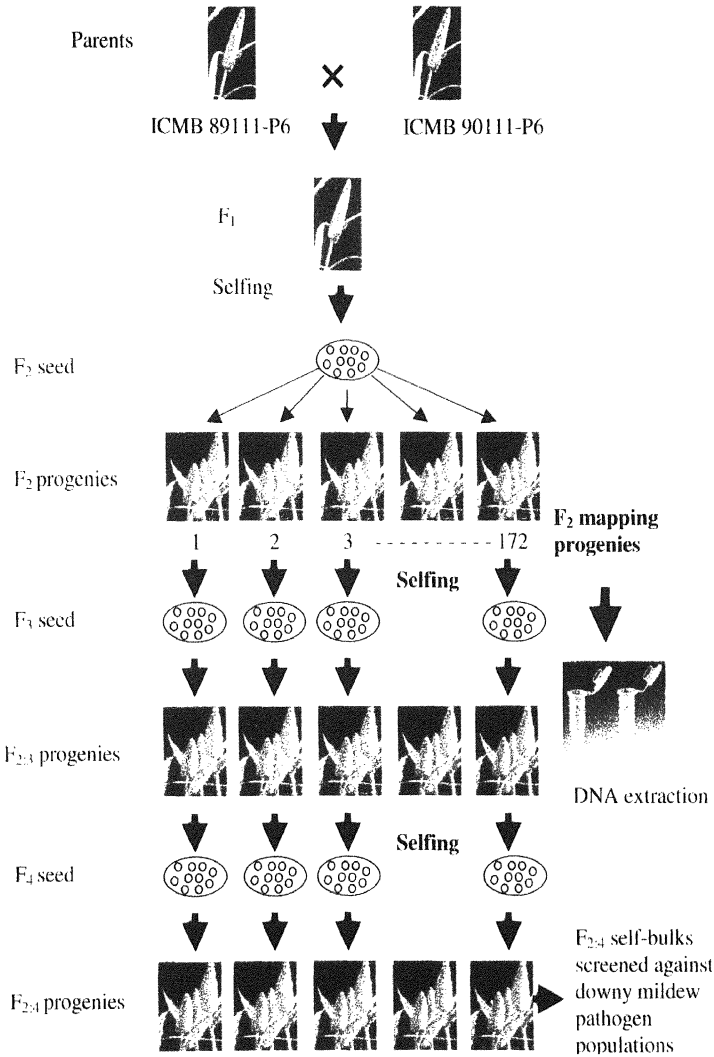
3.1.2. Mapping population-generation advancement

Two pearl millet inbred parental lines, ICMB 89111-P6 and ICMP 423-P6 (=ICMB 90111-P6), were crossed, plant × plant, to produce F_1 hybrids. The F_1 progenies from each plant × plant cross were selfed to produce F_2 seed. Each selfed F_1 plant produced large numbers of F_2 seeds for development of a mapping population. Selfed parents of each of nine plant × plant crosses were screened against six Indian populations of *Sclerospora graminicola* and differential responses were observed. One of the nine available F_1 progenies was selected for further study on the inheritance of these differential reactions.

In 2001, one F_2 segregating population of approximately 172 plants was raised. This F_2 population was derived from a plant × plant cross of ICMB 89111-P6 × ICMP 423-P6 having parental plants differing in their disease resistance to six Indian populations of *Sclerospora graminicola.* F_2 seed of this population was produced previously by selfing single F_1 plants from plant × plant cross. Seed from each of the selfed F_2 plants of this population were in the turn used to produce head-rows of F_3 plants, selfing of which provided F_4 self-bulk seed, representative of a single F_2, plant for use in the downy mildew screens.

During *kharif 2000* and *rabi* 2001/02, 30-35 selfed seeds harvested from each of about 172 individual F_2 plants in the mapping population were sown in pots (one pot per F_2 plant) to produce small bulks of F_2-derived F_3 ($F_{2:3}$) plants, which were used as sources of tissue for DNA isolation for each of F_2 plants in this mapping population. After collecting leaf tissues from each bulk of 10-14 days old seedlings, the seedlings of each F_2-derived F_3 progeny were transplanted to single rows in plot RL 18 at ICRISAT, Patancheru during late *kharif* 2001 and approximately 25-30 F_3 plants were selfed in each of the circa 172 $F_{2:3}$ families to produce $F_{2:4}$ self-bulk seed progenies. The remnant F_3 seed produced by selfing the original F_2 plants of this mapping population was maintained as a nucleus stock for use in further multiplication of F_4 self-bulk ($F_{2:4}$) seed (Figure 2) as described by Hash and Witcombe (1994). During *summer* and *kharif* 2002, the F_4 self-bulk progenies were screened against Indian pathogen populations under greenhouse conditions at ICRISAT, Patancheru to generate the phenotypic data required for QTL mapping. During *kharif* and late *kharif* 2002, these progenies were screened under similar

Figure 2. Advancement of mapping population based on pearl millet cross ICMB 89111-P6 x ICMB 90111-P6, used for genotyping and phenotyping



conditions by Dr. Wendy A. Breese at the University of Wales, Bangor, UK, against two African pearl millet downy mildew pathogen populations.

Single 4-m rows of each of the $F_{2:4}$ self bulk seed progenies of this population were sown in ICRISAT-Patancheru field RL 18 during *kharif* 2003. In the progeny rows, observations were recorded for segregating plant height (both qualitative and quantitative), glume color, basal node and internode color and downy mildew incidence.

3.2. DOWNY MILDEW PATHOGEN POPULATIONS

Indian Isolates	African Isolates
ICRISAT, Patancheru (*Sg*153)	Maiduguri, Nigeria (Screen 43)
MAHYCO, Jalna (*Sg*150)	Bamako, Mali (Screen 45)
GAU MRS, Jamnagar (*Sg*200)	
CAZRI, Jodhpur (*Sg*139)	
RAU ARS, Durgapura, Jaipur (*Sg*151)	
IARI, New Delhi (*Sg*298)	

3.2.1. Preparation of pathogen inoculum

3.2.1.1. Collection and inoculum

Single infected leaves were collected from downy mildew infected plants that did not show extensive chlorosis. Each leaf was washed and gently rubbed with moist cotton wool to remove old sporangia and sporangiophores and then placed into a moist box. Separate boxes were used for each pathogen population. The leaves in the boxes were incubated overnight in the dark at 20°C and 100% relative humidity. The following morning, leaves with the most profuse sporulation were selected. Using a camel hairbrush, for each pathogen population spores were removed from the leaves and collected into ice-cold water (below 2°C). The concentration of spore suspensions were adjusted to approximately 1.5×10^5 sporangia mL^{-1}.

3.2.1.2. Maintenance of pathogen isolates

A wide diversity of populations of this pathogen has been identified from India and samples of these are being maintained at ICRISAT, Patancheru (Thakur and Rao, 1993).

The pathogen populations are maintained on plants of highly downy mildew susceptible pearl millet genotypes 7042(S) [a selection from landrace germplasm accession IP 2696 from Chad (Singh *et al.*, 1994)] and F_1 hybrid NHB 3, both of which show >80% infection under heavy inoculum pressure. The infected plants are grown in sterilized soil in covered pots in a greenhouse room maintained at slightly above atmospheric pressure to prevent the entry of air-borne spores. Seedlings were inoculated at the two or three-leaf stage by spray application of a freshly prepared, chilled suspension of a particular pathogen population. The pots are then covered with polythene bags and incubated at 20°C to promote infection. After 12 hours, the bags were removed and the pots of seedlings infected with a particular pathogen population were maintained at 20-25°C in plexiglass-covers on benches in the greenhouse.

3.2.2. Screening mapping population progenies

3.2.2.1. Screening at ICRISAT, Patancheru

The mapping population (from plant × plant crosses of ICMB 89111-P6 × ICMB 90111-P6) of 172 $F_{2:4}$ entries along with their parental lines and control entries such as 7042(S), 7042(R) = ICML 22 (Singh *et al.*, 1994), 700651 = ICML 16 (Singh *et al.*, 1990), 843B (Stegmeier *et al.*, 1998) P1449-P2, PT 732B (Appadurai *et al.*, 1982) and HB 3, were evaluated under greenhouse conditions during early *kharif* 2002, at ICRISAT, Patancheru. Seedlings were grown in 12 cm diameter plastic pots. Pots were three-quarters filled with a potting mixture consisting of equal proportions of Alfisol, farmyard manure and fine sand. Thirty-five to forty seeds of an entry were sown at a uniform distance on a well-leveled soil surface in a single pot and covered with a two cm layer of potting mixture, irrigated and maintained at 25-30°C in the greenhouse. Seedlings at the coleoptile-to-one-leaf growth stage are considered optimal for inoculation. When seedlings in the majority of pots had reached this stage (normally 3–4 days after sowing), all pots of seedlings were sprayed with an aqueous suspension of sporangia (about 10^5 sporangia mL^{-1}) using a hand sprayer (Figure 3). Care was taken to cover uniformly all of the seedlings and to keep the sporangial suspension adequately chilled (Jones *et al.*, 2001). The pots were then covered with a polythene sheet and incubated in the dark at 20°C for 16 hours to promote infection. After 16 hours, the pots were shifted from the incubation room to greenhouse benches. Counts of total seedlings per pot were taken just before inoculation and those of diseased seedlings per pot were taken 14 days after

inoculation. The same inoculation procedures were repeated three times [time replications in completely randomized block designs for each of the six pathogen populations (a total of 18 different screens].

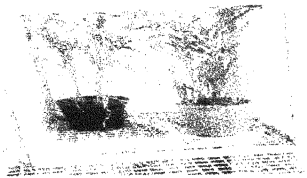
3.2.2.2. Screening at Bangor, UK (Wendy A. Breese, pers. comm.)

Seeds of two parental lines, appropriate control entries, and a circa of 172 (data scored from 164) skeleton-mapped $F_{2:4}$ pearl millet progenies from a cross (ICMB 89111-P6 × ICMB 90111-P6)-based mapping population were sown (43 seeds/pot using a vacuum planter) in low-nutrient peat and sharp sand compost (Chempak Seed Base, Chempak Products, UK: NPK 25-39 mg L^{-1}). Each pot represented a replicate of the pearl millet genotype. For each pathogen population a single inoculation date with three pots (replicates) per entry was used, with pots arranged in a randomized complete block design prior to inoculation. Pots were placed on flood benching in a controlled environment (greenhouse) providing a 16 hour day length (0600-2200 h) with a light intensity of between 500 and 1200 $\mu E\ m^{-2}\ s^{-1}$, and a temperature of 25 to 30°C from 0600-1800 h and 20°C from 1800-0600 h. The benches were flooded daily to an approximate depth of 1 cm for 30 minutes and then drained. When the seedlings were at the coleoptile-to-one-leaf stage the inoculum was prepared. Leaves from 2-3 month-old infected plants were wiped using moist laboratory roll (Kimwipes Roll, Kimberly Clark, Kent, UK) and incubated in sealed plastic boxes lined with moist laboratory roll for 8 h at 20°C in the dark. The resulting sporangia were collected in chilled (below 2°C) distilled water and their concentration assessed. Each pot of seedlings was sprayed with approximately 4 mL of inoculum using a compressed air cylinder fed sprayer (Kestrel Eqpt. Ltd., London). The inoculum was maintained on ice throughout inoculation to prevent zoospore release and so ensure a uniform inoculum concentration over time (Jones *et al.*, 2001). The pots were then covered with a polythene sheet to maintain a high level of humidity and incubated in the greenhouse at 20°C for 15 hours to promote infection. Downy mildew disease was assessed 14 days later based on the percentage of infected seedlings within each pot. The disease score for each genotype was the mean of infection percentages for individual pot replicates.

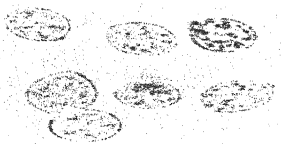
Figure 3. DM screening procedure used to evaluate disease reactions of the 172 $F_{2:3}$ mapping population progenies, parents and control entries against eight pathogen populations from India and Africa

Planting of $F_{2:4}$ progenies for DM Screening

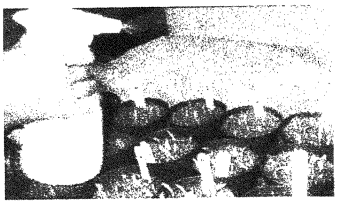
Coleoptile-to-one leaf stage optimal for counting total number of seedlings and spraying inoculum



Inoculum maintained separately for each pathogen population

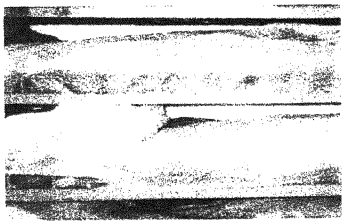


Harvested sporangia used for spraying inoculum



Spraying suspension @ 10^5 sporangia mL^{-1}

Post-infection counting of diseased plants two weeks after inoculation



Post-inoculation incubation cover under polythene sheet at 20°C for 16 hours

3.3. GENOMIC DNA ISOLATION

Several procedures for genomic DNA isolation have been reported (Dellaporta *et al.*, 1983; Murray and Thompson, 1984; Tai and Tanksley, 1990). The procedure based on Sharp *et al.* (1988) and re-optimized by substituting S-buffer with CTAB extraction buffer (Mace *et al.*, 2003) was used for pearl millet genomic DNA isolation in this study. Dark-grown, young seedlings or soft, non-green, stem internodes tissues are generally used to isolate genomic DNA as they yield better quality DNA with good restriction digestion with restriction endonuclease enzymes. These soft tissues respond well because of lower concentrations of phenolics and other adhering compounds compared to older green leaves. However, in this study young green leaves were used as the source of tissue for DNA isolation.

3.3.1. Grinding

Normally two methods are used to grind leaf tissues for DNA isolation. The process of DNA isolation requires lysis of the cell wall and cell membrane to release the DNA into an extraction buffer.

3.3.1.1. Method I

The leaf material is ground to powder with liquid nitrogen using pestle and mortar. The powder is then transferred to centrifuge tubes.

3.3.1.2. Method II

A small coffee grinder serves the purpose of grinding tissue samples, particularly when preparing tissue samples for DNA extraction from a population of more than 172 single plants. In this method, powdered dry ice (solid phase CO_2) is used along with the leaf samples that have been dipped in liquid nitrogen. The ground leaf samples were then kept at 4°C overnight to permit evaporation/sublimation of any remaining dry ice.

3.3.2. Extraction

Extraction of the ground leaf samples using a buffer having CTAB (cetyltrimethyl ammonium bromide), EDTA (ethylene diamine tetra acetic acid) and β-mercaptoethanol, is followed by differential centrifugation to isolate genomic DNA from cell debris, precipitation of CTAB-protein-carbohydrate complexes with an acetate-isopropanol

solution, followed by phenol and phenol-chloroform extractions; and a second precipitation of DNA with absolute alcohol.

In the present study, tissue grinding method I, and a Millet CTAB maxi-prep DNA extraction protocols were followed. CTAB maxi-prep DNA extraction differs from the SDS maxi-prep protocol outlined above in terms of avoiding excessive use of phenols (a highly corrosive chemical) and skipping some of the steps, thus saving money and time. DNA quality is obtained on par with that from the SDS maxi-prep protocol but the quantity is comparatively less.

3.3.2.1. First day extraction

DNA was isolated from 2-3 grams of green F_3 seedling leaf tissue produced from the selfed-bulk seed of a given F_2 plant, 10-14 days after emergence. These seedling leaves were quick-frozen in liquid nitrogen and ground to a fine powder in liquid nitrogen with the help of a pestle and mortar. The ground tissue was transferred to a 50 µL propylene tube containing 15-20 mL extraction buffer (2-3% w/v CTAB, 1.4 NaCl, 20 mM EDTA, 100 mM Tris-HCL pH 8.0, 0.17% β-mercaptoethanol) preheated to 65°C. These tubes were incubated at 65°C in a water bath for 30 minutes with occasional gentle inversions for mixing. An equal volume of chloroform-isoamyl alcohol (24:1 v/v) was added to the ground material and buffer and mixed well by a number of inversions on a shaking platform for 10 minutes to form an emulsion. The emulsion was separated into an aqueous phase and cell debris by centrifugation at 6000 rpm for 10 minutes at 4°C in a Sorvall HB 7 rotor. The upper aqueous phase was transferred to a sterile tube and 0.7 volume of cold iso-propanol was added and the mixture then incubated at –20°C for 20 minutes. Precipitated DNA was spooled out with a glass hook and rinsed twice in 70% ethanol (if no precipitation was seen then the solution was centrifuged at 6000 rpm for 5 minutes at 4°C) and lastly the pellets were air dried under a fan for 20-30 minutes (avoiding over-drying of the pellet to help the dried pellet dissolve quickly in $T_{50}E_{10}$). The dried DNA pellet was then dissolved in 500-700 µL $T_{50}E_{10}$ (50 mM Tris-HCL, 10 mM EDTA, 2 M NaCl, pH 8.0) and incubated overnight at room temperature.

3.3.2.2. Second day purification

To the completely dissolved DNA samples from the first day extraction 10 µL RNase (10 mg/mL) was added and the samples were incubated at 37°C for 1 hour, during which they were gently stirred every 15 minutes. To purify the samples (removing the degraded RNA), an equal volume (500-700 µL) of cold phenol:chloroform:isoamyl alcohol (25:24:1) was added, gently mixed and separated as two phases by centrifugation in a microfuge at 14000 rpm for 5 minutes. The supernatant was transferred into a clean and sterile eppendorf microtube and the extraction was repeated with cold chloroform:isoamyl alcohol (24:1). To the final supernatant (aqueous phase), 3 M sodium acetate (one tenth volume) and two volumes of absolute ethanol was added and mixed by gentle inversions. The jelly-fish-like precipitated DNA was carefully spooled-out with a glass hook and rinsed twice in chilled 70% ethanol, then placed in a clean sterile eppendorf tube. After briefly centrifuging at 10,000 rpm for 2-4 minutes, excess ethanol was poured off and DNA samples were air-dried in dust free conditions for 1-2 hours. The DNA samples were finally suspended in an appropriate volume of $T_{10}E_1$ (10 mM Tris-HCl and 1 mM EDTA, pH 8.0), to make a volume of 250-350 µL (according to the size of the individual pellets) and stored at 4°C.

3.3.3. DNA quantification and purity check

DNA concentration of each sample was quantified by spectrophotometer measurements of UV absorption at 260 nm, assuming 1 OD at 260 nm is equal to 50 µg of DNA (Maniatis *et al.,* 1982). The ratio of OD_{260} to OD_{280} was calculated to check the purity of each DNA sample. Pure DNA preparations show an OD_{260} to OD_{280} ratio between 1.7 and 1.8 (Maniatis *et al.*, 1982).

Each DNA sample was analyzed in a 0.8% TAE-agarose gel to test its integrity (Maniatis *et al.*, 1982). Gels were stained with ethidium bromide and viewed on a UV-transillumninator, then photographed with a camera fitted with a UV filter.

For preparing working solutions of uniform DNA concentration from the above samples, the following formulae were used:

DNA concentration (µg/µL) of stock solution:

= [OD value (260 nm) × 50 μg/mL × DF]

where

DF = Dilution factor (200 in case of 5 μL of newly extracted plant DNA dissolved in 1 mL of DDW (double distilled water) or 500 in case of 2 μL of newly extracted DNA dissolved in 1 mL of DDW for spectrophotometery)

DNA concentration (1 μg/μL) for digestion and blotting:

= (Total volume to be made × 20 μg/μL) ÷ DNA concentration of stock solution

DNA concentration (5 ng/μL) for total dilution of volume of 1000 μL for PCR reactions:

= (5 ng/μL × 1000 μL) ÷ DNA concentration of stock solution

DNA degradation and contamination with other substances were checked by electrophoresis of aliquot of samples in mini agarose gels (0.8%). It was assumed that large molecular weight DNA appears as a band with sharp edges, whereas partially degraded material forms a smear of long to small fragments. The amount of DNA was also approximated utilizing UV-induced fluorescence emitted by ethidium bromide molecules intercalated into the DNA. Because the amount of fluorescence is proportional to the total mass of DNA. The quantity of DNA sample was estimated by comparing the fluorescent yield of the sample with that of a series of *lambda* DNA standards.

3.4. SIMPLE SEQUENCE REPEATS (SSRs) MARKER

3.4.1. Testing parental polymorphism using SSR primers

According to Caetano-Anolles (1997) the parameters of DNA amplification (*viz.*, specificity, efficiency, and fidelity) are strongly influenced by the different components of the reaction and by thermal cycling. Therefore, the careful optimization of these parameters will ultimately result in reproducible and efficient amplification. To achieve the optimization, the range of values for components of PCR reaction was varied in the protocol below for different primers used in the present study.

PCR conditions for pearl millet SSR primer pairs (Allouis *et al.*, 2001; Qi *et al.*, 2001, 2004) were optimized using a grid with various amounts and concentrations of chemicals used for the PCR master mix, at different annealing temperatures. To identify

SSR primer pairs detecting polymorphism between parents, initial screening of parental lines was conducted before actual genotyping of individuals in segregation F_2 mapping population. For this, DNA from ICMB 89111-P6 (taken as first parent *i.e.* P_1) and ICMB 90111-P6 (taken as second parent *i.e.* P_2) and their corresponding F_1 hybrids were subjected to PCR amplification with each of the available SSR primer pairs. A total of 80 SSR primers pairs were used to screen the parents of the population. Line 81B (Anand Kumar *et al.*, 1984) was the source of the DNA used in generation of an SSR-enriched library (Qi *et al.*, 2001) and a mapping parent. Its seed parent Tift $23D_2B$ (Burton, 1969) was the genotype used in the construction of a pearl millet BAC library from which additional pearl millet SSRs were identified (Allouis *et al.*, 2001).

The sequence information of forward and reverse primers used for genotyping pearl millet SSR loci included within study is given in Table 2. From this screening, SSR primer pairs detecting scorable polymorphism between the parents were noted and used for further screening of the F_2 mapping population.

3.4.2. PCR reaction mix and conditions for SSR amplification

The PCR reactions were conducted in a Peltier Thermocycler DNA Engine PTC 200 model and Dyad machine from MJ Research. The standard PCR reagents in a total reaction volume of 20 µL were:

Genomic DNA template (5 ng/µL)	3.0 µL
PCR buffer (10X)	2.0 µL
Mg^{2+} (50 mM)	0.5 µL
DNTP mix (2 mM)	2.0 µL
Primer (30 ng/µL)	1.0 µL
Taq DNA polymerase (5 U/µL)	0.2 µL
De-ionized distilled water	11.3 µL

3.4.2.1. PCR cycling

The cycling conditions for SSR primers were set up using touchdown PCR thermo cycling (Don *et al.*, 1991) with minor modifications. The details of the standard touchdown PCR program are as follows:

95°C – 5 minutes hold (initial denaturation)

95°C – 15 second (denaturation)

*61°C – 20 second (primer annealing)

* = 1°C drop per cycle for 10 cycles

72°C – 30 second (primer extension)

95°C – 10 second (denaturation)

54°C – 20 second (primer annealing) for 35 cycles

72°C – 30 second (primer extension)

72°C – 2 minutes hold

04°C – storage

Optimization started with the above-mentioned PCR conditions. However, changes in annealing temperatures (from 48°C to 63°C) were followed for different primer pairs per the temperature range required for the respective primer pairs to achieve optimization of the amplified products. The most common annealing temperature for a majority of the SSR primers for PCR reactions was 61°C in 9-step touchdown PCR protocol SSR61:

94°C – 4 minutes hold

94°C – 1 minute (initial template denaturation)

Ramping @ – 0.5°C/second to 61°C

61°C – 1 minute (primer annealing)

Ramping @ +0.5°C/second to 72°C

72°C – 1 minute (extension)

Go to step 2 for 34 times

72°C – 4 minutes (final extension)

04°C – storage

In the course of genotyping of the F_2 population, the above-mentioned total volume 20 µL of the PCR master-mix was reduced to half (10 µL) by reducing the volumes of all PCR master-mix ingredients (especially dNTPs, primers and *Taq* DNA polymerase) following re-optimization of the reaction conditions for each primer pairs. This was done to use resources more efficiently and save on consumables costs. Before loading, 5 µL of loading buffer (5X) containing orange dye was added to each reaction sample.

Table 2. List of 26 polymorphic pearl millet SSR primers used for screening of 172 F_2 mapping population progenies from the cross based on parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer name	Linkage group	BAC origin	Primer sequence	Microsatellite motif	PCR product size (bp) based on 81B	Published PIC value	Reference
1	PSMP2030 F	1	--	5' ACC AGA GCT TGG AAA TCA GCA C 3'	(CA)11(GA)10	107	0.49	Qi *et al.* (2004) TAG
	PSMP2030 R			5' CAT AAT GCT TCA AAT CTG CCA CAC 3'				
2	PSMP2080 F	1	--	5' CAG AAT CCC CAC ATC TGC AT 3'	(AC)14	181	0.77	Qi *et al.* (2004) TAG
	PSMP2080 R			5' TGC AAC TGA GCG AAG ATC AA 3'				
3	PSMP2273 F	1	B320P11	5' AAC CCC ACC AGT AAG TTG TGC TGC 3'	(GA)12	169	0.75	Allouis et al. (2001) TAG 102: 1200- 1205.
	PSMP2273 R			5' GAT GAC GAC AAG ACC TTC TCT CC 3'				Qi et al. (2004) TAG
4	PSMP2072 F	2	--	5' GAA ATC TAC ACA AGG GTC TCC A 3'	(CA)24	165	0.90	Qi et al. (2004) TAG
	PSMP2072 R			5' GTA CGG AGC AAT GAC ATC TGA A 3'				
5	PSMP2077 F	2	--	5' GCC AA ATT ATT CCC AAG TGA ACA 3'	(CA)15(TA)8	180	0.50	Qi et al. (2004) TAG
	PSMP2077 R			5' CTC TTG GTT GCA TAT CTT TCT TTT 3'				
6	PSMP2089 F	2	--	5' TTC GCC GCT GCT ACA TAC TT 3'	(AC)15	127	0.80	Qi et al. (2004) TAG
	PSMP2089 R			5' TGT GCA TGT TGC TGG TCA TT 3'				
7	PSMP2201 F	2	B320O08	5' CCC GAC GTT ATG CGT TAA GTT 3'	(GT)6	364	0.69	Qi et al. (2001) BioTechniques 31: 355- 362.
	PSMP2201 R			5' TCC ATC CAT CCA TTA ATC CAC A 3'				Qi et al. (2003) Genetics
8	PSMP2225 F	2	B320N23	5' CCG TAC TGA TGA TAC TGA TGG TT 3'	(GT)12	238	multiple loci	Qi et al. (2001) BioTechniques 31: 355-362.
	PSMP2225 R			5' TGG GAG GTA AGC TCA GTA GTG T 3'		250 in 841B		Qi et al. (2004) TAG
9	PSMP2237 F	2	B320F05	5' TGG CCT TGG CCT TTC CAC GCT T 3'	(GT)8	233	0.60	Allouis et al. (2001) TAG 102: 1200-1205.
	PSMP2237 R			5' CAA TCA GTC CGT AGT CCA CAC CCC A 3'				Qi et al. (2004) TAG

.....contd. Table 2. List of 26 polymorphic pearl millet SSR primers used for screening of 172 F_2 mapping population progenies from the cross based on parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer name	Linkage group	BAC origin	Primer sequence	Microsatellite motif	PCR product size (bp) based on 81B	Published PIC value	Reference
10	PSMP2070 F	3	--	5' ACA GAA AAA GAG AGG CAC AGG AGA 3'	(CA)25(TA)6	226	0.90	Qi et al. (2004) TAG
	PSMP2070 R			5' GCC ACT CGA TGG AAA TGT GAA A 3'				
11	PSMP2267 F	3	B320K05	5' GGA AGG CGT AGG GAT CAA TCT CAC 3'	(GA)16	241	0.79	Allouis et al. (2001) TAG 102: 1200-1205.
	PSMP2267 R			5' ATC CAC CCG ACG AAG GAA ACG A 3'				Qi et al. (2004) TAG
12	PSMP2084 F	4	--	5' AAT CTA GTG ATC TAG TGT GCT TCC 3'	(AC)42	245	0.80	Qi et al. (2004) TAG
	PSMP2084 R			5' GGT TAG TTT GTT TGA GGC AAA TGC 3'				
13	PSMP2078 F	5	--	5' CAT GCC CAT GAC AGT ATC TTA AT 3'	(CA)42	172	0.85	Qi et al. (2004) TAG
	PSMP2078 R			5' ACT GTT CGG TTC CAA AAT ACT T 3'				
14	PSMP2202 F	5	B320D05	5' CTG CCT GTT GAG AAT AAA TGA G 3'	(GT)8	161	0.42	Qi et al. (2001) BioTechniques 31: 355-362.
	PSMP2202 R			5' GTT CCG AAT ATA GAG CCC AAG 3'				Qi et al. (2004) TAG
15	PSMP2208 F	5	B320E09	5' GGA AGA GCA AAC TGA ACA ATC CC 3'	(GT)10	253	0.78	Qi et al. (2001) BioTechniques 31: 355-362.
	PSMP2208 R			5' ACT TTG CCC TGG ATG ATC CTC 3'				Qi et al. (2004) TAG
16	PSMP2220 F	5	B320I12	5' GCA TCC TTC ACC ATT CAA GAC A 3'	(GT)11	128	0.66	Qi et al. (2001) BioTechniques 31: 355-362.
	PSMP2220 R			5' TGG GAA ACA GAA TGG AGA AAA GAG 3'				Qi et al. (2004) TAG
17	PSMP2274 F	5	B320P12	5' CAC CTA GAC TCT ACA CAA TGC AAC 3'	(GA13)	265	0.85	Allouis et al. (2001) TAG 102: 1200-1205.
	PSMP2274 R			5' AAT ATC AAG TGA TCC ACC TCC CAA 3'				Qi et al. (2004) TAG
18	PSMP2276 F	5		5' TGT GGC AAT TAC GGT CGA GC 3'	(CA)16	700 in 841B		None
	PSMP2276 R			5' CTA CCT CTA TCT TAC TTC ACC 3'				

.....contd. Table 2. List of 26 polymorphic pearl millet SSR primers used for screening of 172 F_2 mapping population progenies from the cross based on parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer name	Linkage group	BAC origin	Primer sequence	Microsatellite motif	PCR product size (bp) based on 81B	Published PIC value	Reference
19	PSMP2277 F	5		5' GGA ATG CTC ATC CAA TAC CCT CC 3'	(GT)16	650 in 841B		None
	PSMP2277 R			5' CCA GGA CTG ATG AGG TGT GGC 3'				
20	PSMP2018 F	6	–	5' CGC AAG ACA TTT TAG TAT CAC C 3'	(GT)30	203	0.40	Qi et al. (2004) TAG
	PSMP2018 R			5' ACA GTC ATC CTC AGT CGT CC 3'				
21	PSMP2074 F	7	--	5' AGG ACT GTA GGA GTG TGG ACA A 3'	(AC)11	227	0.75	Qi et al. (2004) TAG
	PSMP2074 R			5' CCA GAC CTA CCA GTG AAT GAG A 3'				
22	PSMP2203 F	7	B320O12	5' GAA CTT GAT GAG TGC CAC TAG C 3'	(GT)18 imperfect	357	0.80	Qi et al. (2001) BioTechniques 31: 355-362.
	PSMP2203 R			5' TTG TGT AGG GAG CAA CCT TGA T 3'				Qi et al. (2004) TAG
23	PSMP2224 F	7	B320L19	5' GGC GAA ATT GGA ATT CAG ATT G 3'	(TG)10	155	0.58	Qi et al. (2001) BioTechniques 31: 355-362.
	PSMP2224 R			5' CGT AAT CGT AGC GTC TCG TCT AA 3'				Qi et al. (2004) TAG
24	PSMP2263 F	7	B320D16	5' AAA GTG AAT ACG ATA CAG GAG CTG AG 3'	(AG)33	238	0.72	Allouis et al. (2001) TAG 102: 1200-1205.
	PSMP2263 R			5' CAT TTC AGC CGT TAA GTG AGA CAA 3'				Qi et al.(2004) TAG
25	PSMP2232 F	1 and 2	B320D05	5' TGT TGT TGG GAG AGG GTA TGA G 3'	(TG)8	233	0.68	Allouis et al. (2001) TAG 102: 1200-1205.
	PSMP2232 R			5' CTC TCG CCA TTC TTC AAG TTC A 3'				Qi et al. (2004) TAG
26	PSMP2261 F	Unlinked	B320B11	5' AAT GAA AAT CCA TCC CAT TTC GCC 3'	(GA)16	193	0.78	Allouis et al. (2001) TAG 102: 1200-1205.
	PSMP2261 R			5' CGA GGA CGA GGA GGG CGA TT 3'				

3.4.3. Separation of PCR products containing SSRs

For separation and visualization of PCR products both agarose (1%) as well as polyacrylamide gels (6%) were used. Agarose gels were used only for initial visualization/checking of amplification considering two limitations in their use. Firstly, exact sizing of microsatellite alleles cannot be accomplished on agarose. Secondly, it is difficult to distinguish two, three or four base-pair differences in DNA fragment length on agarose (Cregan and Quigley, 1998). Therefore, scoring of allele size differences of PCR amplified products was performed in polyacrylamide gels.

3.4.3.1. Agarose gel electrophoresis

Agarose 1% gels were cast in TBE buffer (1X). Gels were cast in a horizontal gel frame (GIBCO BRL or Bio-Rad sub cell or Owl separation systems) and products were visualized by incorporating 1 µL (10 mg/mL) ethidium bromide (EB) per 20 mL of gel solution and viewed in a gel documentation system. Here the purpose of running agarose gels (for 15-30 minutes at 100 V) was to check whether amplification of PCR product has taken place or not before attempting to separate this PCR product on PAGE as a PAGE run takes more time than an agarose run.

3.4.3.2. Polyacrymide gel electrophoresis (PAGE)

For separation and visualization of PCR products, 6% polyacrylamide gels were used. The details of gel preparation, electrophoresis and visualization of separated DNA bands are given below.

3.4.3.2.1. Gel preparation and electrophoresis

Polyacrylmide gels allow high resolution of amplified products from PCR. For separating amplified products of SSR primers, non-denaturing polyacrylmide gels (6%) were used. Before preparation of gel solution, glass plates were cleaned thoroughly with ethanol. A few drops of repel-silane-ES were applied to the back plate and rubbed over the surface. This makes it easier to separate the plate from the gel. To the front glass plate a few drops of bind silane were applied and rubbed over its entire surface. This prevents the gel from dislodging during staining.

Cautions:

i) If plates are not thoroughly cleaned prior to use, bubbles can get trapped while pouring the gel, which adversely affects movement of the PCR-amplification of a product resulting in ambiguous scoring of the bands.

ii) Silane is a carcinogen, so gloves and a facemask should be worn when applying this to the glass plates.

1). For a 6% gel (Bio-Rad plates) 75 mL of gel solution was prepared by mixing

08.0 mL TBE buffer (10X)

15.0 mL 29:1 (w/w) acrylamide/bis-acrylamide solution

52.0 mL distilled water

Caution:

1). Acrylamide is a neurotoxin. Always wear gloves, goggles and a facemask when working with acrylamide powder, solutions and/or gels.

2). The contents of the gel solution were mixed vigorously. TEMED (90 µL) was added and mixed by swirling the flask.

3). Immediately added 400 µL of 10% (w/v) APS (ammonium per-sulphate) and mixed by swirling the flask.

4). Acrylamide solution was poured into the syringe, which feeds it between the glass plates, and the comb was inserted to produce track-loading wells at the top of the gel.

Note: Polymerization is catalyzed by the addition of freshly prepared APS, so be quick in pouring the solution into the plates after adding this catalyst to the gel solution.

5). After polymerization the gel was set up for running electrophoresis. The comb was removed. The lower tank was filled with TBE (0.5X; approximately 250-300 mL) and the back of the plate and upper reservoir with the same (approximately 400 mL), ensuring that the well was covered. Each well was aspirated with TBE buffer using a Pasteur pipette to remove small fragments of gel and tiny bubbles. The gel was pre-run for at least 10 min at 5 V/cm (approximately 400 V, 9 W) to warm it.

6). The samples were made up for loading in 5X loading buffer to give a final concentration of 1X. Between 2 and 5 µL were loaded into each well on the gel. Lambda size markers (2 µL of 50 ng/µL) were loaded along with the samples.

7). The gel was run at approximately 5 V/cm (400 V, 9 W) until the desired resolution was reached. This was determined by the dye front.

8). After the run, the plates were carefully pulled apart so that the gel remained attached to the front glass plate.

3.4.3.2.2. Silver staining and visualization of bands

Electrophoresed DNA fragments were detected with silver nitrate staining (Goldman and Merril, 1982). Several protocols for silver staining can be used, most of which require approximately 2 hours. Although commercial kits for silver staining were available from several manufacturers (*e.g.*, Bio-Rad), lab-made solutions were used in the present study. Each solution was prepared in a separate container. The same solutions were used twice over a 30 h period except for silver nitrate solution and developer, which were freshly prepared during the staining process.

The steps followed for silver staining are:

1). The gel was rinsed in distilled water for 3-5 minutes.
2). The gel was soaked in 2 L of 0.1% CTAB (2 g in 2 L of water) for 20 minutes.
3). The gel was incubated in 0.3% ammonia (26 mL in 2 L) for 15 minutes with shaking.
4). Silver nitrate solution was prepared (2 g silver nitrate, 8 mL of 1M NaOH, 8 mL 25% ammonia) and titrated with ammonia until the solution became clear after which a further 1 mL of ammonia solution was added.
5). The gel was placed in the silver nitrate solution for 15 minutes and was gently agitated.
6). The gel was then rinsed in water for 1 minute.
7). The gel was placed in developer (30 g sodium carbonate, 0.4 mL formaldehyde, 2 L of water) until the bands became visible.
8). The plate was rinsed in water for 1 minute to stop staining.
9). The gel was placed in fixer (30 mL glycerol in 2 L of water).
10). The gel was kept for air-drying overnight and then scanned.

Note: To remove the dried gel from the plate, the plate was soaked in concentrated sodium hydroxide (NaOH) solution (40 g flakes in 1 L of distilled water) for a few hours.

DNA polymorphism among the parents was observed based on length of amplified fragments in terms of number of base pairs by comparing with a 100 base pair ladder (50 ng/μL). The images showing parental polymorphism for some SSR primer

pairs on a polyacrylamide gel visualized by silver staining are presented in Figure15-16. Among the different bands observed in each lane, the smallest base pair size of a band was considered for scoring.

3.4.4. Genotyping F_2 population with SSR primers

3.4.4.1. Monoplex PCR product separation and visualization

Subsets (depending on size of comb) of the mapping population were screened with the SSR primer pairs showing polymorphism between mapping population parents. The PCR products (2 µL) of each individual in the mapping population were loaded along with those of the parents and standard size ladder (1.5-2.0 µL of 100 bp ladder of concentration 50 ng/µL) using a custom 50- or 69- or 100-toothed comb (0.4 cm well-center-to-well center with thickness of 0.4 mm). The electrophoretically separated DNA bands were visualized by silver staining. The gels were cleaned well before scanning. More than 90% of the SSR loci were genotyped using monoplex PCR product separation and visualization. An image of a silver-stained PAGE gel in which PCR products of a single primer pair were loaded is presented in Figure 19-21.

3.4.4.2. Multiplex PCR product separation and visualization

Individuals of the mapping population were screened with more than one primer simultaneously on a single PAGE gel. This is based on the concept of differences in size of amplified products with different primers; the information of which was previously known from the parental screening. While choosing the primers, it was made sure that the amplified products of selected primers do not co-migrate with each other. In this procedure instead of running the PCR reaction with a number of primer pairs simultaneously (pre-amplification multiplex PCR), which requires a lot of time for optimization, the PCR reaction for each primer pair was set up separately and these monoplex products were pooled together, *i.e.*, post-amplification multiplexing (also called multi-loading). These samples were loaded in the PAGE and bands were visualized with silver staining procedures. The alleles for each primer were scored separately by comparing banding patterns with those of parental alleles for the respective primer pairs. An example for two-primer multiplex *(Xpsmp*2203 and *Xpsmp*2202, *Xpsmp*2202 and *Xpsmp*2274*)* and same primer loaded twice with a time interval of 15 minutes on a urea

denaturating gel and 30-45 minutes on a 6% non-denaturating polyacrylamide gel are given in Figure 18.

3.4.4.3. Scoring of SSR amplified bands and genotyping

The banding patterns obtained from PCR amplification of various SSR primers in the F_2 individuals were scored as follows:

A = Homozygote for allele a from parental strain P_1 at this locus

B = Homozygote for allele b from parental strain P_2 at this locus

H = Heterozygote carrying alleles from both P_1 and P_2 parental strains *i.e.* genotype comparable to the F_1

C = Not a homozygote for allele a (*i.e.* either B or H)

D = Not a homozygote for allele b (*i.e.* either A or H)

- = Missing data for the individual at this locus

After scoring, the individual progeny genotypes were typed in a Microsoft Excel spread sheet in a format suitable for linkage analysis by MapMaker/Exp. (*i.e.*, rows = genotype score at a given locus; columns = F_2 individual of the mapping population).

3.5. RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) MARKER

3.5.1. Restriction enzyme digestion

For each mapped F_2 plant from the population, 20 µg of DNA in sterile distilled water (SDW) was digested with *Dra*I, *Eco*RI, *Eco*RV and *Hind*III restriction endonucleases following the supplier's instructions (Amersham Pharmacia Biotech, Ltd.). The digestion was conducted in a total volume of 30 µL and incubated overnight at 37°C. The reaction was terminated by addition of 5 µL of loading buffer (25% sucrose, 0.1% bromophenol-blue and 20 mM EDTA) to each 30 µL sample.

3.5.2. Electrophoresis

Fragments of digested DNA obtained after restriction enzyme digestion were separated by electrophoresis in 0.8% TAE-agarose on a horizontal slab gel (Bio-Rad DNA Sub Cell^{TM}) electrophoresis unit (Owl Separation Systems Model No.A-1) for 16 hours at 38 V cm^{-1} in TAE (0.04 M Tris-acetate, 0.001 M EDTA, pH 7.8) buffer. Agarose gels were prepared in the same buffer that was used for electrophoresis. *Hind*III-digested lambda

DNA (λ DNA) was used as molecular size markers with fragment sizes of 23.1 kb, 9.4 kb, 6.6 kb, 4.4 kb, 2.3 kb and 2.0 kb. Gels were stained in 0.5 μg mL^{-1} ethidium bromide for 15 minutes, destained for 30 minutes in distilled water, viewed on a UV-transilluminator and photographed to assess digestion quality (Figure 4).

3.5.3. Southern blot hybridization

3.5.3.1. Preparation of southern blots

DNA fragments, separated electrophoretically after digestion, were transferred from agarose gels onto nucleic acid nylon transfer membranes (Hybond-N^+, Amersham Pharmacia Biotech, Ltd.) following the procedure of Southern (1975). The sponge was partially dipped in an alkali solution of 0.4 M NaOH. This solution served as a denaturing agent and the vehicle for capillary transfer of DNA fragments from the gel to the nylon membrane. As the alkali solution passes through the gel on its way to be absorbed in the paper towel, the DNA fragments are carried out of the gel and bound to the nylon membrane. Transferred membranes were soaked in 2X SSC for 2 minutes to neutralize the alkali, air dried, wrapped with cling film and then stored at –20°C for future use.

3.5.3.2. Probes used

The clones used for probing were selected from a *Pst*I genomic library (named as PgPSM1 to PgPSM1000) constructed from total DNA of pearl millet genotype 7042(S) by Liu *et al.* (1994). Initially around 40 clones identified as detecting single copy or low copy loci were evaluated and from this a set of 20 detecting polymorphism between parental lines ICMB 89111-P6 and ICMB 90111-P6 were selected for use (Table 3).

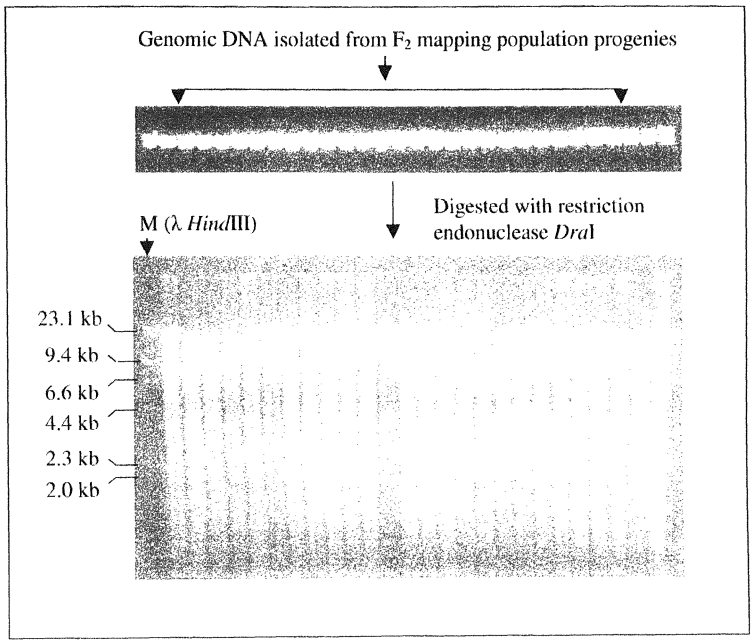
3.5.3.2.1. *Pst*I genomic library (Liu *et al.*, 1994)

Pearl millet *Pst*I genomic library clones were constructed with total-plant DNA extracted from the leaves of pearl millet genotype 7042(S). Fifty micrograms of DNA was digested with 100 units of *Pst*I enzyme and subjected to electrophoresis. Fragments in the size range of 500-3000 bp were collected using DEAE membrane. The purified fragments were then ligated into the *Pst*I site of pUC 18. The *Escherichia coli* bacterial strain DH5 was transformed and plated out on LB media (NaCl, trypton and yeast extract) with ampicilin (10 mg mL^{-1}). Individual colonies were picked out and grown in 2 mL of LB media containing cafbenicillin (10 mg mL^{-1}) to produce stab cultures (Liu *et al.*, 1994).

Table 3. List of 20 polymorphic probe-enzyme combinations used for screening of segregating 172 F_2 mapping population progenies from the cross based on parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Locus	Probe name	Restriction enzyme	Linkage group	Insert size	Copy number
1	*Xpsm*280	PgPSM280	*Hind*III	1	0.8	Single-copy
2	*Xpsm*492.1	PgPSM492	*Eco*RI	1	0.3	Multi-copy
3	*Xpsm*17	PgPSM17	*Eco*RV	1	0.8	Single-copy
4	*Xpsm*196.1	PgPSM196	*Eco*RI	1 & 4	2.2	Multi-copy
5	*Xpsm*708.1	PgPSM708	*Eco*RV	2	Not known	Multi-copy
6	*Xpsm*37	PgPSM37	*Eco*RV	3	0.7	Single-copy
7	*Xpsm*18	PgPSM18	*Hind*III	3	2.0	Single-copy
8	*Xpsm*409.1	PgPSM409	*Dra*I	4	3.0	Multi-copy
9	*Xpsm*648	PgPSM648	*Hind*III	4	0.6	Single-copy
10	*Xpsm*344	PgPSM344	*Eco*RI	4	2.0	Single-copy
11	*Xpsm*84	PgPSM84	*Eco*RI	4	1.3	Single-copy
12	*Xpsm*837.2	PgPSM837	*Dra*I	1 & 4	1.6	Multi-copy
13	*Xpsm*416.3	PgPSM416	*Eco*RI	3 & 4	1.0	Multi-copy
14	*Xpsm*588	PgPSM588	*Eco*RV	6	1.8	Single-copy
15	*Xpsm*696	PgPSM696	*Eco*RV	6	2.7	Single-copy
16	*Xpsm*575	PgPSM575	*Eco*RV	6	0.8	Single-copy
17	*Xpsm*718	PgPSM718	*Hind*III	7	0.7	Single-copy
18	*Xpsm*269	PgPSM269	*Hind*III	7	0.6	Single-copy
19	*Xpsm*160	PgPSM160	*Hind*III	7	0.4	Single-copy
20	*Xpsm*190	PgPSM190	*Hind*III	7	0.4	Single-copy

Figure 4. Image of pearl millet genomic DNA isolated from F_2 mapping population progenies restricted with *Dra*I on ethidium stained 1.0 % agarose gel electrophoresis



ICRISAT received the pearl millet RFLP probes as stab culture stocks, from Drs. Michael D. Gale and Katrien M. Devos of the John Innes Centre, Cambridge Laboratory, Norwich, UK.

3.5.3.2.2. Plasmid DNA extration

From a stab culture, one loop full of culture was taken and transferred to a culture tube containing 5 mL of LB media with 10 µL ampicilin (10 mg mL^{-1}). The inoculated cultured tubes were kept overnight in a shaker at 270 rpm at 37°C. Centrifugation was done at 6000 rpm for 10 minutes to get the pellet. Then 200 µL of solution A (4 mg lysozyme mL^{-1} of GTE solution), 300 µL of solution B (10% SDS and 1 N NaOH) and 300 µL of solution C (7.5 M ammonium acetate) were added to the pellet which was re-dissolved in this solution and incubated for 10 minutes in ice. Again centrifugation was performed at 10,000 rpm for 10 minutes. To the supernatant, 30 µL of RNase (10 mg mL^{-1}) was added and incubated at 37°C in an oven for an hour. The solutions were then centrifuged at 5000 rpm for two minutes with equal volumes of phenol:chloroform:isoamyl alcohol (25:24:1 v/v). After collecting the supernatant, chloroform mixture was added, mixed gently and the solution again centrifuged at 5000 rpm for two minutes. To the supernatant, an equal volume of isopropanol was added, mixed thoroughly but gently, and kept for 20 minutes at –20°C. After one centrifugation at 10,000 rpm, the plasmid DNA pellet was washed two times with 70% ethanol, and diluted with 30 µL of $T_{10}E_1$ for storage at –20°C.

3.5.3.2.3. Purification of the inserts-NA 45 membrane method

The gene inserts of the clones were cleaved from their vectors using appropriate endonuclease enzymes such as *Pst*I and *Pst*II and fractioned by electrophoresis on a mini-gel of 0.8% agarose in TAE buffer for 3 hours at 6 V cm^{-1}. The gel was observed on a UV-transilluminator and the desired fragment was transferred onto NA 45 membrane in a slit just behind the band of the interest and allowing the electrophoresis to resume for a further 30 minutes. The DNA was eluted from the membrane by addition of sufficient (250 µL) high salt buffer (1 M NaCl, 0.01 mM EDTA, 20 mM Tris, pH 8.0) to cover the membrane, followed by incubation at 65°C for 10 minutes. Ethidium bromide was removed by extraction with TE saturated n-butanol and the DNA was precipitated with 0.5 volume of isopropanol at –80°C for 30 minutes and pelleted in a Sorvall

microcentrifuge at 10,000 rpm for 10 minutes. The pellet was washed in 70% ethanol, dried under vacuum and dissolved in $T_{10}E_1$.

3.5.3.2.4. Using PCR amplification for insert purification

Alternatively, we used PCR for amplification and then inserts were purified using Sephadex® 6-50 or Spin Column Elutips® or similar size exclusion media. In this method, the extracted plasmid DNA was diluted 100 times by mixing 1 µL plasmid DNA in 99 µL of water and 5 µL of this solution was used in a PCR reaction using M13 forward and M13 reverse primers. The following recipe was used to make the PCR reaction mixture:

PCR components	Volume
Distilled water	32.5 µL
10X PCR buffer	05.0 µL
2.5 mM dNTP	02.0 µL
Farward primer	01.0 µL
Reversel primer	01.0 µL
Taq poymerase	00.5 µL
Template DNA	05.0 µL
Total	50.0 µL

The details of the PCR cycling conditions used for insert amplification are as follows:

94°C – 3–5 minutes (initial denaturation)

94°C – 1 minute (denaturation)

40°C – 1 minute (annealing)

70°C 2 minutes for 32 cycles (annealing)

72°C – 5 minutes (extension)

04°C – Storage

After amplification, the amplified insert was purified using the Geneclean II DNA purification kit from BIO 101, Vista California or the GFx™ PCR DNA and gel band purification kit from Amersham Pharmcia Biotech, NJ, USA. After purification of the

insert, a minigel of 0.8% agarose was run to determine the concentration of the insert based on band intensity and probe volume taken at the time of hybridization.

3.5.3.3. Labeling of probes

The random-primed method of Feinberg and Vogelstein (1983a) was used for labeling DNA with α-^{32}P. A purified insert DNA sample of 5 µL was denatured by heating at 95°C for 5 minutes, then quenched on ice for 3 minutes before the labeling reaction mixture was added and incubated at 37°C for 2-3 hours. The reaction was terminated by adding 5 µL of 0.5 M EDTA to use in the hybridization step.

The labeling reaction mixture consists of 5 µL of oligo-labeling buffer (Amersham Phamacia Biotech), 2 µL equimolar concentrations of dCTP, dGTP and dTTP each, 2 µL of 50 µCi ^{32}P-dATP, and 2 units of Klenow enzyme.

Alternatively, the NE Blot® kit from New England Biolab Inc. can also be used for labeling following the method of Feinberg and Vogelstein (1983a & b). In this method, random sequence octa-deoxinucleotides serve as primers for DNA synthesis *in vitro* from denatured double stranded template DNA.

3.5.3.4. Hybridization to labeled probe

3.5.3.4.1. Pre-hybridization

Southern blots were pre-hybrized at 65°C with 15–20 mL of pre-hybridization solution (3 mL of 5X HSB, 1.0–1.5 mL of denatured salmon sperm DNA, 1.0 mL of Denhardt's solution and sterile distilled water to 15 mL) for 6 hours in case of new blots and 2–4 hours for stripped blots. Pre-hybridization was performed in a Techne Hybridizer (HB-1D).

3.5.3.4.2. Hybridization

Labeled probe was added to the hybridization bottles containing blots and pre-hybridization mixture and incubated at 65°C in a hybridization oven for at least 16 hours. Care was taken to remove air bubbles present between the blots and the wall of the hybridization bottle.

3.5.3.4.3. Washing of blots

Following hybridization, the blots were washed using four changes of 50–60 mL each of ^{32}P-wash solution. Each wash was carried out for 15 minutes at 65°C in hybridization bottles using the hybridization oven. The first two washes were done using wash 1 solution (100 mL 20X SSC, 25 mL 20% SDS and diluted to a volume of 1 L with distilled water) followed by two washes with wash 2 solution (10 mL 20X SSC, 25 mL 20% SDS and diluted to a volume of 1 L with distilled water). Membranes were air dried and enclosed in cling films.

3.5.3.5. Autoradiography

Autoradiography was conducted at –80°C by exposing the membrane to photographic film (Kodak, X-OMAT™, XK-5) using Kodak intensifying screens in a cassette for various exposure times depending on radioactivity counts. The X-ray films were developed with Kodak developer for 2 minutes followed by a stop bath (1% acetic acid) treatment for 1 minute, fixed with Kodak fixer for 2 minutes, washed in running tap water and air dried. The autoradiograms (Figures 17 and 22–25, given in Chapter 4 Experimental Results) were scanned/photographed using Kodak 100 ASA color print films.

3.5.3.6. Filter stripping and reuse

After results were scored, incorporated ^{32}P-α-dATP was stripped off from the filters so they could be used with the next probe. Filters were placed in the plastic box and boiling stripping solution (0.1X SSC, 0.5 SDS) was poured in until it covered the top filter. The box was placed on a shaking platform and kept there for 5 minutes while shaking intensively. The solution was poured off and the procedure was repeated three more times. After stripping, filters were wrapped in Saran-Wrap® and kept at –20°C, or hybridized again immediately. Filters were reused for 4–5 times before the resulting images became too weak to score confidently.

3.5.4. Scoring RFLP bands and genotyping

The banding patterns obtained from RFLP procedures were also scored in the same fashion as given above (section 3.4.4.3) in SSR banding pattern scoring.

3.5.5. Probe-enzyme DNA polymorphism between parents

To identify polymorphic combinations of probes and restriction enzymes, initial screening of parental lines was conducted before the actual genotyping of individuals in the F_2 mapping population. For this, the DNA from parental plants of ICMB 89111-P6 and ICMB 90111-P6 for population under study was restricted with four endonuclease restriction enzymes and probed against the available pearl millet PgPSM probes. From this screening, the polymorphic combinations were noted and used for further screening of the segregating F_2 population (Table 3).

3.6. LINKAGE MAPPING

Linkage analysis was accomplished using the software MapMaker/Exp version 3.0b supplied by E.S. Lander, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA (Lincoln *et al.*, 1992a). CentiMorgan (cM) distances were calculated using the Haldane function. The base map of pearl millet constructed by Liu *et al.*, (1994) from a cross of LGD × ICMP 85410 was used for comparison. The "sequence", "group" and "map" command were performed for linkage mapping and "build" command to place new markers from genotypic data set in the most appropriate position within the identified linkage group. Then software Mapchart was used to draw all linkage groups of the genetic linkage map.

3.7. QTL MAPPING

3.7.1. Using MapMaker/QTL

Trait data from $F_{2:4}$ self-bulks was averaged for each entry and sorted to correspond with the progeny order of the genotypes (marker data). The total number of progeny individuals from the cross (ICMB 89111-P6 × ICMB 90111-P6) with both trait and genotype information was 172. QTL mapping was performed using the program MapMaker/QTL version 1.1b (Lander and Botstein, 1989; Lincoln *et al.*, 1992b). MapMaker/QTL calculates additive and dominance effect from the change in phenotype resulting from the substitution of B parent alleles for A parent alleles. In the cross under study, ICMB 89111-P6 was susceptible downy mildew (scored as 'A) and the male parent ICMB 90111-P6 was resistant to downy mildew (scored as 'B').

The measured phenotype of F_2 individual number 1 is calculated as follows:

F_2 Trait = Mean + (Weight × Num_i) + (Dominance × Het_i) + Noise

where

Mean = the mean value of the components of the traits that was not controlled by this locus (in effect, the average trait value for A/A individuals)

Weight = the additive component of the B allele effect at this locus

Num_i = the number of B alleles carried by the individual number I; 0, 1, or 2

Dominance = the dominance component of the QTL B allele effect

Het_j = 1 if individual i is an A/B hetrozygote, and 0 otherwise

Noise = variation in the trait not controlled by this QTL (a normally distributed random variable)

3.8. MULTILOCATIONAL TRIALS

3.8.1. Development of different versions of hybrid HHB 94-like hybrid

Versions of ICMA 89111 corresponding to each of three sub-selections of ICMB 89111 have been produced by two generations of backcrossing (one generation each in *summer* and *kharif* 2001). During *summer* 2002, different versions of HHB 94 were developed by producing hybrid of the A-line corresponding to the mapping population parent versions of ICMB 89111, and the original ICMA 89111 with pollinator G73/107 (Table 4).

3.8.2. Layout out the experiment

The resulting nine different version of HHB 94 hybrids based on sub-selections of ICMA 89111, ICMB 89111 and ICMB 90111 with pollinator G 73/107, the original HHB 94 and control entry HHB 181 were evaluated in replicated trials conducted across different rainy season locations over 2 years (*kharif* 2002 and *kharif* 2003). In each environment, the 11 entries were evaluated in 5 replications of 4 rows by 4 m plots arranged in completely randomized block designs. Between and within rows spacing was 45-75 cm and 15 cm (Appendix 6), respectively, and 1 m alleys separated the plots. In *kharif* 2002, all 11 entries were evaluated at HAU Hisar, HAU RRS Bawal, and ICRISAT Patancheru (two locations RP 6 and RCE 24E). During *kharif* 2003, all entries were again evaluated at Patancheru (RP 9A and RCE 24C), HAU Hisar, HAU RRS Bawal (low fertility conditions), RAU RRS Durgapura, and RAU RRS Nagaur.

Table 4. Development of different versions of HHB 94-like hybrids using sub-selections of ICMA 89111, ICMB 89111 and ICMB 90111 as seed parent pollinated by G73/107

Sr. No.	Seed parent	Pollinator	Hybrid
1	ICMA 89111-P2	G 73/107	HHB 94-P2A
2	ICMA 89111-P5	G 73/107	HHB 94-P5A
3	ICMA 89111-P6	G 73/107	HHB 94-P6A
4	ICMA 89111-Original	G 73/107	HHB 94-Original
5	ICMB 90111-P2 or ICMP 423-P2	G 73/107	ICMH 02002
6	ICMB 90111-P5 or ICMP 423-P5	G 73/107	ICMH 02005
7	ICMB 90111-P6 or ICMP 423-P6	G 73/107	ICMH 02006
8	ICMB 89111-P2	G 73/107	HHB 94-P2B
9	ICMB 89111-P5	G 73/107	HHB 94-P5B
10	ICMB 89111-P6	G 73/107	HHB 94-P6B
11	ICMA 89111-original	H 77/833-2	HHB 181 (Control)

Plant counts were taken after thinning from the central two rows of each plot to determine the actual plant population. Flowering time, plant height, panicle girth and panicle length were assessed before harvesting as described below. At physiological maturity, panicles from the two central rows were harvested, counted, dried (oven dried at 60°C for 24 hours at Patancheru and sun dried at other locations), weighed and threshed to determine grain yield/plot and 1000-grain mass. Stover of the two harvested rows was cut and fresh yield was observed. Fresh straw samples were dried in the same fashion mentioned for panicles above to assess dry straw yield. Straw moisture content, average number of grains per panicle, effective number of tillers, total above-ground-biomass yields and harvest index were calculated using the above-mentioned information. All the observed plot data were transformed to a per square meter basis prior to statistical analysis.

3.8.3. Observations and measurements in multilocational trials

The observations and measurements taken during the trial were as follows:

1). Time to 50% flowering (TF): Time to 50% flowering was recorded as the number of days from sowing until 50% of the plants in each plot produced stigmas on their main stem panicles.

2). Plant height (PH): Plant height (cm) was measured from the base of the main stem to the tip of the panicle at maturity. Data was recorded on 5 random plants from the central two rows of each plot.

3). Panicle length (PL): Length of the panicle (cm) was measured for main the stem of the same plants considered for plant height in each plot.

4). Panicle diameter/girth (PD): Panicle diameter (mm) was measured using venire calipers on the panicles for which panicle length was recorded.

5). Effective plant stand (EPS): Number of plants was counted from the central two rows from each plot and then transformed into EPS per square meter.

6). Effective Tiller number (ETN): Number of productive panicles was counted from the central two rows from each plot and then transformed into ETN per square meter.

7). Fresh straw yield (FSY): After panicles were harvested, the stems and the tillers were cut and weighed of each plot, and then plot values transformed into per square meter.

7.1). Sub-sample fresh straw weight (SSFSW): Samples of fresh straw were then collected from each entry and chopped and fresh weight of these samples was taken (only at Patancheru and Nagaur).

7.2). Sub-sample dry straw weight (SSDSW): The chopped samples were kept in a drier for two days at temperature of 60°C and their dry weights were then recorded (only at Patancheru).

8). Dry straw yield (DSY): Dry straw yield was calculated using FSY, SSFSW and SSDSW per plot as follows:

DSY= (FSY*SSDSW) / (SSFSW)

and then transformed into dry matter yield per square meter.

9). Straw moisture content (SMC in %): Straw moisture content was computed as follows:

SMC= 100*(FSY-DSY)/(FSY)

10). Panicle yield (PY): After harvesting was completed, panicles were dried in an oven for 24 hours at 60°C at Patancheru and sun-dried at other locations. The dry weight of the panicles was then recorded before threshing and then this was transformed into panicle yield per square meter

11). Grain yield (GY): Dried panicles harvested from the two central rows of each plot were threshed and their grain cleaned. The weight of the grains from each plot was recorded and then transformed into grain yield per square meter.

12). 1000-grain mass (TGM): One thousand grains were counted and their mass was recorded for each plot.

13). Panicle grain number (PGN): It was calculated for each plot as follows:

PGN = (1000*GY)/ (TGM*ETN)

14). Total above ground biomass yield (TAGBY): Above-ground-biomass yield was calculated for each plot as the sum of PY and DSY.

15). Harvest Index (HI%): It was calculated for each plot using total above ground, biomass yield and grain yield in percentage as

HI= 100*GY/ (TAGBY)

3.8.4. In addition to the above characteristics, the analysis of other single degree of freedom contrasts were carried out as follows

Test of potential in ICMB 90111 to improve HHB 94 (in B-cytoplasm) was computed as

$\overline{X}_{5,6,7,} - \overline{X}_{8,9,10}$

where

$\overline{X}_{5,6,7}$ = trait average of entries 5-7 (ICMB 89111-based sub-selections × G 73/107),

$\overline{X}_{8,9,10}$ = trait average of entries 8-10 (ICMB 90111-based sub-selections × G 73/107)

Test of A_1 cytoplasm effect on GY within HHB 94 background was computed as

$\overline{X}_{1,2,3} - \overline{X}_{8,9,10}$

where

$\overline{X}_{1,2,3}$ = trait average of entries 1-3 (ICMA 89111-based sub-selections × G 73/107)

$\overline{X}_{8,9,10}$ = trait average of entries 8-10 (ICMB 90111-based sub-selections × G 73/107)

Test effect of selection within ICMB 89111 was computed as $\overline{X}_{1,2,3} - \overline{X}_4$

Test for comparison between HHB 94 and HHB 181 was computed as $\overline{X}_{11} - \overline{X}_4$

3.9. STATISTICAL ANALYSIS OF THE DM SCREENS

3.9.1. Frequency distributions

To identify clear breakpoints for resistance and susceptibility to downy mildew disease incidence, frequency distributions with class values of 0-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, 50-55, 55-60, 60-65, 65-70, 70-75, 75-80, 80-85, 85-90, 90-95 and 95-100% disease incidence were used. These class values were plotted on the X-axis against the interval frequency on the Y-axis to produce a frequency graph/histogram for disease reactions of the progenies from this pearl millet mapping population screened against each of the eight pathogen populations.

3.9.2. Chi-square tests

Chi-square goodness of fit tests is widely applicable to numerous problems of significance in frequency data. If the calculated Chi-square value is zero, the observed distribution shows complete agreement with the hypothetical distribution. Chi-square table values are compared at (n-1) degrees of freedom with the calculated Chi-square value.

$$\chi^2 = \sum_{i=1}^{n} (O - E)/E$$

where

O = observed frequencies

E = expected frequencies

Σ = summation over all the classes

n = number of independent classes in the hypothetical distribution.

All possible Mendelian ratios for monogenic, digenic, and trigenic (polygenic) segregations and their epsitatic interactions were taken as a expected frequencies and compared against the observed frequencies of downy mildew incidence resistance and susceptible behavior using distribution breakpoints in the disease incidence frequency histograms to assign groups to "resistant" or "susceptible" classes for estimation of observed frequencies.

3.9.3. Spearman rank correlation coefficients

Spearman rank correlation coefficients were calculated for disease incidence of the progenies in a given mapping population against each of the possible pairs of pathogen populations and a correlation table was formed to study the relationship between the different pathogen populations from India and Africa using the ranks of entry replicated mean downy mildew incidence (%) values of this mapping population progeny against each of a pair of pathogen isolates as the x and y inputs and summing across all progenies in a given mapping population (Azhaguvel, 2001).

Spearman rank correlation coefficient r = cov $(x, y) / (\sigma_x, \sigma_y)$

where

$\text{cov}(x, y) = \sqrt{\sum x_i y_i - xy / N}$

$\sigma_x = \sqrt{\sum x_i^2 - x^2 / N}$

$\sigma_y = \sqrt{\sum y_i^2 - y^2 / N}$

r = correlation coefficient

σ_x = standard deviation of x

σ_y = standard deviation of y

x = rank of variable 1 (DM incidence in screen against pathogen population 1)

y = rank for variable 2 (DM incidence in screen against pathogen population 2)

N = total number of mapping population progenies (from a single mapping population) scored against the two pathogen populations

i = mapping population progeny *i*....*N*

3.9.4. Cluster analysis

Cluster analysis was done with a hierarchical clustering technique–Euclidean distance with average link analysis–using NTSYSpc ver. 2.1d from Applied Biostatistics Inc. and Genstat version 6.0 from Rothamsted, UK (GENSTAT, 2002) to group the pathogen populations from India and Africa, using first, the ranks of entry replicated mean disease reaction and then entry replicated mean disease reactions of the 172 progenies in screens against each of the eight pathogen populations as the input data.

3.10. STATISTICAL ANALYSIS OF MULTILOCATIONAL FIELD TRIALS

All statistical analyses were performed using Genstat version 6 from Rothamsted, UK. Analysis was performed using the plot data recorded from multilocational trials conducted in two different *kharif* seasons. For the traits measured on individual plants, the phenotypic data was analyzed as means of 5 individual plants from each plot. Other traits were measured on plot basis and transformed to a per square meter basis prior to analysis.

3.10.1. Statistical analysis of individual trial environment data sets

The data obtained form *kharif* 2002 and *kharif* 2003 for different phenotypic traits were statistically analyzed on the basis of a model described by Panse and Sukhatme (1967):

$$Y_{ij} = \mu + a_i + b_j + e_{ij}$$

where,

Y_{ij} = observation in the i^{th} treatment and j^{th} block

μ = general mean

a_i = i^{th} treatment effect

b_j = j^{th} block effect, and

e_{ij} = random error associated with the i^{th} treatment and the j^{th} block.

The assumptions of the model are:

a. all the observations should be independent;

b. different effects in the model should be additive; and

c. error in the population should be normally and independently distributed with mean zero and variance σ_e^2.

3.10.1.1. Analysis of variance (ANOVA)

Analysis of variance for all characters under study were carried out for individual trial environments separately, as follows:

Source	d.f.	M.S.	Expected M.S.	F ratio
Replication	(r-1)	MSr	$\sigma^2_e + t\,\sigma^2_r$	MSr/MSe
Treatment	(t-1)	MSt	$\sigma^2_e + r\,\sigma^2_g$	MSt/MSe
Error	(r-1) (t-1)	MSe	σ^2_e	
Total	(rt-1)			

where,

r = number of replications;

t = number of treatments or genotypes;

σ^2_g = genotypic variance of character x;

σ^2_r = replication variance of character x;

σ^2_e = error variance of character x and

MSr, MSt and MSe stand for mean sums of squares due to replications, treatments and error, respectively.

3.10.1.2. Genotypic and phenotypic variances

The Genotypic and phenotypic variances for each trial environment were calculated as follows

Genotypic variance of character x = σ^2_g = (MSt – MSe) / r

Phenotypic variance of character x = $\sigma^2_p = \sigma^2_g + \sigma^2_e$

3.10.1.3. Parameters of variability

3.10.1.3.1. Mean:

Mean value ($\overline{X}$) of each character in each trial environment was worked out dividing the sum of the observed values by the corresponding number of observations:

$$\overline{X} = \sum X_{ij} / N$$

where,

X_{ij} = observation in the i_{th} treatment and j_{th} replication, and

N = total number of observations.

3.10.1.3.2. Range:

Lowest and highest entry mean values for each character were recorded for each trial location.

3.10.1.3.3. Standard error:

Standard errors of means were calculated for each character for each trial location from the corresponding mean square error values from the analysis of variance tables as:

$$S.E.\ (m) = \sqrt{\sigma^2_e / r}$$

where

σ^2_e is estimated mean sum of squares

S.E. is the standard error of the mean, and

r is the number of replications.

3.10.1.3.4. Honestly significant difference (hsd):

For all the characters, hsd values were calculated to compare treatment means within each trial location as suggested by Tukey (1953), using the equation:

$$W = q_\alpha\ (p, n_2)\ s_{\bar{x}}$$

where,

q_α is obtained from table for $\alpha = 0.05$ or 0.01 (Steel and Torrie, 1960)

p is the number of treatments,

n_2 is the error degrees of freedom,

$s_{\bar{x}}$ is the standard error of the mean (SEM) and

w is used to judge the significance of each of the observed pair-wise differences between treatment means.

3.10.1.3.5. Coefficient of Variation:

Genotypic and phenotypic coefficients of variation were estimated for each observed or calculated trait in each trial environment by the formula suggested by Burton (1952) for each character as:

Genotypic coefficient of variation (G.C.V.) = $\frac{\sqrt{\sigma^2_g \times 100}}{\bar{x}}$

Phenotypic coefficient of variation (P.C.V.) = $\frac{\sqrt{\sigma^2_p \times 100}}{\bar{x}}$

where

$\bar{x}$ is the mean of that particular trait in that particular trial environment.

3.10.1.3.6. Heritability (in broad sense):

Heritability in broad sense was calculated according to the formula suggested by Hanson (1956) for each character as given below

$$H = \frac{\sigma^2_g \times 100}{\sigma^2_p}$$

3.10.2. Statistical analysis of genotypic × environment interactions

Analyses of variance were first performed on the data from individual and pairs of locations/environment using micro across location randomized block design (acrrbd) program and restricted maximum likelihood (REML) from Genstat version 6, from Rothamsted Experimental Station. The observation Y_{ijkl} on genotype i recorded in block j of the replication k in environment l was modeled as:

$$Y_{ijkl} = \mu + e_l + (r_{kl} + b_{jkl}) + g_i + (ge)_{il} + e_{ijkl}$$

where

μ denotes the general mean;

e_l denotes the effect of environment, l;

r_{kl} denotes the effect of replication k within environment l;

b_{jkl} denotes the effect of block j within replication k within environment l;

g_i denotes the effect of genotype i;

$(ge)_{il}$ denotes effect of interaction of genotype i within environment l; and

e_{ijkl} denotes residual effect.

3.10.2.1. Pooled analysis of variance

Pooled analysis of variance (Macintosh, 1983) for all characters across all multiple-test environment clusters and all ten test environments was carried out using Genstat version 6 (2002) as follows:

Source of variation	d.f.	M.S.	Expected M.S.	F ratio
Environment	(e-1)	MSe	$\sigma^2_e + r\sigma^2_{ge} + g\sigma^2_{r(e)} + rg\sigma^2_e$	MSe/MSE
Blocks/Test Environment	e(r-1)	MSr	$\sigma^2_e + r\sigma^2_{r(e)}$	MSr/MSE
Genotype	(g-1)	MSg	$\sigma^2_e + r\sigma^2_{ge} + re\sigma^2_g$	MSg/MSE
G × E	(e-1) (g-1)	MSge	$\sigma^2_e + r\sigma^2_{ge}$	MSge/MSE
Poled error	r(e-1)(g-1)	MSE	σ^2_e	

where,

r = number of replications;

g = number of treatments or genotypes;

σ^2_g = genotypic variance of character x;

σ^2_{ge} = variance of character x due to g×e;

σ^2_e = error variance of character x, and

MSr, MSg, MSge and MSE stand for mean sums of squares due to replications, genotypes, genotype × environment interactions and error, respectively.

CHAPTER 4

CHAPTER 4

EXPERIMENTAL RESULTS

4. EXPERIMENTAL RESULTS

4.1. DOWNY MILDEW SCREENING

4.1.1 Downy mildew reaction by parental and control entries

The parental lines of the P6 mapping population (ICMB 89111-P6 × ICMB 90111-P6) along with resistant and susceptible control entries were screened against six Indian and two African populations of *Sclerospora graminicola* under greenhouse conditions at ICRISAT, Patancheru, India (six Indian pathogen populations) in *kharif* 2002 and at the University of Wales, Bangor, UK (two African pathogen populations) in late *kharif* 2002 in three replications each. Parental line ICMB 90111-P6 was highly resistant and exhibited no symptoms of infection against three pathogen populations, one from India (Patancheru) and two from Africa (Maiduguri, Nigeria and Bamako, Mali), but was resistant to moderately susceptible to five pathogen populations [Jamnagar (4% downy mildew incidence), New Delhi (6%), Jodhpur (9%), Durgapura (10%) and Jalna (24%)] from India, as presented in Table 5.

Very high downy mildew incidence (DMI %) levels were observed on susceptible parental line ICMB 89111-P6 in DM screens against Indian pathogen populations from Durgapura (95%), Jamnagar (94%), and Patancheru (91%). Moderately high DMI level were observed for this parental line in screens against the African pathogen population from Bamako, Mali (82%), as well as those from Jodhpur (73%) and New Delhi, (69%). But surprisingly, a different pattern was recorded from the screen against the Jalna pathogen population where parental line ICMB 89111-P6 exhibited a low level of DMI (14%). This parental line also exhibited a relatively moderate DMI level in its screen against pathogen population from Maiduguri, Nigeria (35%). DMI was comparatively higher on ICMB 89111-P6 in screens against the six Indian pathogen populations than against the two African pathogen populations. Parental line ICMB 90111-P6 showed 0% DMI against the two African pathotypes for which data were generated, where as differential disease reactions were observed on the parents in its screens against the six Indian pathogen populations. Among various control entries 7042 (S) showed 95-100% DMI across all Indian and 75-85% DMI in screens against the two African pathogen

Table 5. The mean downy mildew reactions of pearl millet mapping population parental lines ICMB 89111-P6 and ICMB 90111-P6, and resistant and susceptible control inbreds, against six Indian and two African populations of *Sclerospora graminicola* under greenhouse conditions at Patancheru, India (*kharif*, 2002) and at Bangor, UK (Late *kharif* 2002)

Parents/controls	CAZRI, Jodhpur, *Sg*139			ICRISAT, Patancheru, *Sg*153			IARI, New Delhi, *Sg*298			JAU MRS, Jamnagar, *Sg*200		
	TPC	DMC	DMI%	TPC	DMC	DMI%	TPC	DMC	DMI%	TPC	DMC	DMI%
ICMB 89111-P6	32	23	72	20	18	91	25	18	69	36	34	94
ICMB 90111-P6=ICMP 423-P6	23	2	9	12	0	3	21	1	6	26	1	4
7042(S)	32	32	100	31	30	99	33	32	96	32	32	99
700651 = ICML 16	18	4	22	22	4	18	23	5	23	24	4	16
7042R = ICML 22	29	17	59	25	15	63	26	15	58	32	14	44
843B	40	38	95	40	39	97	33	31	96	39	35	90
P1449-2	20	1	5	17	1	9	31	1	4	32	0	0
PT 732B	32	0	0	33	27	81	32	30	92	34	1	3

Parents/controls	RAUARS, Durgapura, *Sg*151			MAHYCO, Jalna, *Sg*150			Maiduguri, Nigeria, Screen 43			Bamako, Mali, Screen 45		
	TPC	DMC	DMI%	TPC	DMC	DMI%	TPC	DPC	DMI%	TPC	DPC	DMI%
ICMB 89111-P6	25	24	95	23	3	14	27	9	35	36	29	82
ICMB 90111-P6=ICMP 423-P6	12	1	10	13	3	24	20	0	0	27	0	1
7042(S)	26	25	99	26	25	95	40	30	75	38	32	85
700651 = ICML 16	18	3	17	17	6	35	-	-	-	-	-	-
7042R = ICML 22	32	17	53	32	17	53	-	-	-	-	-	-
843B	35	32	92	38	7	19	-	-	-	-	-	-
P1449-2	26	0	0	24	1	5	-	-	-	-	-	-
PT 732B	27	0	0	26	24	90	-	-	-	-	-	-
ICMB 89111-P2	-	-	-	-	-	-	29	0	1	37	28	75
ICMB 90111-P2=ICMP 423-P2	-	-	-	-	-	-	14	7	52	23	11	50
HB 3	-	-	-	-	-	-	39	33	82	40	35	88
P7-3	-	-	-	-	-	-	34	0	0	36	11	30

DMI (%) = downy mildew incidence = 100×DMC/TPC; DMA = arcsin (DMC/TPC); TPC = total plant count; DMC = downy mildew count;
- = indicates non-availability of data

populations. Another susceptible control entry (843B) exhibited DMI values ranging from 90% to 97% across the Indian pathogen populations against which it was screened except that from Jalna (19%). In contrast, a very highly resistant reaction was observed for resistant control P1449-2 in screens against all six Indian pathogen populations (DMI values ranges from 0% to 9%). Resistant control 700651 was also found to possess moderate levels of resistance that was effective across most of Indian pathogen populations. Another control genotype, PT 732B exhibited high levels of resistance to pathogen population from Jodhpur, Durgapura and Jamnagar (0-3% each) but high levels of susceptibility to those from New Delhi, Jalna and Patancheru (81-92% DMI). This differential disease reaction of PT 732B is a clear case of pathogen population-specific host-plant resistance as described by van der Plank (1963, 1968), Day (1974) and many others. A high degree of susceptibility was observed in susceptible control hybrid HB 3 when it was screened against both the African pathogen populations. In contrast, very high to moderate levels of resistance were recorded for resistant control inbred P7-3 against downy mildew African pathogen populations from Maiduguri and Bamako, respectively. For all other resistant and susceptible genotypes used in this study, variable DMI levels were observed across both Indian and African pathogen populations of *S. graminicola*.

4.1.2. DM reaction by 172 $F_{2:4}$ self-bulks against individual pathogen populations

Table 6 presents a summary of analysis of variance for screens against these eight individual pathogen populations from India and Africa. The individual comparisons of average downy mildew disease incidence of 172 $F_{2:4}$ self-bulks and eight parents and control entries, made among Indian as well as African pathogen populations, revealed that the pathogen population of *S. graminicola* from Jodhpur (*Sg*139) caused the greatest disease incidence (37.31%) followed by the pathogen populations from Bamako, Mali (Africa) and Durgapura (India). The pathogen population from Jalna (India) with mean DMI 13.91% and that from Maiduguri, Nigeria (Africa) with mean DMI 14.24% were observed to be the least virulent pathogen populations used in this study. A general trend of very high operational heritability estimates for DMI was observed for screens against the Maiduguri (0.83 and 0.94) and Jamnagar (0.82 and 0.93) pathogen populations. The lowest operational heritability values for DMI were recorded from screens against pathogen populations from Jalna (0.58 and 0.80) and Jodhpur (0.64 and 0.84).

Table 6. Individual summaries of DM screens of 164 to 172 $F_{2:4}$ mapping population progenies with their parents and control inbreds against eight pathogen populations of *S. graminicola* from India and Africa

Summary	CAZRI, Jodhpur, *Sg*139				ICRISAT, Patancheru, *Sg*153				IARI, New Delhi, *Sg*298				JAU MRS, Jamnagar, *Sg*200			
	DMI%	DMA	TPC	DMC	DMI%	DMA	TPC	DMC	DMI%	DMA	TPC	DMC	DMI%	DMA	TPC	DMC
Mean	37.31	0.43	28.37	10.02	24.27	0.28	27.91	6.50	21.05	0.23	29.61	5.95	28.30	0.32	30.71	8.21
SE (+/-)	9.57	0.13	3.49	2.50	7.31	0.10	2.78	2.05	6.99	0.09	3.11	1.95	6.46	0.08	2.53	2.00
LSD	13.54	0.19	4.94	3.53	10.33	0.14	3.93	2.90	9.89	0.12	4.40	2.76	9.13	0.12	3.58	2.83
CV (%)	44.43	53.29	21.33	43.22	52.16	63.60	17.26	54.71	57.51	65.52	18.21	56.80	39.51	45.72	14.26	42.24
F-ratio	6.25	6.40	5.27	9.04	12.00	10.81	7.84	13.88	7.37	7.71	6.03	9.00	14.27	15.26	8.92	13.83
h^2 (mean basis)	0.64	0.64	0.59	0.73	0.79	0.77	0.70	0.81	0.68	0.69	0.63	0.73	0.82	0.83	0.73	0.81
h^2 (plot basis)	0.84	0.84	0.81	0.89	0.92	0.91	0.87	0.93	0.86	0.87	0.83	0.89	0.93	0.93	0.89	0.93

Summary	RAU ARS, Durgapura, *Sg*151				MAHYCO, Jalna, *Sg*150				Maiduguri, Nigeria, Screen 43				Bamako, Mali, Screen 45			
	DMI%	DMA	TPC	DMC	DMI%	DMA	TPC	DMC	DMI%	DMA	TPC	DMC	DMI%	DMA	TPC	DMC
Mean	31.87	0.35	29.39	8.74	13.91	0.15	29.22	3.78	14.24	0.15	30.95	4.35	34.85	0.38	31.41	10.78
SE (+/-)	7.60	0.10	2.73	2.31	5.97	0.07	2.60	1.48	4.74	0.06	2.11	1.49	6.78	0.09	2.03	2.16
LSD	10.75	0.14	3.86	3.26	8.44	0.10	3.68	2.09	6.71	0.09	2.98	2.11	9.59	0.12	2.87	3.05
CV (%)	41.31	47.83	16.09	45.71	74.26	82.38	15.43	67.82	70.57	11.80	59.51	57.70	33.72	38.60	11.18	34.66
F-ratio	8.98	9.61	8.88	8.17	5.06	5.44	9.80	5.94	15.82	12.57	11.56	16.95	12.86	11.59	11.70	14.41
h^2 (mean basis)	0.73	0.74	0.72	0.71	0.58	0.60	0.75	0.62	0.83	0.79	0.78	0.84	0.80	0.78	0.78	0.82
h^2 (plot basis)	0.89	0.90	0.89	0.88	0.80	0.82	0.90	0.83	0.94	0.92	0.91	0.94	0.92	0.91	0.91	0.93

DMI (%) = downy mildew incidence = 100 × (DMC/TPC); DMA = arcsin (DMC/TPC); TPC = total plant count; DMC = downy mildew count

4.1.3. Genotype by pathogen population interactions

Replicated data of 172 $F_{2:4}$ self-bulks, two hybrid parental lines and six control entries screened against six Indian and two African pathogen populations of *S. graminicola* were subjected to analysis of variance. The 172 $F_{2:4}$ self-bulk showed significant variation in downy mildew screens against all pathogen populations, as indicated by significant values of F-ratios. Table 7 presents a summary of this analysis of variance showing interactions between genotypes and their screens against pathogen populations from India (Asia) and Africa.

No significant variation was recorded between pathogen populations from different continents, but significant variations between pathogen populations from Asia and between pathogen populations from Africa were observed. Also significant effects of entries and entries × pathogen population interactions were observed for DMI, DMA and DMC. Total plant count (TPC) differed significantly across the two continents ($P>0.01$, level of significance), indicating that the seedling numbers used in screens at ICRISAT-Patancheru (against the six pathogen isolates from India) and the University of Wales, Bangor (against the two pathogen populations from Western Africa) were significantly different. Significant differences were recorded for all four variables (DM%, DMA, TPC and DMC) among the six Indian pathogen populations and among the two African pathogen populations using the 172 $F_{2:4}$ self-bulk mapping population progenies (except for TPC within two African pathogen populations). Significant differences were observed among genotypes ($F_{2:4}$ self-bulks) across all pathogen populations for all four variables. In addition to this, genotype × pathogen population interactions including pathogen population across continents, Indian pathogen populations × genotypes, and African pathogen populations × genotypes were significant for all four variables.

REML variance component analysis (Table 8) revealed significant contributions of differences among genotypes (F_2-derived F_4 self bulks), and genotypes × screens (pathogen populations) interactions, observed variation downy mildew incidence (DMI%), DMA, TPC, DMC across all the eight screens; however pathogen populations themselves do not contribute significantly to observed variation. No significant differences were observed across pathogen populations for any of the variables (DMI, DMA, TPC and DMC) when tested at 5% and 1% levels of significance. Almost the same pattern was followed across Indian and African pathogen populations for all four

Table 7. Analysis of variance showing interactions between genotypes (172 $F_{2:4}$ self-bulks and eight parents and controls) and their screens against eight pearl millet pathogen populations of *S. graminicola* from India and Africa against which 180 common host entries were screened

Source of variation	d.f. (m.v.)	Mean sums of squares			
		DMI%	DMA	TPC	DMC
Continent	1	573.20	0.16	1418.01*	51.38
Asia	5	36603.70**	5.20**	523.42*	2713.25**
Africa	1	118390.60**	13.34**	54.86	11552.71**
Error	16	5315.10	0.76	172.64	453.96
Entries	179	8146.90**	1.38**	1254.01**	735.54**
Continent × Entry	170(9)	861.30**	0.15**	72.03**	113.15**
Asia × Entry	895	393.10**	0.07**	25.96**	34.13**
Africa × Entry	170(9)	665.60**	0.10**	13.28	73.99**
Error	2818(46)	149.10	0.03	21.77	12.25
Total	4255(64)				

DMI (%) = downy mildew incidence = 100 × (DMC/TPC)
DMA (radians) = arcsin (DMC/TPC)
TPC = total plant count
DMC = downy mildew count

Table 8. Summary of REML variance components analysis showing the interactions between genotypes and pathogen populations across continents

	Variance Component Analysis											
	Indian pathogen populations				African pathogen populations				All the eight screens			
Source of variation	DMI%	DMA	TPC	DMC	DMI%	DMA	TPC	DMC	DMI%	DMA	TPC	DMC
Replication	0.30	0.00	0.01	0.01	11.30	0.00	0.96	1.37	0.40	0.00	0.07	0.06
Host genotype	347.00**	0.06**	53.11**	32.01**	251.90**	0.03**	45.34**	27.74**	316.70**	0.05**	51.05**	28.55**
Pathogen population	67.50	0.01	0.92	4.94	208.80	0.03	0.08	20.36	78.20	0.01	0.99	6.43
Geno × Patho	66.60**	0.01**	0.00	6.36**	181.20**	0.03**	0.13	20.59**	105.40**	0.02**	2.36**	11.97**
Error	192.60	0.03	34.46	15.13	119.90	0.02	12.91	12.11	178.00	0.03	29.51	14.69
Grand mean	26.12	0.29	29.24	7.20	24.38	0.26	31.22	7.53	25.70	0.29	29.72	7.27
Heritability (bs)	0.94	0.94	0.97	0.94	0.69	0.69	0.95	0.69	0.94	0.94	0.97	0.93
CV (%)	53.14	61.48	20.07	54.06	44.92	52.24	11.51	46.23	51.90	60.20	18.28	52.68
SE (+/-)	3.22	0.04	1.36	0.91	4.81	0.06	1.56	1.53	3.22	0.04	1.27	0.94
LSD	8.94	0.12	3.77	2.51	13.34	0.17	4.34	4.26	8.94	0.12	3.52	2.61

DMI (%) = downy mildew incidence = 100×DMC/TPC; DMA = arcsin (DMC/TPC); TPC = total plant count; DMC = downy mildew count

variables except for TPC where no significant differences were recorded for genotype × pathogen population interactions for pathogen populations from within India (screens conducted at Patancheru) or pathogen population from within Africa (screen conducted at Bangor).

The grand means of 29.24 for total plant count (TPC) and 7.20 for downy mildew diseased plant count (DMC) resulted in a grand mean DMI value of 26.12% across all six Indian pathogen populations of pearl millet downy mildew for all 172 $F_{2:4}$ self-bulks, two parents and six checks. Significant and very high broad sense heritability values for all the four variables were observed (>0.94). The analysis of screens against the two African pathogen populations indicated a mean DMI value of 24.38% from a TPC mean value of 31.22 and DMC mean value of 7.53. Very high heritability for TPC (0.95) and moderately high heritability values (0.69) for DMI (%), DMC and DMA were observed. The grand means of all four variables and their heritabilities were also calculated from DM screen data against eight pathogen populations. This too revealed very high heritability values for DMI, DMA and TPC and DMC (>0.93), with a grand mean of 25.70% (DMI) resulting from a mean downy mildew plant count of 7.27 out of the mean 29.72 total plant count (Table 8).

4.1.4. Inheritance of downy mildew disease resistance

The Chi square (χ^2) estimates for goodness of fit among 172 $F_{2:4}$ self-bulks screens against six Indian and two African pearl millet pathogen populations of *S. graminicola* under greenhouse conditions revealed monogenic to oligogenic types of gene action governing the downy mildew disease resistance exhibiting classical Mendelian ratios from 3:1 (single gene action) to 255:1 epistatic gene interaction) for resistant : susceptible disease reactions of the mapping population progenies against different pathogen populations (Table 9) at natural downy mildew disease incidence (DMI%) break points observed in frequency distributions (Figures 5-12). The details of DMI against each pathogen population resistance and inheritance of DMR for each pathogen population is explained below:

4.1.4.1. Pathogen population from CAZRI, Jodhpur, Rajasthan, India (*Sg*139)

DM screening of the 172 $F_{2:4}$ mapping population progenies revealed a good fit to the Mendelian ratio of 1 resistant to 3 susceptible (monogenic recessive resistance) against

Table 9. Chi square estimates for goodness of fit to various classical Mendelian ratios among 164 to 172 $F_{2:4}$ self-bulks under greenhouse conditions against eight pearl millet pathogen populations of Indian and African origin

Pathogen Population	DMI (%) break point at	Mendelian segregation ratio	Chi square value
CAZRI, Jodhpur, India (*Sg*139)	15	1:3	1.74
	55	13:3	0.00
	65	55:9	0.35
	95	63:1	1.24
ICRISAT, Patancheru, India (*Sg*153)	30	3:1	0.63
	40	13:3	0.54
	80	15:1	0.16
IARI, New Delhi, India (*Sg*298)	25	3:1	0.63
	50	15:1	0.01
	60	63:1	5.49
	85	255:1	0.04
JAU MRS, Jamnagar, India (*Sg*200)	50	13:3	0.86
	85	15:1	1.05
RAU ARS, Durgapura, India (*Sg*151)	40	3:1	0.19
	50	13:3	0.86
	80	15:1	0.50
	90	63:1	2.99
MAHYCO, Jalna, India (*Sg*150)	30	15:1	0.06
	40	63:1	2.99
	55	255:1	0.04
Maiduguri, Nigeria West Africa Screen 43	20	3:1	2.61
	50	15:1	0.29
	75	63:1	0.37
Bamako, Mali West Africa Screen 45	5	9:55	0.66
	10	3:13	0.29
	50	3:1	0.30
	60	13:3	0.51
	80	15:1	1.07

the CAZRI, Jodhpur pathogen population using a breakpoint of 15% DMI (Figure 5). Using a breakpoint of 55% DMI, the digenic epistatic ratio of 13:3 (resistant : susceptible) was observed, whereas trigenic epistatic segregation ratios of 55:9 and 63:1 (triplicate dominant resistance) were observed at DMI breakpoints of 65% and 95%, respectively. The majority of the $F_{2:4}$ self-bulks exhibited DMI values in the range of 20% to 40% with the mapping population frequency distribution displaying a moderate skewedness toward the resistant parent value.

4.1.4.2. Pathogen population from ICRISAT, Patancheru, India (*Sg*153)

Segregation among the 172 $F_{2:4}$ self-bulks for disease reaction against the pathogen population from ICRISAT, Patancheru gave a good fit to the Mendelian monogenic dominant resistance ratio (3 resistant : 1 susceptible) at a natural breakpoint of 30% DMI. At breakpoints of 40% and 80% DMI the Mendelian segregation ratios (among progenies of the mapping population) of 13:3 (one basic gene and one inhibitory gene action) and 15:1 (duplicate dominant resistance) respectively, were observed to fit best to observed resistant : susceptible segregation patterns. Figure 6 indicates that distribution of the 172 $F_{2:4}$ mapping population progeny self-bulks was skewed towards the DMI value of the resistant parent, ICMB 90111-P6, with a substantial majority of the progenies exhibiting a DMI below 35%.

4.1.4.3. Pathogen population from IARI, New Delhi, India (*Sg*298)

The 172 $F_{2:4}$ mapping population progenies screened against the pearl millet downy mildew pathogen population from IARI, New Delhi recorded the best fit to a Mendelian segregation ratio of 3:1 (resistant : susceptible) at a DMI breakpoint level of 25% (Figure 7). It also fit well a digenic dominant resistance gene ratio of 15:1 at the DMI breakpoint level of 50% DMI. However, it did not fit trigenic ratio of 63 resistant to 1 susceptible at the natural breakpoint level of 80% DMI. At a high DMI breakpoint of 85%, for the pathogen population from New Delhi, this mapping population progeny set also exhibited a good fit to the Mendelian segregation ratio of 255:1 (quadruplicate dominant resistance genes). A relatively high resistance level was observed among the mapping population progenies against this pathogen population, with low DMI value ranging between 5% and 20% for the majority of the 172 F_2-derived F_4 self-bulks, the distribution strongly skewed towards the value of the resistant parent ICMB 90111-P6, as shown in Figure 7.

Figure 5. Frequency distribution of downy mildew disease incidence (%) among 172 $F_{2:4}$ self-bulks from the pearl millet cross (ICMB 89111-P6 x ICMB 90111-P6) when screened under greenhouse conditions in ICRISAT-Patancheru against a *S. graminicola* population (*Sg*139) from CAZRI, Jodhpur, Rajasthan, India. The arrows in red color indicate DMI (%) natural break points

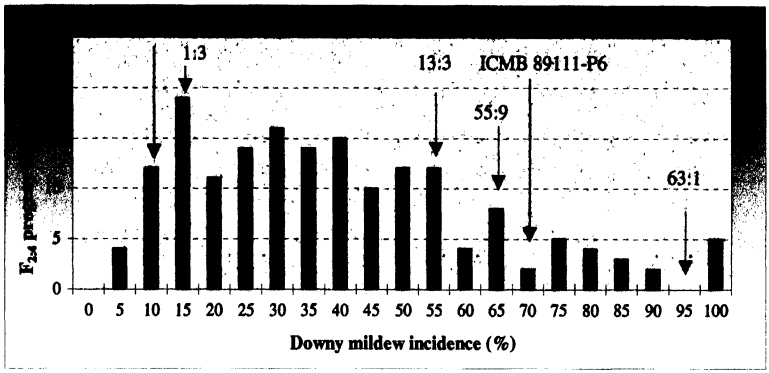
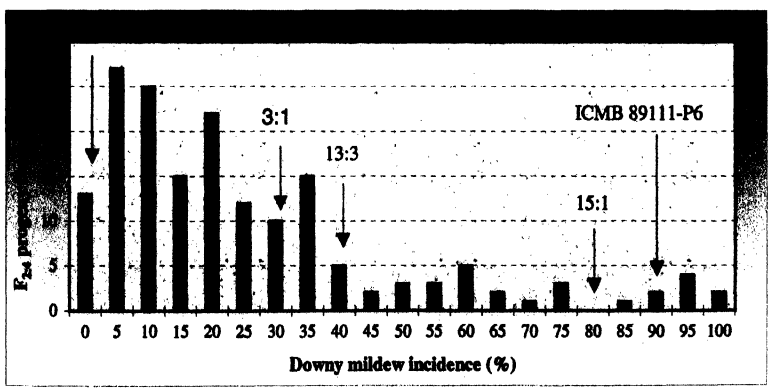


Figure 6. Frequency distribution of downy mildew disease incidence (%) among 172 $F_{2:4}$ self-bulks from the pearl millet cross (ICMB 89111-P6 x ICMB 90111 P6) when screened under greenhouse conditions in ICRISAT-Patancheru against a *S. graminicola* population (*Sg*153) from ICRISAT, Patancheru, A.P., India. The arrows in red color indicate DMI (%) natural break points



4.1.4.4. Pathogen population from JAU MRS, Jamnagar, Gujarat, India (*Sg*200)

The segregating pattern observed for downy mildew disease reactions among the 172 $F_{2:4}$ mapping population progenies when screened against the pathogen population from Jamnagar, Gujarat, India, suggested that two epistatic host plant resistance genes were largely responsible (Figure 8). Good fits to Mendelian digenic segregation ratios 13:3 and 15:1 were recorded at DMI breakpoints of DMI 50% and 85%, respectively, as shown in the frequency distribution histogram. The frequency distribution histogram drawn for this pathogen population depicts a very consistent disease reaction pattern with large numbers of progenies having low DMI values and small numbers of progenies having high levels of DMI, showing a strong skewedness of the distribution toward values of the resistant parent ICMB 90111-P6.

4.1.4.5. Pathogen population from RAU ARS Durgapura, Rajasthan, India (*Sg*151)

The frequency distribution of DMI values observed among 172 F_2-derived F_4 self-bulks from pearl millet cross ICMB 89111-P6 × ICMB 90111-P6 in their screen against downy mildew pathogen population *Sg*151 from RAU, ARS Durgapura is presented in Figure 9. The monogenic dominant resistance ratio of 3:1 and the digenic ratio of 13:3 gave good fits at the natural breakpoints of 40% and 50% DMI respectively. Digenic and Trigenic ratios of 15:1 and 63:3 were observed to give best fits to natural breakpoints in frequency distribution at 80% and 90% among a mapping population of 172 self-bulks. The majority of these F_2-derived F_4 self-bulks showed DMI values in the range from 10% to 35%, so the population exhibited substantial skewedness towards the DMI level observed in resistant parent ICMB 90111-P6.

4.1.4.6. Pathogen population from MAHYCO, Jalna, India (*Sg*150)

The DMI screening of 172 $F_{2:4}$ mapping population progenies against this pathogen population displayed a fairly different pattern of DMI as compared to the five screens mentioned above. More than 90% of the 172 $F_{2:4}$ self-bulks showed a downy mildew disease incidence values of 5% to 25% and DMI as high as 60% was observed for only one of the $F_{2:4}$ self-bulks (Figure 10). The *S. graminicola* pathogen population from Jalna was found to much less virulent than others used in screening the mapping population of cross ICMB 89111-P6 × ICMB 90111-P6. At natural DMI breakpoints of 30%, the digenic ratio of 15 resistant : 1 susceptible, which has been fairly consistently observed

Figure 7. Frequency distribution of downy mildew disease incidence (%) among 172 $F_{2:4}$ self-bulks from the pearl millet cross (ICMB 89111-P6 x ICMB 90111-P6) when screened under greenhouse conditions in ICRISAT-Patancheru against a *S. graminicola* population (*Sg*298) from IARI, New Delhi, India. The arrows in red color indicate DMI (%) natural break points

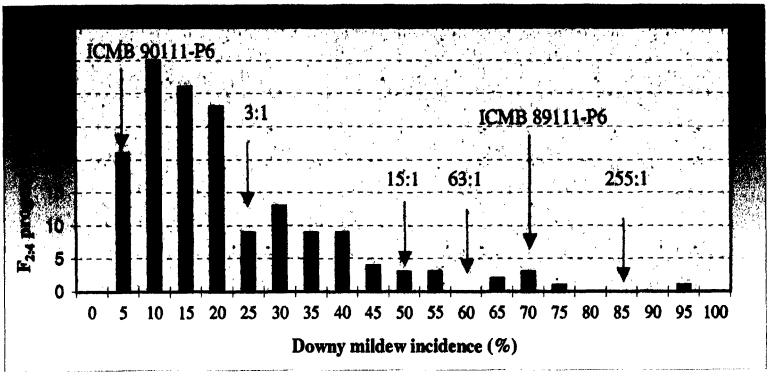


Figure 8. Frequency distribution of downy mildew disease incidence (%) among 172 $F_{2:4}$ progenies from the pearl millet cross (ICMB 89111-P6 x ICMB 90111-P6) when screened under greenhouse conditions in ICRISAT-Patancheru against a *S. graminicola* population (*Sg*200) from JAU MRS, Jamnagar, Gujrat, India. The arrows in red color indicate DMI (%) natural break points

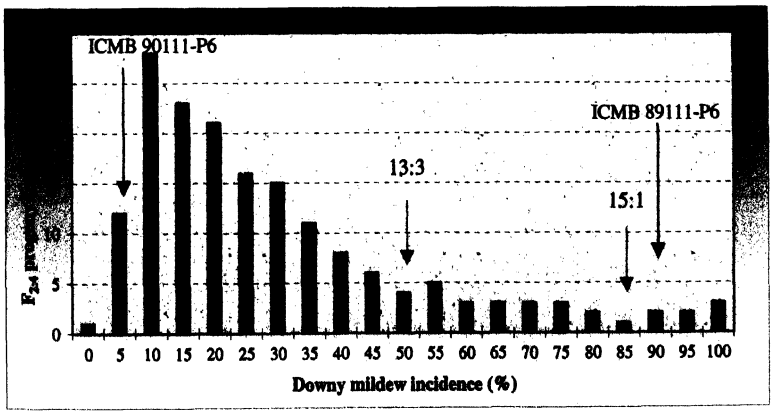


Figure 9. Frequency distribution of downy mildew disease incidence (%) among 172 $F_{2:4}$ self-bulks from the pearl millet cross (ICMB 89111-P6 x ICMB 90111-P6) when screened under greenhouse conditions in ICRISAT-Patancheru against a *S. graminicola* population (*Sg*151) from RAU ARS, Durgapura, Jaipur, Rajasthan, India. The arrows in red color indicate DMI (%) natural break points

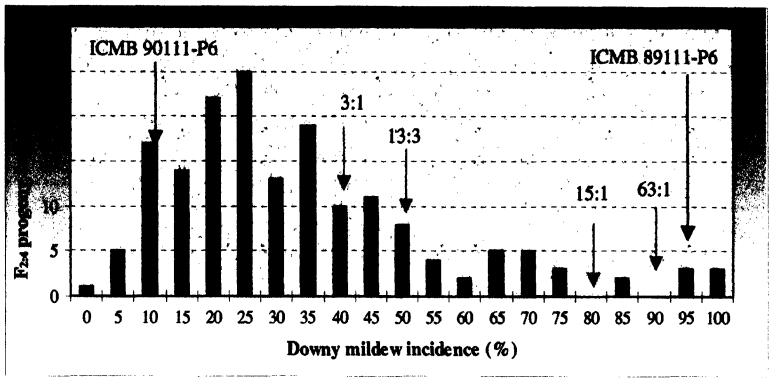
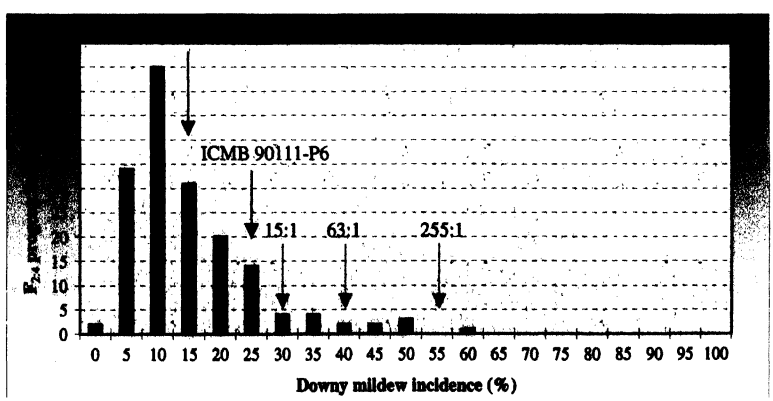


Figure 10. Frequency distribution of downy mildew disease incidence (%) among 172 $F_{2:4}$ progenies from the pearl millet cross (ICMB 89111-P6 x ICMB 90111-P6) when screened under greenhouse conditions in ICRISAT-Patancheru against a *S. graminicola* population (*Sg*150) from MAHYCO, Jalna, Maharashtra, India. The arrows in red color indicate DMI (%) natural break points



across majority of screens in present study, was also recorded against this pathogen population. Pearl millet downy mildew screening of 172 $F_{2:4}$ mapping population progenies against the pathogen population (*Sg*150) from Jalna also exhibited 63:1 (three dominant resistance genes) and 255:1 (four dominant resistance genes) Mendelian segregation ratios at 40% and 55% of natural DMI breakpoints respectively. However perhaps the most interesting results from this screen was that the parent ICMB 891111-P6 appeared to be more resistance to the MYHCO-Jalna pathogen population than the "resistant" parent ICMB 90111-P6.

4.1.4.7. Pathogen population from Maiduguri, Nigeria (West Africa)

The frequency distribution of pearl millet downy mildew incidence among 172 $F_{2:4}$ self-bulks from cross ICMB 89111-P6 × ICMB 90111-P6, when screened against this pathogen population from Maiduguri, Nigeria (Africa) showed that a majority of the self-bulks had DMI values of 0% to 20% and their distribution was thus skewed strongly towards the DMI level observed in resistant parent ICMB 90111-P6. The monogenic segregation ratio of 3:1 and the digenic segregation ratio of 15:1 were observed to fit well at natural DMI frequency distribution breakpoints of 20% and 50%, respectively (Figure 11). However, trigenic dominant gene action with a segregation ratio of 63:1 (resistant : susceptible) also gave a good fit when using the natural DMI breakpoint of 75%. A single major R-gene from ICMB 90111-P6 appears responsible for most of the observed variation in DMI with two additional genes of smaller effect also likely to be present.

4.1.4.8. Pathogen population from Bamako, Mali (West Africa)

It is evident from the pearl millet DM screening results that the Bamako Mali pathogen population from West Africa was more virulent than the six Indian pathogen populations and the first African (Maiduguri, Nigeria) pathogen population used in this study. Digenic Mendelian segregating ratios of 13:3 and 15:1 were observed to fit well the observed segregation pattern among the 172 $F_{2:4}$ self-bulks using natural breakpoints in DMI frequency distribution of 60% and 80%, respectively (Figure 12). Interestingly, a digenic segregation ratio (3 resistant : 13 susceptible) and a trigenic segregation ratio (9 resistant : 55 susceptible) were also observed, both ratios fitting good to Mendelian segregation pattern at natural breakpoint of 10% DMI from screen of the 172 mapping population progenies under study against pathogen population from Bamako (Africa). More than half

Figure 11. Frequency distribution of downy mildew disease incidence (%) among 172 $F_{2:4}$ self-bulks from the pearl millet cross (ICMB 89111-P6 x ICMB 90111-P6) when screened under greenhouse conditions at the University of Wales, in Bangor, UK, against a *S. graminicola* population from Maiduguri, Nigeria, in West Africa. The arrows in red color indicate DMI (%) natural break points

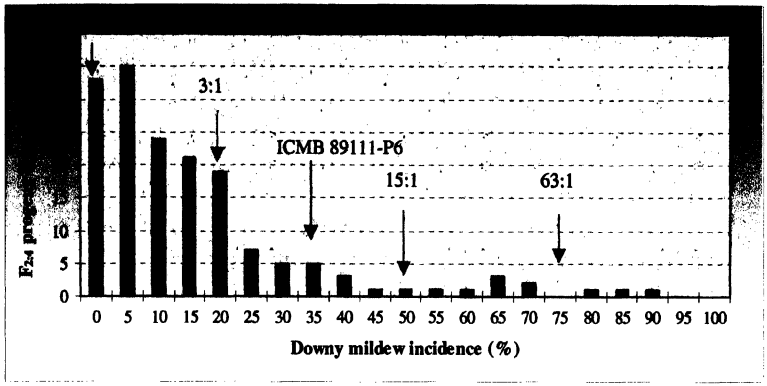
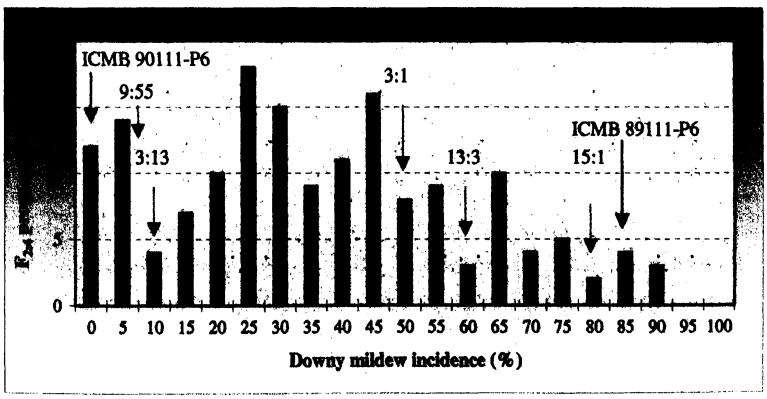


Figure 12. Frequency distribution of downy mildew disease incidence (%) among $F_{2:4}$ self-bulks from the pearl millet cross (ICMB 89111-P6 x ICMB 90111-P6) when screened under green house conditions at the University of Wales, in Bangor, UK, against a *S. graminicola* population from Bamako, Mali, in West Africa. The arrows in red color indicate DMI (%) natural break points



of the total of 172 $F_{2:4}$ self-bulks of mapping population progenies from cross ICMB 89111-P6 × ICMB 90111B-P6 displayed DMI values between 20% and 45% in the screen against this highly virulent pathogen population.

4.1.5. Spearman rank correlation coefficient

Spearman rank correlation coefficient values (significant at $p<0.01$ level) were calculated based on ranks of entry mean DMI (%) among 172 $F_{2:4}$ self-bulks screened against each of six Indian pathogen populations, and are presented in Table 10. Separate Spearman rank correlation coefficient values were also calculated based on ranks of entry mean DMI (%) among 164 $F_{2:4}$ mapping population progenies self-bulks screened against each of the six Indian and two African pathogen populations and these values are presented in Table 11. Very strong correlations were observed in disease reactions of the mapping population progenies to pathogen populations of *S. graminicola* from Patancheru and New Delhi both, from India ($r = 0.823$), and between Patancheru (India) and Maiduguri (Nigeria) ($r = 0.822$), despite the apparent differences in frequency distributions of DMI values (compare Fig. 6 vs. Fig. 7 and Fig. 6 vs. Fig. 11). The general trend of correlation was stronger amongst pathogen populations from India and between Indian and African populations than between the two African pathogen populations. A relatively lower correlation ($r = 0.539$) was observed between the two African pathogen populations. Pathogen populations from northern India (Jodhpur, New Delhi, Durgapura and Jamnagar) were more highly correlated among themselves than with the pathogen populations from southern regions of India (Patancheru and Jalna). Two highly virulent pathogen populations from Jodhpur (India) and Bamako (Mali) exhibited a significant positive correlation ($r = 0.713$) where as a correlation of $r = 0.707$ was observed between the two most highly virulent Indian pathogen populations (Jodhpur and Patancheru). In contrast, mapping population progeny disease reactions against the Jalna pathogen population exhibited a very poor correlation with those against the rest of the Indian and African pathogen populations used in this study. Among Indian pathogen populations (Table 10), very strong correlations were also recorded for Jamnagar with Patancheru (0.810), New Delhi with Patancheru (0.805) and both New Delhi and Durgapura with Jamnagar. For example, the relationship between pathogen populations from Jalna and Patancheru was observed to be relatively poor [(0.449 and 0.433 based on 172 (six Indian) and 164 $F_{2:4}$ progenies (two African pathogen populations), respectively)].

Table 10. Spearman rank corrleation coefficients for greenhouse seedling downy mildew reaction among 172 $F_{2:4}$ self-bulks derived from a single selfed plant of pearl millet cross (ICMB 89111-P6 × ICMB 90111-P6) when screened against six Indian pathogen populations of *S. graminicola*

Jodhpur	1					
Patancheru	0.695**	1				
New Delhi	**0.776****	**0.805****	1			
Jamnagar	0.724**	**0.810****	0.797**	1		
Durgapura	0.766**	0.739**	0.773**	0.797**	1	
Jalna	0.450**	0.449**	0.467**	0.546**	0.543**	1
	Jodhpur	Patancheru	New Delhi	Jamnagar	Durgapura	Jalna

Table 11. Spearman rank corrleation coefficients for greenhouse seedling downy mildew reaction among 164 $F_{2:4}$ self-bulks derived from a single selfed plant of pearl millet cross (ICMB 89111-P6 × ICMB 90111-P6) when screened against six Indian and two African pathogen populations of *S. graminicola*

Jodh-Pur	1							
Patan-Cheru	0.707**	1						
New Delhi	0.765**	0.823**	1					
Jam-Nagar	0.722**	0.814**	0.802**	1				
Durga-Pura	0.762**	0.738**	0.766**	0.789**	1			
Jalna	0.430**	0.433**	0.448**	0.527**	0.522**	1		
Maidu-guri	0.571**	0.822**	0.732**	0.724**	0.669**	0.411**	1	
Bam-ako	0.713**	0.558**	0.684**	0.585**	0.647**	0.385**	0.539**	1
	Jodh-pur	Patan-cheru	New Delhi	Jam-nagar	Durga-pura	Jalna	Maidu-guri	Bam-ako

These Spearman rank correlation matrix values were then used to construct two separate dendrograms (Figures 14a and 14b). The first dendrogram shows relationships among six pathogen populations of Indian origin and the second dendrogram was constructed based on screens against these six Indian and two additional African pathogen populations.

4.1.6. Cluster analysis

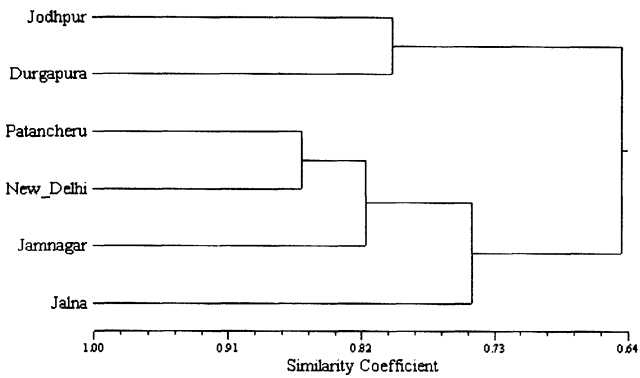
4.1.6.1. Based on entry mean DMI (%)

The entry mean downy mildew incidence (%) of 164 $F_{2:4}$ mapping population progenies from screens against each of eight pathogen populations from India and Africa, were used to construct two separate dendrograms (Figure 13). The first dendrogram was constructed based on screens against six Indian pathogen populations. The second dendrogram was constructed on based on screens from these six Indian and two African pathogen populations. The first dendrogram (Figure 13a) revealed two groups of pearl millet pathogen population of *S. graminicola* used in present study. First group consisted of two more virulent pathogen populations from Jodhpur and Durgapura. The second group consisted of three virulent pathogen populations from Patancheru, New Delhi, Jamnagar and one very less virulent pathogen population from Jalna. Among the six Indian pathogen populations, those from Patancheru and New Delhi were observed to be most similar to each other based on downy mildew reactions exhibited among these 172 $F_{2:4}$ self-bulks. These two similar pathogen populations forming a subgroup within the second group, in which they appeared closer in relationship with the pathogen population from Jamnagar than that from Jalna.

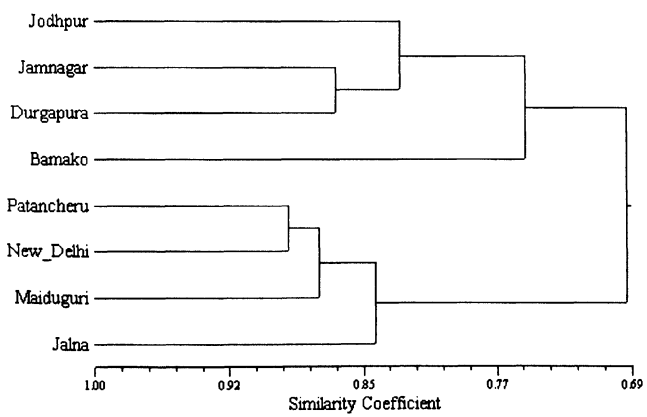
The dendrogram based on entry mean DMI (%) values from screens of 164 common $F_{2:4}$ self-bulks against each of the eight pathogen populations also depicted two clear-cut groups, each group containing four pathogen populations (Figure 13b). The first one included pathogen populations from Jodhpur, Jamnagar, Durgapura (all pathogen populations from India) and Bamako (a pathogen population from Africa). With this group, pathogen populations from Jamnagar and Durgapura exhibited the greatest similarities for DMI among the $F_{2:4}$ mapping population progenies under study. The degree of relatedness decreased as pathogen populations from Jodhpur and Bamako were added in first group. The second group included two less virulent pathogen populations

Figure 13. Dendrograms showing relationships among eight *S. graminicola* populations from India and Africa based on % similarity in mean disease incidence for $F_{2:4}$ self bulks derived from pearl millet cross (ICMB 89111-P6 × ICMB 90111-P6), when screened against severe inoculum pressure of the individual pathogen populations under greenhouse conditions

a. Based on entry mean DMI (%) values from screens against six Indian pathogen populations (172 $F_{2:4}$ self-bulks)



b. Based on entry mean DMI (%) values from screens against eight Indian and African pathogen populations (164 $F_{2:4}$ self-bulks)



— one from Maiduguri (Africa) and the other from Jalna (India) — along with two more virulent Indian pathogen populations from Patancheru and New Delhi. Within this second group, the closest similarities were between the two pathogen populations from Patancheru and New Delhi, while relatedness decreased as Maiduguri and Jalna were added. Two most virulent pathogen populations included in this study, (Jodhpur and Bamako) were relatively distanced from each other in the first group of this dendrogram, while the two least virulent pathogen populations observed (Maiduguri and Jalna) were found relatively closer/similar in causing DMI among mapping population progenies in second group of the dendrogram.

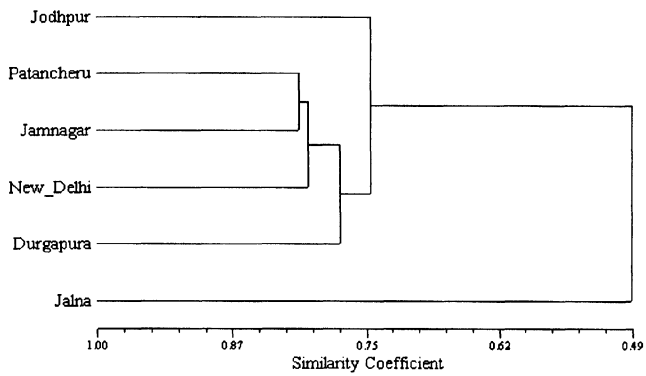
4.1.6.2. Based on spearman rank correlations

Spearman rank correlations calculated from ranks of entry mean downy mildew incidence (%) among $F_{2:4}$ self-bulks from in each of eight screens (see Tables 6, 10 and 11) were used to construct two separate dendrograms. The first dendrogram (Figure 14a) was constructed using Spearman correlation matrix values based on 172 $F_{2:4}$ self-bulks screened against each of the six Indian pathogen populations. The second dendrogram (Figure 14b) was constructed using Spearman correlation matrix values based on ranks of entry mean DMI values among 164 $F_{2:4}$ mapping population progenies screened against each of these six Indian and two additional African pathogen populations of *S. graminicola*. The first dendrogram depicted two clusters, the first consisting of five more virulent pathogen populations (Durgapura, New Delhi, Jamnagar, Patancheru and Jodhpur), and second cluster containing only one less virulent pathogen population (Jalna). Among Indian pathogen populations those from Patancheru and Jamnagar were found to be the most similar. The degree of relatedness decreased as the pathogen population from New Delhi, Durgapura, and Jodhpur were added to this first cluster, with increased dissimilarity in ranks of mean disease reactions of the 172 $F_{2:4}$ mapping population progenies.

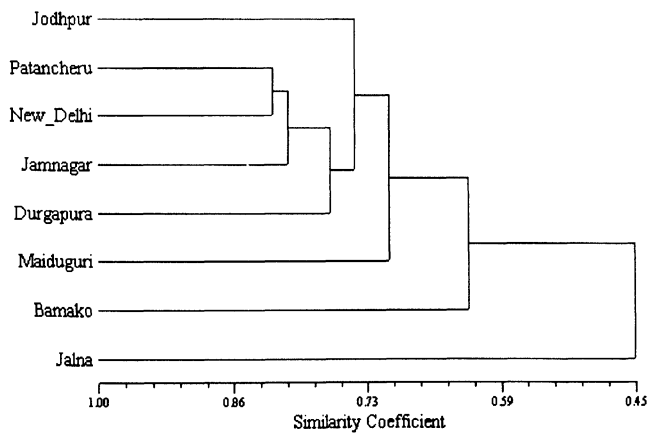
Clustering of Indian and African pathogen population using Spearman rank correlation matrix values based ranks of DMI (%) for 164 $F_{2:4}$ mapping population progenies from screens against each of eight pathogen populations depicted a continuum (Figure 14b). The core of this consists of five more closely related Indian (Durgapura, Jamnagar, New Delhi, Patancheru, and Jhodhpur). Two African (Maiduguri and Bamako) pathogen populations and the less Indian virulent Indian pathogen population from Jalna

Figure 14. Dendrograms showing relationships among eight *S. graminicola* populations from India and Africa based on % similarity in rank order of entry mean disease incidence for 172 $F_{2:4}$ self bulks derived from pearl millet cross (ICMB 89111-P6 × ICMB 90111-P6), when screened against severe inoculum pressure of the individual pathogen populations under greenhouse conditions

a. Based on Spearman rank correlations among six Indian pathogen populations (172 $F_{2:4}$ self-bulks)



b. Based on Spearman rank correlations among eight Indian and African pathogen populations (172 $F_{2:4}$ self-bulks)



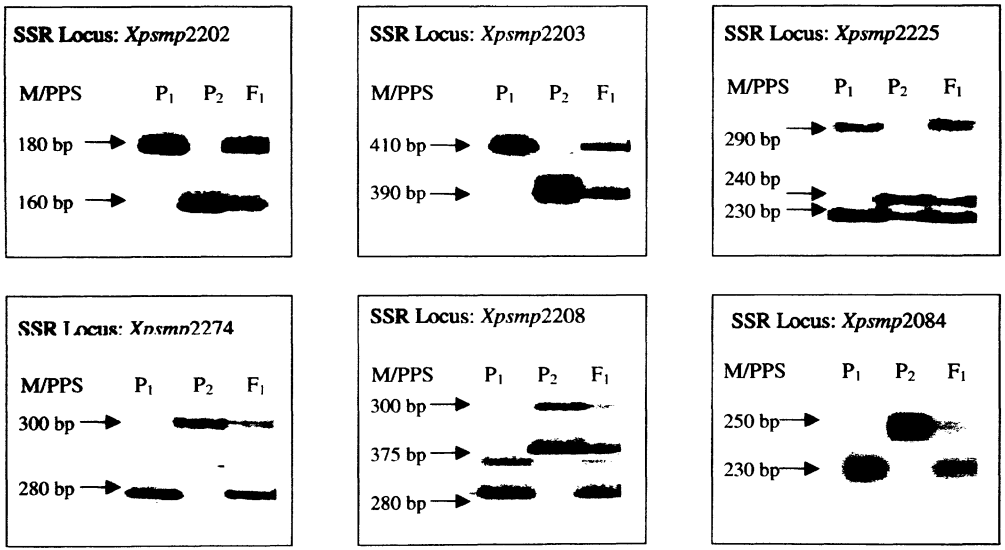
rounded out the continuum. In the core group, pathogen populations from Patancheru and New Delhi (both from India) exhibited considerable similarity and the degree of relatedness decreased as those from Jamnagar, Durgapura and Jodhpur (all from India) were added. Maiduguri and Bamako (both from Africa), and the very less virulent pathogen populations from Jalna were placed very far from the pathogen population from Patancheru in this dendrogram. The two most highly virulent pearl millet downy mildew pathogen populations used in this study, one from Jodhpur (India) and the other from Bamako (Mali), were observed to be relatively dissimilar to each other based upon Spearman rank correlations, so it is expected that the host resistance genes effective against these two pathogen population are different.

4.2. PARENTAL POLYMORPHISM

Pearl millet mapping population parental lines ICMB 89111-P6 and ICMB 90111-P6 were screened against 80 SSR (microsatellite) and 35 selected RFLP (140 probe-enzymes combinations from 35 probes × 4 restriction enzymes) markers to identify polymorphic combinations. This study included both single-copy and multi-copy markers, the majority of which were single-copy SSR and RFLP markers that had been mapped previously (Liu *et al.*, 1994 and Qi *et al.*, 2004). PAGE gels and auto-radiograms showing results from screens of these parental lines against a set of SSR markers and RFLP probe-enzyme combinations have been given as Figures 15-17.

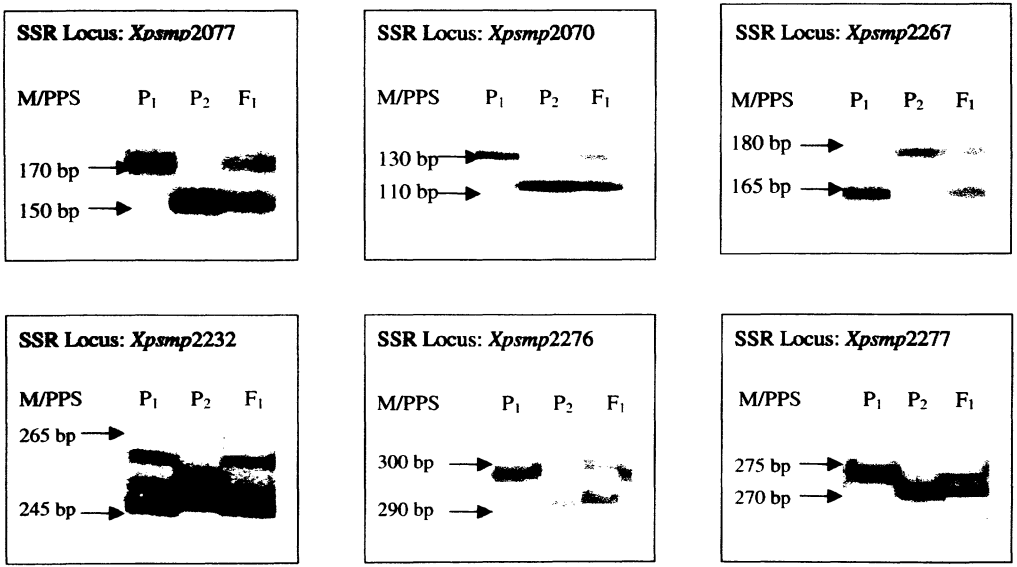
Out of the total 115 markers screened (80 SSRs and 35 RFLPs), approximately 45% showed good polymorphism between these two parental lines. The restriction enzyme *Hind*III gave more polymorphic combinations (35%) with the set of 35 RFLP probes used in study followed by *Eco*RV (30%), *Eco*RI (25%) and *Dra*I (10%). Larger numbers of polymorphic probe-enzyme combinations were obtained with *Dra*I, but most of these gave highly distorted segregation ratios or produced autoradiograms for which scoring was difficult, so these were not pursued further. A total of 26 SSR markers (Table 2) and 20 RFLP markers (Table 3), found polymorphic between two parental lines were used to screen the mapping population of 172 $F_{2:4}$ self-bulks.

Figure 15. Screening of parental lines of the cross ICMB 89111-P6 × ICMB 90111-P6 and their F_1 for SSR (microsatellite) marker polymorphism using various primer pairs



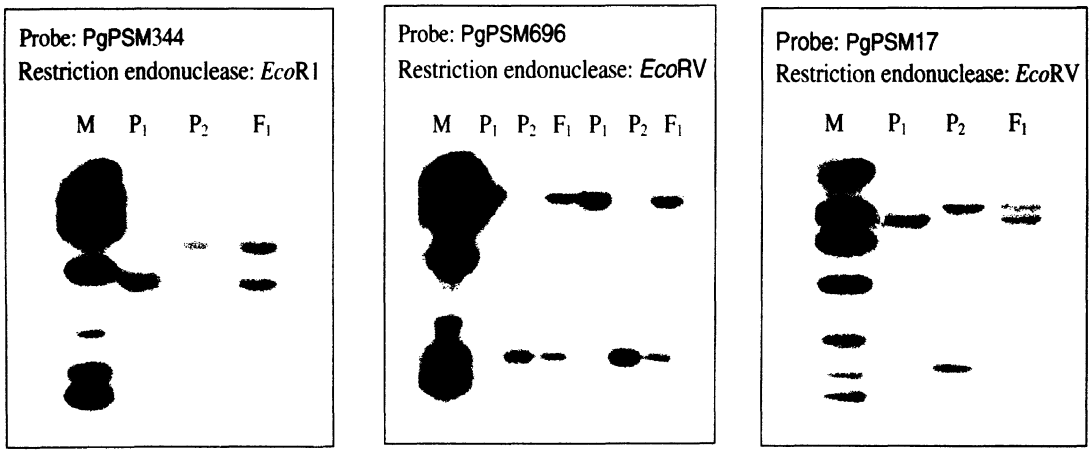
P_1 = Homozygous for susceptible parent ICMB 89111-P6 allele, denoted as A
P_2 = Homozygous for resistance parent ICMB 90111-P6 allele, denoted as B
F_1 (=P_1 × P_2) = Heterozygote, denoted as H
M/PPS = Molecular marker ladder (100 base pair)/PCR product size

Figure 16. Screening of parental lines of the cross ICMB 89111-P6 × ICMB 90111-P6 and their F_1 for SSR (microsatellite) marker polymorphism using various primer pairs



P_1 = Homozygous for susceptible parent ICMB 89111-P6 allele, denoted as A
P_2 = Homozygous for resistance parent ICMB 90111-P6 allele, denoted as B
F_1 (=P_1 × P_2) = Heterozygote, denoted as H
M/PPS = Molecular marker ladder (100 base pair)/PCR product size

Figure 17. Screening of parental lines of the cross ICMB 89111-P6 x ICMB 90111-P6 and their F_1 against different probe-enzyme combinations used in the present study



P_1 = Homozygous for susceptible parent ICMB 89111-P6 allele, denoted as A
P_2 = Homozygous for resistance parent ICMB 90111-P6 allele, denoted as B
F_1 (=P_1 x P_2) = Heterozygote, denoted as H
M = Molecular marker ladder of *Hind*III-digested lambda (λ) DNA

4.3. SEGREGATION OF MARKERS AND THEIR DISTORTION

The segregation ratios of the marker loci were calculated using Chi square (χ^2) formula [$\chi^2 = \sum_{i=1}^{n}$ (Observed - Expected)/Expected. The calculated χ^2 values were then compared with tabulated values for 1 degree of freedom. The segregation pattern of marker loci (SSRs and RFLPs) for the mapping population of 172 F_2 progenies was compared with the expected ratio of 1:2:1 (1 homozygote from Parent P_1 : 2 hetrozygote : 1 homozygote from P_2). The calculated χ^2 values using observed frequency of A : H : B and its expected frequency for each and every individual marker locus are presented in the Table 12. The calculated χ^2 values were compared with tabulated values for 5% and 1% probability levels at 1 degree of freedom. Eighteen out of 46 co-dominant marker loci (including both SSR and RFLP) revealed non-significant χ^2 values when compared with table values of 3.84 at the 5% and 6.64 at the 1% probability levels. The remaining 28 markers deviated significantly from expected ratios at both probability levels. The significant χ^2 values ranging from 6.66 to 20.81 recorded the highest degree of distortion at 1% level for 21 marker loci on LG1, LG2, LG4, LG5, LG6 and LG7. The most distorted marker loci were placed in an order of (from the most deviated to the least deviated) *Xpsm*588 > *Xpsmp*2018 > *Xpsm*696 > *Xpsm*575 > *Xpsmp*2080 > *Xpsmp*2263 > *Xpsmp*2072 in terms of higher χ^2 values to lower ones.

All four marker loci on LG6 exhibited highly significant segregation distortion while none of four marker loci on LG3 showed such segregation distortion. Ten RFLP loci and eighteen SSR loci showed segregation distortion. RFLP loci on LG6 showed the highest degrees of segregation distortion. The silver-stained PAGE gels of PCR products and the autoradiograms from Southern hybridization showing examples of scoring of mapping population progenies with some of the SSR and RFLP markers are presented in Figures 18-25.

4.4. GENETIC LINKAGE MAP BASED ON CROSS ICMB 89111-P6 × ICMB 90111-P6

Genotypic data generated for a total of 46 marker loci (26 SSRs and 20 RFLP probe-enzyme combinations) were used to construct a linkage map of the pearl millet mapping population of 172 F_2-derived F_4 progenies based on the cross ICMB 89111-P6 × ICMB 90111-P6. A previously constructed integrated consensus pearl millet linkage map (Qi *et*

Figure 18. Multiplex PAGE gel obtained from genotyping of the segregating F_2 mapping population progenies using two different SSR loci differing in size of PCR-amplified DNA of plant entries

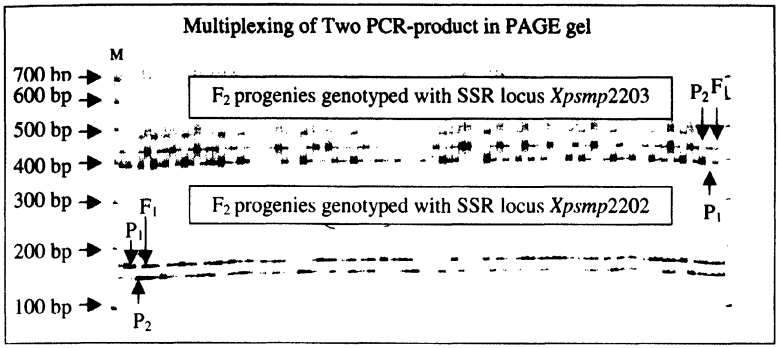
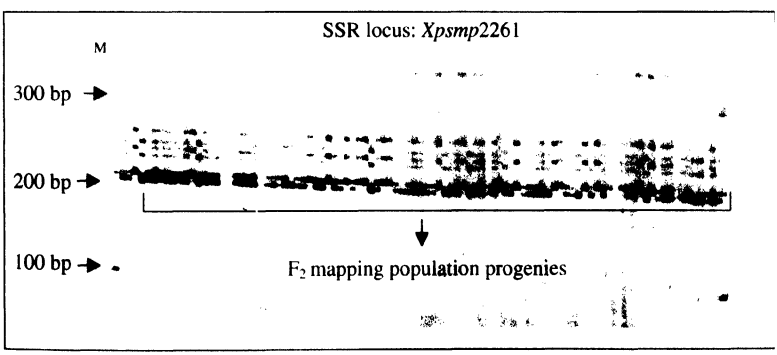


Figure 19. Monoplex PAGE gel obtained from genotyping of the segregating F_2 mapping population progenies using PCR-amplified DNA of plant entries from single SSR locus *Xpsmp*2261



P_1 = Homozygous for susceptible parent ICMB 89111-P6 allele, denoted as A
P_2 = Homozygous for resistance parent ICMB 90111-P6 allele, denoted as B
F_1 (=$P_1 \times P_2$) = Heterozygote, denoted as H
M = Molecular marker ladder (100 base pair)

Figure 20. Monoplex PAGE gel obtained from genotyping of the segregating F_2 mapping population progenies using PCR-amplified DNA of plant entries from single SSR locus *Xpsmp*2261

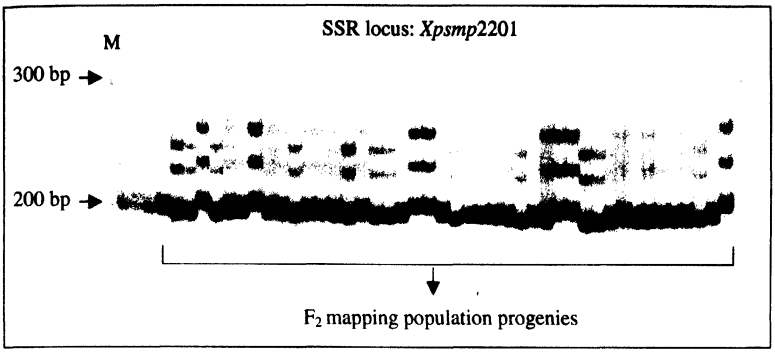
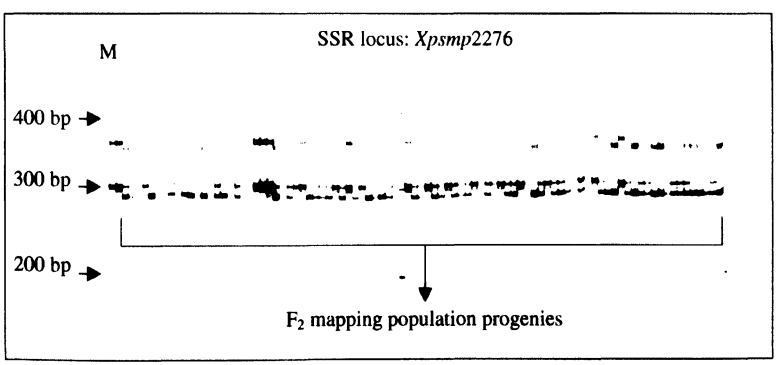


Figure 21. Monoplex PAGE gel obtained from genotyping of the segregating F_2 mapping population progenies using PCR-amplified DNA of plant entries from single SSR locus *Xpsmp*2276



P_1 = Homozygous for susceptible parent ICMB 89111-P6 allele, denoted as A
P_2 = Homozygous for resistance parent ICMB 90111-P6 allele, denoted as B
F_1 (=$P_1 \times P_2$) = Heterozygote, denoted as H
M/PPS = Molecular marker ladder (100 base pair)/PCR product size

Figure 22. Autoradiogram obtained from genotyping of the segregating F_2 mapping population progenies based on cross ICMB 89111-P6 x ICMB 90111-P6 with RFLP locus *Xpsm*344.

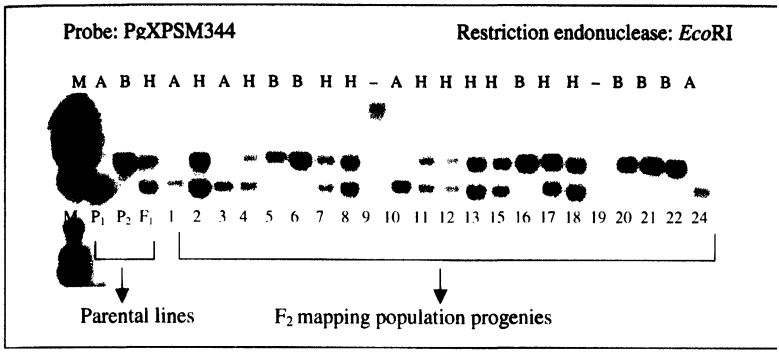
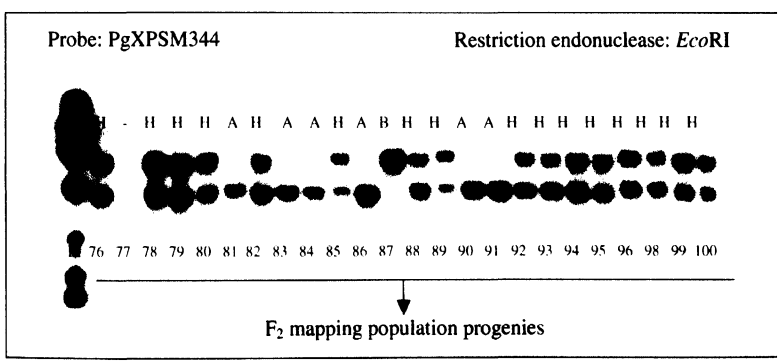


Figure 23. Autoradiogram obtained from genotyping of the segregating F_2 mapping population progenies based on cross ICMB 89111-P6 x ICMB 90111-P6 with RFLP locus *Xpsm*344



P_1 = Homozygous for susceptible parent ICMB 89111-P6 allele, denoted as A
P_2 = Homozygous for resistance parent ICMB 90111-P6 allele, denoted as B
F_1 (=P_1 x P_2) = Heterozygote, denoted as H
M = Molecular marker ladder of *Hind*III-digested lambda (λ) DNA

Figure 24. Autoradiogram obtained from genotyping of the segregating F_2 mapping population progenies based on cross ICMB 89111-P6 × ICMB 90111-P6 with RFLP locus *Xpsm*344

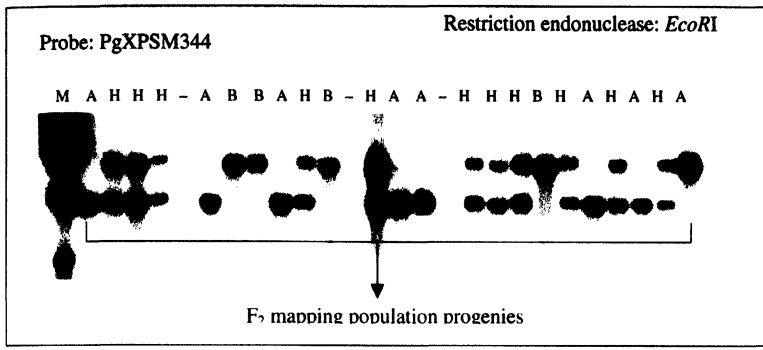
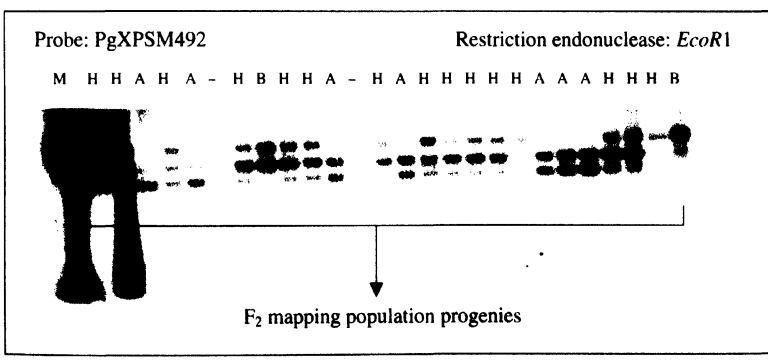


Figure 25. Autoradiogram obtained from genotyping of the segregating F_2 mapping population progenies based on cross ICMB 89111-P6 × ICMB 90111-P6 with RFLP locus *Xpsm*492



P_1 = Homozygous for susceptible parent ICMB 89111-P6 allele, denoted as A
P_2 = Homozygous for resistance parent ICMB 90111-P6 allele, denoted as B
F_1 (=P_1 × P_2) = Heterozygote, denoted as H
M = Molecular marker ladder of *Hind*III-digested lambda (λ) DNA

Table 12. Chi square estimates and segregation distortion observed among 172 F_2 pearl millet mapping population progenies based on cross ICMB 89111-P6 × ICMB 90111-P6 across 46 polymorphic marker loci (including SSRs and RFLPs) as compared to expected Mendelian 1:2:1 segregation ratios

Linkage group	Marker locus	Observed F_2 data segregation						Total	Chi square calculated	Expected F_2 data segregation			Excess (+) / Deficit (-) of		
		A	H	B	C	D	Missing			A	H	B	A	H	B
LG 1	*Xpsm*280	39	74	31	2	4	22	172	1.00	37.5	75.0	37.5			
	*Xpsm*492	27	88	33	5	0	19	172	5.78*	38.3	76.5	38.3	-	+	
	*Xpsmp*2273	25	89	43	1	0	14	172	6.94**	39.5	79.0	39.5	-	+	+
	*Xpsm*17	19	64	42	1	0	46	172	8.54**	31.5	63.0	31.5	-	+	+
	*Xpsmp*2080	22	87	52	0	0	11	172	12.23**	40.3	80.5	40.3	-	+	+
	*Xpsmp*2030	22	82	48	2	0	18	172	9.84**	38.5	77.0	38.5	-	+	+
	*Xpsm*196.1	25	89	40	2	4	12	172	6.66**	40.0	80.0	40.0	-	+	
LG 2	*Xpsm*708.1	35	77	32	5	3	19	171	0.82	38.0	76.0	38.0			
	*Xpsmp*2237	35	91	25	4	13	4	172	7.69**	42.0	84.0	42.0	-	+	-
	*Xpsmp*2072	47	94	24	1	2	4	172	9.62**	42.0	84.0	42.0	+	+	-
	*Xpsmp*2077	47	90	25	0	2	8	172	7.98**	41.0	82.0	41.0	+	+	-
	*Xpsmp*2201	52	79	33	1	4	3	172	4.62*	42.3	84.5	42.3	+		-
	*Xpsmp*2232.2	38	66	42	5	4	17	172	1.56	38.8	77.5	38.8			
	*Xpsmp*2225	24	82	41	6	3	16	172	5.90*	39.0	78.0	39.0	-		
	*Xpsmp*2089	28	79	46	7	3	9	172	4.40*	40.8	81.5	40.8	-		+
LG 3	*Xpsm*37	43	78	41	2	6	2	172	0.27	42.5	85.0	42.5			
	*Xpsm*18	36	73	27	5	15	16	172	1.93	39.0	78.0	39.0			
	*Xpsmp*2070	42	85	36	1	1	7	172	0.74	41.3	82.5	41.3			
	*Xpsmp*2267	47	72	32	5	2	14	172	3.30	39.5	79.0	39.5			
LG 4	*Xpsm*409.1	46	75	24	1	3	23	172	6.85**	37.3	74.5	37.3	+		-
	*Xpsm*648	24	91	39	7	4	7	172	8.01**	41.3	82.5	41.3	-	+	-
	Xpsm 344	34	94	44	0	0	0	172	2.65	43.0	86.0	43.0			
	*Xpsm*84	35	85	38	5	4	5	172	1.03	41.8	83.5	41.8			
	*Xpsmp*2084	37	83	38	3	1	10	172	0.42	40.5	81.0	40.5			
	*Xpsm*837.2	30	70	30	1	6	35	172	0.77	34.3	68.5	34.3			
	*Xpsm*416.3	33	90	31	1	1	16	172	4.44*	39.0	78.0	39.0	-	+	-

...contd. Table 12. Chi square estimates and segregation distortion observed among 172 F_2 pearl millet mapping population progenies based on cross ICMB 89111-P6 × ICMB 90111-P6 across 46 polymorphic marker loci (including SSRs and RFLPs) as compared to expected Mendelian 1:2:1 segregation ratios

Linkage group	Marker locus	Observed F_2 data segregation						Total	Chi square calculated	Expected F_2 data segregation			Excess (+) / Deficit (-) of		
		A	H	B	C	D	Missing			A	H	B	A	H	B
LG 5	*Xpsmp2202*	36	98	30	0	0	8	172	6.68**	41.0	82.0	41.0	-	+	-
	Xpsmp2274	33	98	29	1	4	7	172	8.30**	41.3	82.5	41.3	-	+	-
	Xpsmp2220	34	90	26	2	4	16	172	6.85**	39.0	78.0	39.0	-	+	-
	Xpsmp2078	33	92	28	2	3	14	172	6.61*	39.5	79.0	39.5	-	+	-
	Xpsmp2208	35	83	28	3	1	22	172	3.41	37.5	75.0	37.5			
	Xpsmp2276	34	90	33	0	2	13	172	3.38	39.8	79.5	39.8			
	Xpsmp2277	40	84	37	4	0	7	172	0.42	41.3	82.5	41.3			
	Xpsmp2261	33	95	32	0	0	12	172	5.64*	40.0	80.0	40.0	-	+	-
LG 6	*Xpsm588*	15	90	52	9	4	2	172	20.81**	42.5	85.0	42.5	-	+	+
	Xpsm696	14	92	42	8	12	4	172	19.35**	42.0	84.0	42.0	-	+	
	Xpsmp2018	22	80	62	3	0	5	172	19.61**	41.8	83.5	41.8	-	-	+
	Xpsm575	25	85	57	2	2	1	172	12.32**	42.8	85.5	42.8	-		+
LG 7	*Xpsm718*	33	92	36	2	3	6	172	3.40	41.5	83.0	41.5			
	Xpsm269	27	89	28	2	1	25	172	8.04**	36.8	73.5	36.8	-	+	-
	Xpsmp2224	30	91	28	1	2	20	172	7.36**	38.0	76.0	38.0	-	+	-
	Xpsmp2074	28	86	24	6	7	21	172	8.61**	37.8	75.5	37.8	-	+	-
	Xpsmp2263	30	93	23	4	10	12	172	11.63**	40.0	80.0	40.0	-	+	-
	Xpsmp2203	38	93	33	1	2	5	172	3.26	41.8	83.5	41.8			
	Xpsm160	40	64	36	9	5	18	172	1.26	38.5	77.0	38.5			
	Xpsm190	39	86	39	3	1	4	172	0.39	42.0	84.0	42.0			

*, ** significant at 0.05 and 0.01 levels of probability, respectively

al., 2004) based on RFLP and SSR markers was used as a reference for marker linkage group assignment and initial marker ordering in this study.

A linkage map of seven linkage groups with a total map length of 747.9 cM was constructed using data from 46 marker loci for 172 F_2 progenies. The map lengths of individual linkage groups ranged from a minimum of 30.2 cM (LG3) to a maximum of 195.2 cM (LG7), as shown in Figures 26-27. Polymorphic marker loci on the distal ends of several linkage groups were included in this study enhancing the total length of the map. The total mapped genome and linkage group lengths for seven pearl millet mapped populations, including the one used in this study and LGD 1-B-10 × ICMP 85410 (Liu *et al.*, 1994) used pearl millet base linkage maps are presented in Table 48 for their comparison across linkage groups.

4.4.1 MapMaker/Exp

MapMaker/Exp version 3.0 multipoint analysis was used to construct the linkage map using a LOD threshold value of 2.0 and recombination fraction of 0.5. After making seven linkage groups, markers were placed based on "group", "sequence" and "map" commands. Unlinked markers were then placed in appropriate linkage groups using the "build" command. Markers with satisfactory orders were then anchored in each linkage group and the framework command was used. Marker orders with fewer candidate errors and higher log likelihood (LOD) values were preferred for anchoring and frame working of each linkage group. Utmost care was taken particularly to properly place marker loci detected by multiple-copy RFLP probes while assigning them to appropriate linkage groups.

4.4.1.1. Linkage group 1

Linkage group 1 accommodated seven loci detected by three SSRs (*Xpsmp*2273, *Xpsmp*2080 and *Xpsmp*2030) and four RFLPs (*Xpsm*280, *Xpsm*492, *Xpsm*17 and *Xpsm*196.1). The total map length of this linkage group is 139.6 cM with comparatively higher negative LOD value (-354.72) than other linkage groups and a small number of candidate errors (putative double crossovers). The "compare" command of MapMaker predicted the best marker order of *Xpsm*280, *Xpsm*492, *Xpsmp*2273, *Xpsm*17, *Xpsmp*2080, *Xpsmp*2030 and *Xpsm*196.1 in this linkage group (Figure 26).

4.4.1.2. Linkage group 2

The total length of linkage group 2 is 192.3 cM, which is second longest among the seven linkage groups (Figure 26). It consists of seven SSRs and only one RFLP (on the top of this linkage group). The best order predicted by "compare" command of MapMaker is *Xpsm*708.1, *Xpsmp*2237, *Xpsmp*2072, *Xpsmp*2077, *Xpsmp*2201, *Xpsmp*2232.2, *Xpsmp*2225 and *Xpsmp*2089 and this gave the most highly negative LOD value (-429.34) with few putative double crossovers.

4.4.1.3. Linkage group 3

This is the shortest linkage group among the seven in this skeleton map. It has a total length of 30.2 cM and consists of four markers (two SSRs and two RFLPs). These markers were placed in the order of *Xpsm*37, *Xpsm*18, *Xpsmp*2070 and *Xpsmp*2267 with inter-marker distances of 17.9, 7.1 and 5.2 cM, respectively (Figure 26). A negative log likelihood value of -183.21 was obtained for this locus order, with only a few candidate errors.

4.4.1.4. Linkage group 4

Seven markers (six RFLPs and one SSRs) were placed in this linkage group with an order of *Xpsm*409.1, *Xpsm*648, *Xpsm*344, *Xpsm*84, *Xpsmp*2084, *Xpsm*837.2 and *Xpsm*416.3 (Figure 26). This linkage group has a total map length of 98.3 cM with a log likelihood value of -285.12 and a few putative double crossovers. Marker locus *Xpsm*409.1 was placed on top of this linkage group, with a very large map distance of 65.9 cM to the nearest marker, *Xpsm*648. The remaining loci in this linkage group were located close to each other with optimum inter-marker intervals (ranging from 2.4 cM to 9.6 cM) for QTLs detection.

4.4.1.5. Linkage group 5

This linkage group consisted of eight SSRs markers. A total Haldane map length of 50.1 cM was observed with the best marker order of *Xpsmp*2202, *Xpsmp*2274, *Xpsmp*2220, *Xpsmp*2078, *Xpsmp*2208, *Xpsmp*2276, and *Xpsmp*2261. This marker order gives a negative LOD value of -285.12 with a number of putative double crossovers. Marker locus *Xpsmp*2261 (which was not mapped earlier) was placed at the bottom of this linkage group at map distance of 20.5 cM from *Xpsmp*2277. The inter-marker distance

Figure 26. Genetic linkage map of pearl millet linkage groups (1-4) based on cross ICMB 89111-P6 x ICMB 90111-P6. Left side of the map of each linkage group are inter-marker distances in cM (Haldane) and on right side is the name of markers (green color denotes RFLP probes and blue color denotes SSR primers).

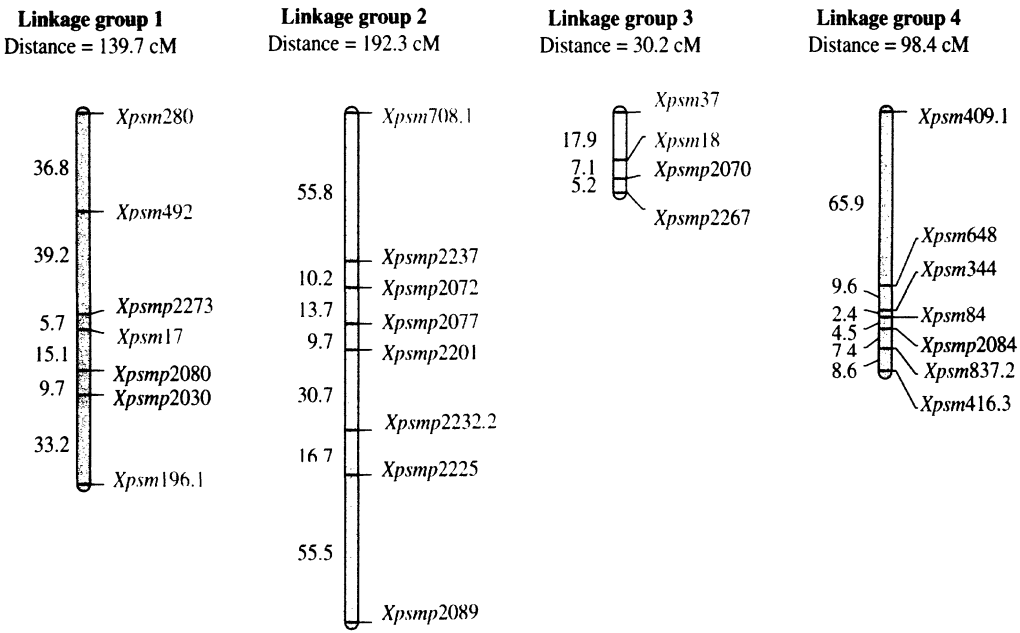
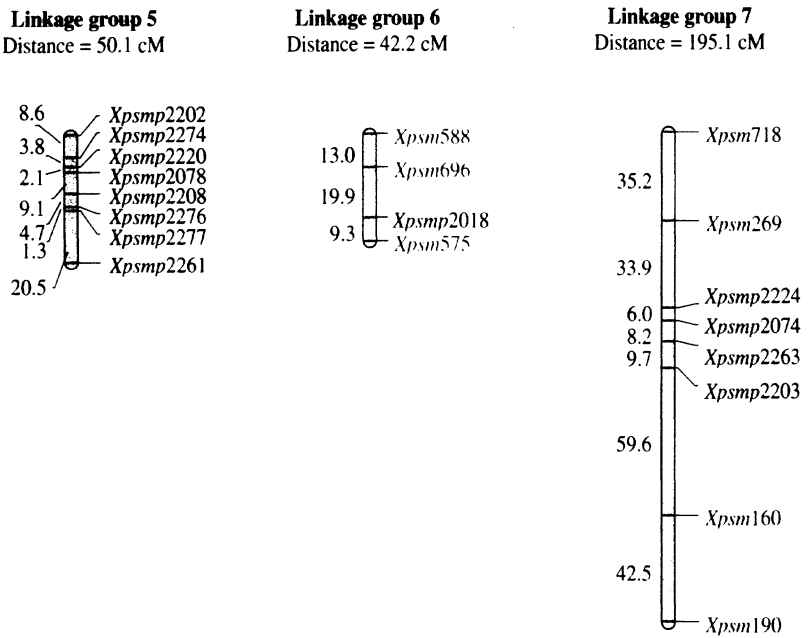


Figure 27. Genetic linkage map of pearl millet linkage groups (5-7) based on cross ICMB 89111-P6 x ICMB 90111-P6. Left side of the map of each linkage group are inter-marker distances in cM (Haldane) and on right side is the name of markers (green color denotes RFLP probes and blue color denotes SSR primers).



ranged from 1.3 cM between *Xpsmp*2276 and *Xpsmp*2277 to 20.5 cM between *Xpsmp*2277 and *Xpsmp*2261 (Figure 27) which are near optimal for QTL detection.

4.4.1.6. Linkage group 6

Linkage group 6 was second smallest linkage group for the mapping population with four markers (including three RFLPs and one SSR), placed in best order of *Xpsm*588, *Xpsm*696, *Xpsmp*2018 and *Xpsm*575 with inter-marker map distances of 13.0, 19.9 and 9.3 cM, respectively (Figure 27). A total map distance of 42.2 cM was obtained with a negative LOD score of -207.18 with a few putative double crossovers.

4.4.1.7. Linkage group 7

This was the largest linkage group for this mapping population with a Haldane map length of 195.2 cM. This linkage group consists of four SSR markers centrally placed and four RFLPs, of which two RFLPs are located on each of the distal ends. The inter-marker distances between the centrally placed SSRs were much lower than the distally located RFLPs. This linkage group recorded a negative log likelihood value of -421.38, with marker order of *Xpsm*718, *Xpsm*269, *Xpsmp*2224, *Xpsmp*2074, *Xpsmp*2263, *Xpsmp*2203, *Xpsm*160 and *Xpsm*190 (Figure 27), a reasonably accepted number of putative double crossovers.

4.5. QTL MAPPING BASED ON CROSS ICMB 89111-P6 × ICMB 90111-P6

The 748 cM (Haldane) linkage map constructed for 172 $F_{2:4}$ self-bulk progenies from the cross ICMB 89111-P6 × ICMB 90111-P6 was used for QTL analysis of phenotypic data for downy mildew incidence (%) generated from seedling green house screen of 164 to 172 of the $F_{2:4}$ self-bulks against eight pearl millet downy mildew pathogen populations. MapMaker/Exp 3.0b was used for constructing linkage map (described above) and then two software packages, first MapMaker/QTL ver. 1.1b (Lincoln *et al.*, 1992b) and second PlabQTL ver. 1.1 (Utz and Melchinger, 2000), were used for QTL mapping. A linkage map output data file from MapMaker/Exp was used for the PlabQTL analysis.

4.5.1. Simple interval mapping as implemented in MapMaker/QTL

MapMaker/QTL was used for simple interval mapping with a LOD score of 2.0 as the threshold value for detecting significant QTLs. As the phenotyping was done in $F_{2:4}$ self-

bulks, this software calculates weight (additive), dominance and recessive genetic effects and gives estimates of the proportion of observed phenotypic variation explained by individual QTLs. The commands "sequence [all]", "list loci"; "show linkage maps", "list traits", "show traits", "scan", "show peaks' and "map" were used for QTL analysis to calculate the above mentioned effects of individual putative QTLs. Based on estimated single QTL map positions, combined effects of two or more QTLs were calculated for two-QTL, three-QTL, four-QTL models etc. The qualifying criteria for accepting a multiple-QTL model was a LOD score of at least 2.0 units more than the highest LOD score of the best model having one less QTL:

$$LOD_n \geq LOD_{(n-1)} + 2.0$$

where

LOD_n = minimum qualifying LOD score for acceptance in a multiple-QTL model with 'n' QTLs,

$LOD_{(n-1)}$ = maximum LOD score for any observed model with n-1 QTLs.

4.5.2. Composite interval mapping as implemented in PlabQTLs

Composite interval mapping as implemented in PlabQTL (Utz and Melchinger, 2000) was used with a threshold likelihood ratio of 2.5 for declaration of QTL significance. Weight (additive) and dominance effects were calculated for each QTL detected by this software package.

In this study, genotypic data was generated in $F_{2:3}$ self-bulks (representing each of 172 F_2 individuals from which the mapping population was derived) and phenotypic data from DM screening of F_2 derived F_4 self-bulk families produced by selfing individual F_2 plants for two successive generations. Therefore, the dominance effect observed in the F_2:F_4 families were reduced to 25% of that expected in the F_2 generation. So for the estimation of the mode of inheritance, the degree of dominance has to be calculated using the following ratio:

$$4D/W$$

where

D = Dominance

W = Weight (= additive effect)

Depending on the absolute value 4D/W ratio, the inheritance patterns were assessed as

00.0-0.20 = Additive inheritance

0.21-0.80 = Partially dominant inheritance

0.80-1.20 = Dominant inheritance

> 1.20 = Over-dominant inheritance

In this mapping population, the male parent (marker allele homozygote scored as B) was ICMB 90111-P6 (downy mildew resistant) and female parent (marker allele homozygote scored as A) was ICMB 89111-P6 (downy mildew susceptible). If weight is negative, the alleles from resistant parent (ICMB 90111-P6) decrease DM incidence and hence contribute to DM resistance. Similarly if weight is positive, the alleles from ICMB 90111-P6 increase disease incidence (*i.e.*, the alleles from ICMB 89111-P6 increase DM resistance or decrease DM susceptibility). This fully depends upon the marker genotype scoring codes and the software used. In this study, the more susceptible female parental line ICMB 89111-P6 was scored as A and the more resistance male parental line ICMB 90111-P6 was scored as B, so the above explanation holds good for both MapMaker/QTL and PlabQTL.

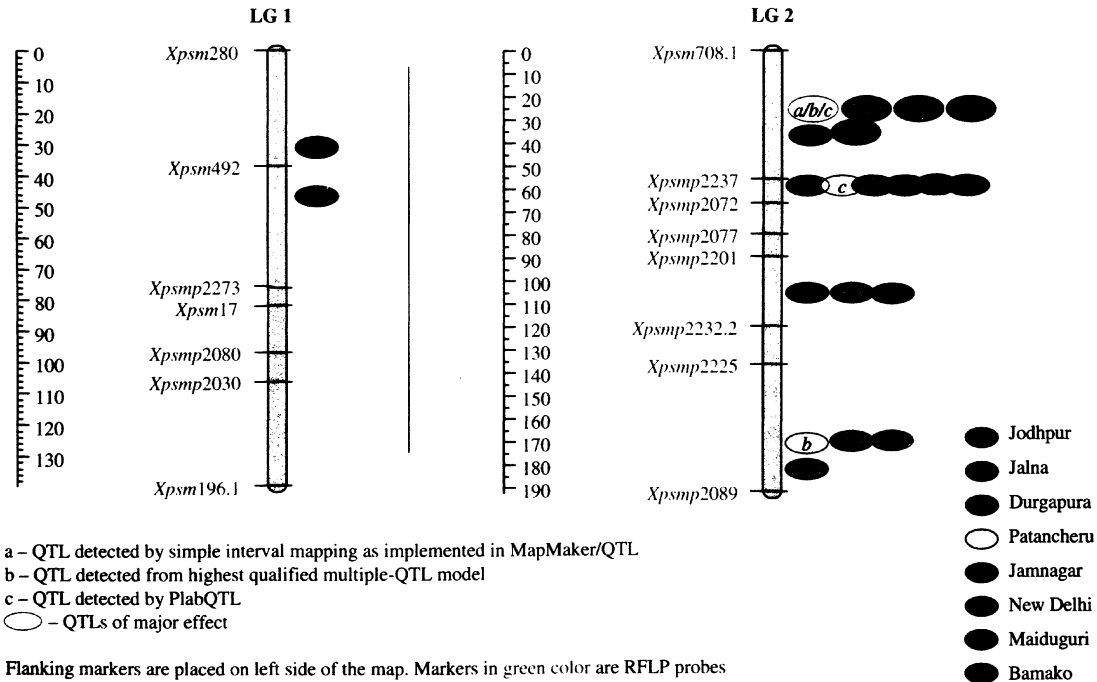
Data on total and diseased plant counts per replication for each pathogen population was converted into disease incidence (%) and across replication mean disease incidence values were used for mapping QTLs for downy mildew resistance. Both analyses (simple interval mapping and composite interval mapping) detected downy mildew resistance QTLs on LG1, LG2, LG3, LG4 and LG7 (Figures 28-30) with major significant effects, which are described in detail below and the comparison of DM resistance QTL LOD peaks for each pathogen populations from India and Africa mapped on each the seven linkage groups of the linkage map using composite interval mapping (CIM) are presented in Figures 31-37.

4.6. QTLS FOR DOWNY MILDEW RESISTANCE (DMR)

4.6.1. Downy mildew resistance QTLs for the Jodhpur (*Sg*139) pathogen population

Four DMR QTLs were identified by PlabQTL, each one on LG2 and LG3, and two on LG4 (Table 13). However, MapMaker/QTL detected five putative QTLs, each one on LG1, LG2, LG3, LG4 and LG7. Simple interval mapping indicated the QTL on LG4 (between *Xpsm*409.1 and *Xpsm*648) to be a major one, with a large phenotypic effect

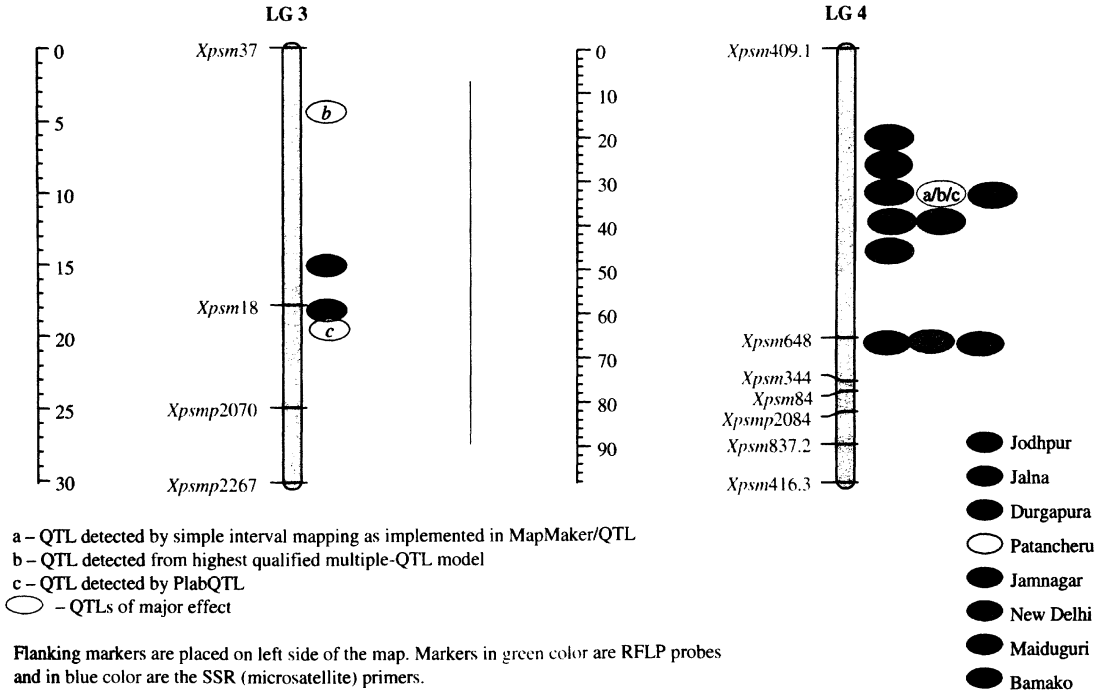
'igure 28. *QTLs* positions on genetic linkage map of pearl millet based on cross the ICMB 89111-P6 x ICMB 90111-P6 detected by simple interval and omposite interval mapping on LG1 and LG2 for resistance to downy mildew pathogen populations from India and Africa



a – QTL detected by simple interval mapping as implemented in MapMaker/QTL
b – QTL detected from highest qualified multiple-QTL model
c – QTL detected by PlabQTL
◯ – QTLs of major effect

Flanking markers are placed on left side of the map. Markers in green color are RFLP probes and in blue color are the SSR (microsatellite) primers.

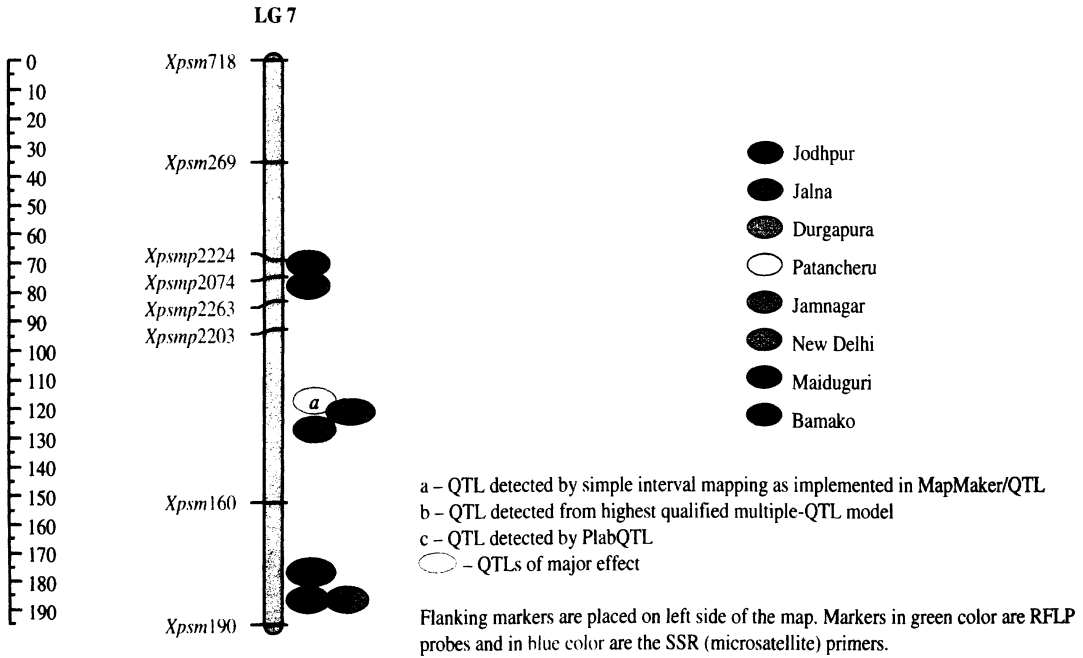
Figure 29. QTLs positions on genetic linkage map of pearl millet based on the cross ICMB 89111-P6 x ICMB 90111-P6 detected by simple interval and composite interval mapping on LG3 and LG4 for resistance to downy mildew pathogen populations from India and Africa



a – QTL detected by simple interval mapping as implemented in MapMaker/QTL
b – QTL detected from highest qualified multiple-QTL model
c – QTL detected by PlabQTL
– QTLs of major effect

Flanking markers are placed on left side of the map. Markers in green color are RFLP probes and in blue color are the SSR (microsatellite) primers.

Figure 30. QTLs positions on genetic linkage map of pearl millet based on the cross ICMB 89111-P6 x ICMB 90111-P6 detected by simple interval and composite interval mapping on LG7 for resistance to downy mildew pathogen populations from India and Africa



a – QTL detected by simple interval mapping as implemented in MapMaker/QTL
b – QTL detected from highest qualified multiple-QTL model
c – QTL detected by PlabQTL
– QTLs of major effect

Flanking markers are placed on left side of the map. Markers in green color are RFLP probes and in blue color are the SSR (microsatellite) primers.

Figure 31. Comparison of downy mildew resistance (DMR) QTL LOD peaks obtained for pathogen populations from India and Africa mapped on linkage group 1 of the newly constructed genetic linkage map based on the cross ICMB 89111-P6 x ICMB 90111-P6 using composite interval mapping method as implemented in PlabQTL

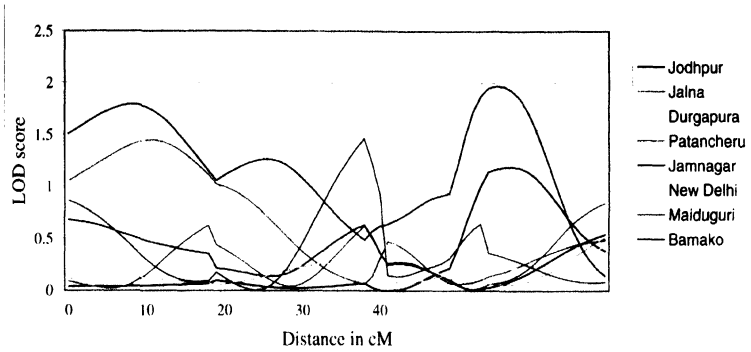


Figure 32. Comparison of downy mildew resistance (DMR) QTL LOD peaks obtained for pathogen populations from India and Africa mapped on linkage group 2 of the newly constructed genetic linkage map based on the cross ICMB 89111-P6 x ICMB 90111-P6 using composite interval mapping method as implemented in PlabQTL

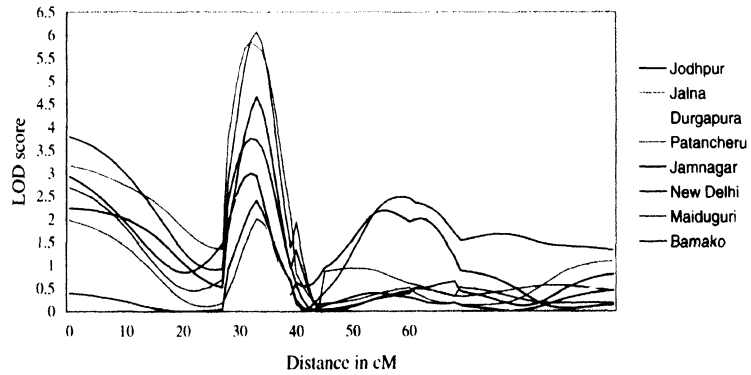


Figure 33. Comparison of downy mildew resistance (DMR) QTL LOD peaks obtained for pathogen populations from India and Africa mapped on linkage group 3 of the newly constructed genetic linkage map based on the cross ICMB 89111-P6 x ICMB 90111-P6 using composite interval mapping method as implemented in PlabQTL

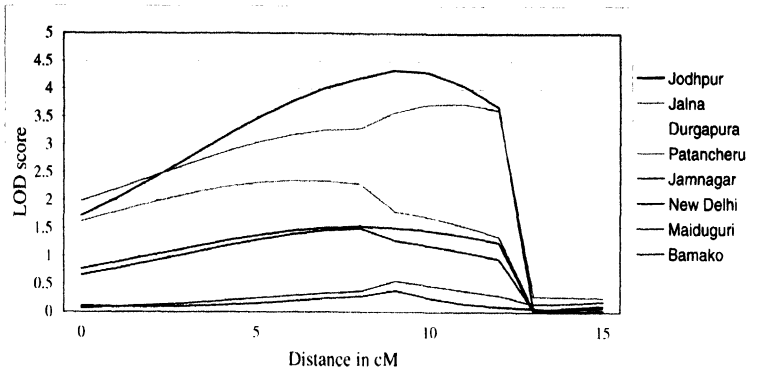


Figure 34. Comparison of downy mildew resistance (DMR) QTL LOD peaks obtained for pathogen populations from India and Africa mapped on linkage group 4 of the newly constructed genetic linkage map based on the cross ICMB 89111-P6 x ICMB 90111-P6 using composite interval mapping method as implemented in PlabQTL

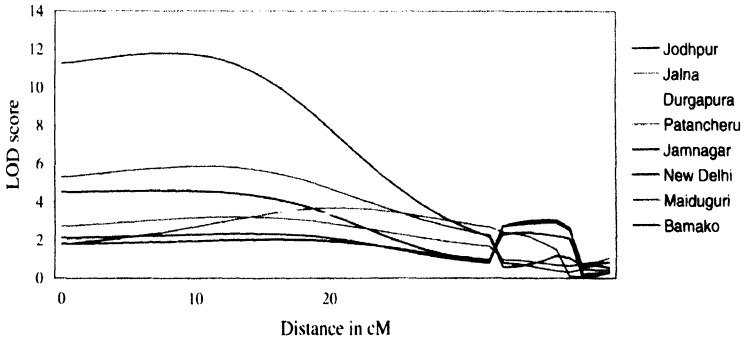


Figure 35. Comparison of downy mildew resistance (DMR) QTL LOD peaks obtained for pathogen populations from India and Africa mapped on linkage group 5 of the newly constructed genetic linkage map based on the cross ICMB 89111-P6 x ICMB 90111-P6 using composite interval mapping method as implemented in PlabQTL

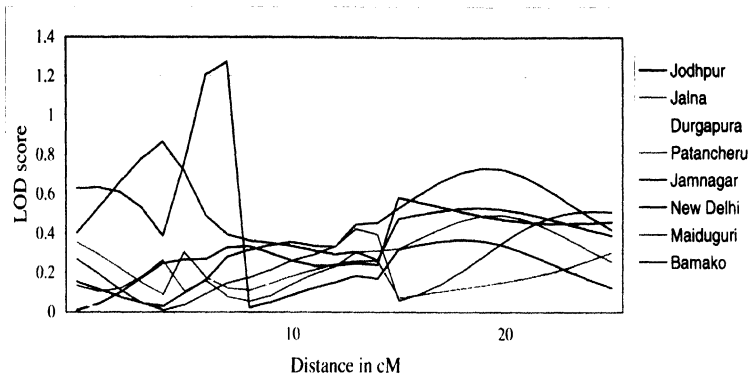


Figure 36. Comparison of downy mildew resistance (DMR) QTL LOD peaks obtained for pathogen populations from India and Africa mapped on linkage group 6 of the newly constructed genetic linkage map based on the cross ICMB 89111-P6 x ICMB 90111-P6 using composite interval mapping method as implemented in PlabQTL

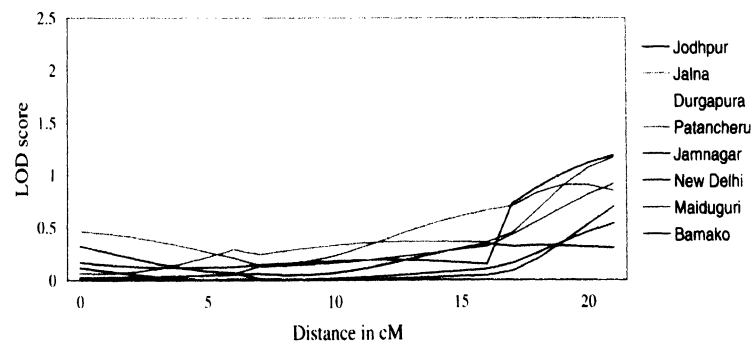
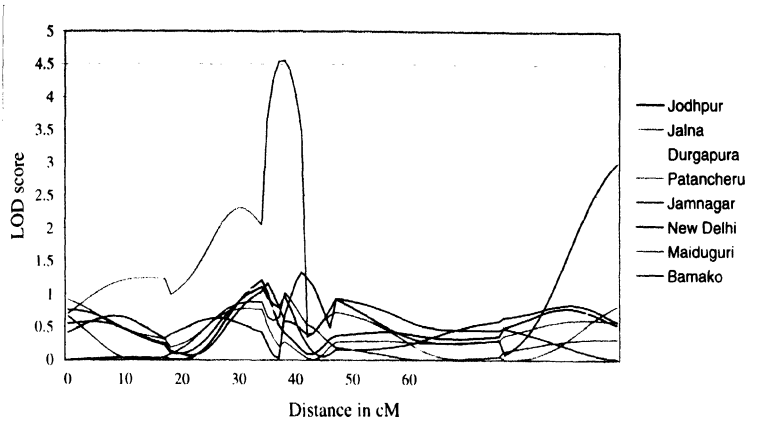


Figure 37. Comparison of downy mildew resistance (DMR) QTL LOD peaks obtained for pathogen populations from India and Africa mapped on linkage group 7 of the newly constructed genetic linkage map based on the cross ICMB 89111-P6 x ICMB 90111-P6 using composite interval mapping method as implemented in PlabQTL



accounting for 65.3% of observed variation and a high LOD score of 11.0. The best two-QTL model which explained 72.1% of observed phenotypic variation and had a high LOD value of 14.6, (>11.0+2.0 = 13.0) was accepted as better than the best single-QTL model. Further to this, a superior three-QTL model, significantly better than the best two-QTL model, explained about 83.2% of phenotypic variation in disease reaction among 172 mapping population progenies and had a very high LOD score of 19.2 (>14.6+2.0 = 16.6). The inheritance of these QTLs showed additive and over-dominant resistance, with susceptible parent ICMB 89111-P6 providing the resistance alleles for the QTL on LG3, and resistant parent ICMB 90111-P6 providing the resistance alleles for the remaining resistance QTLs detected using the Jodhpur pathogen population. In addition inheritance was recessive for putative DMR QTL identified on LG1, LG4 and LG7.

4.6.2. Downy mildew resistance QTLs for the Patancheru (*Sg*153) pathogen population

For this pathogen population a total of five DMR QTLs were placed using both interval mapping method implemented in MapMaker/QTL and composite interval mapping method implemented in PlabQTL. PlabQTL detected four DMR QTLs, two QTLs located on LG2, and one each on LG3 and LG4. MapMaker/QTL identified three putative QTLs, one each on LG2, LG4 and LG7. The best single-QTL model based on the LG4, QTL had a high critical LOD value of 12.9 and explained 70.5% observed phenotypic variation (Table 14).

In contrast, PlabQTL identified as best for DM resistance against Patancheru pathogen population a QTL located on LG2 (8.0 cM proximal to *Xpsmp*2237) with a likelihood of 5.9 that accounted for 14.6 % a less observed phenotypic variation.

The best two-QTL model recorded a higher LOD value of 19.4, significantly better than the best single-QTL model (14.9 = 12.9+2.0). A total of 76.4% of phenotypic variation in downy mildew incidence was explained among the 172 $F_{2:4}$ self-bulk progenies screened against this pathogen population. Among other multiple-QTL models the best three-QTL and four-QTL models had high LOD values of 21.9 (>19.4+2.0) and 25.1 (>21.9+2.0 = 23.9), respectively. The later explained 90.4% of observed phenotypic variation for downy mildew incidence in this particular DM screen. The mode of inheritance for majority of QTLs identified by single- as well as multiple-QTL models

Table 13. QTL results for downy mildew resistance against a pathogen population from the Central Arid Zone Research Institute (CAZRI), Jodhpur (*Sg*139) using PlabQTL composite interval mapping and MapMaker/QTL interval mapping methods for single- and multiple-QTL models

LG	Marker locus interval	Position (cM)	Likelihood ratio/LOD score	Variance explained (%)	Additive effect	Dominance effect	Inheritance
PlabQTL							
2	*Xpsmp*2237 - *Xpsmp*2072	8.0	3.0	7.8	-6.4	-6.9	Over-dominant
3	*Xpsm*18 - *Xpsmp*2070	0.0	4.3	11.0	9.9	0.6	Additive, from ICMB 89111-P6
4	*Xpsm*409.1 - *Xpsm*648	16.0	**4.6**	13.2	-16.2	0.9	Additive
4	*Xpsm*648 - *Xpsm*344	6.0	3.1	7.9	-8.8	2.5	Recessive
MapMaker/QTL							
1	*Xpsm*280 - *Xpsm*492	36.2	3.3	9.7	-10.6	3.4	Recessive
2	*Xpsmp2201* - *Xpsmp*2232.2	17.7	3.9	16.7	-7.3	16.0	???/Artifact
3	*Xpsm*37 - *Xpsm*18	16.3	3.0	9.1	10.1	-0.6	Additive, from ICMB 89111-P6
4	*Xpsm*409.1 - *Xpsm*648	31.0	**11.0**	65.3	-27.2	-10.0	Over-dominant
7	*Xpsm*160 - *Xpsm*190	40.1	4.2	12.2	-10.9	5.5	Recessive
Two-QTL model (acceptable score is 11.0 + 2.0 = 13.0)							
2	*Xpsmp*2237 - *Xpsmp*2072	7.4			-6.4	-7.2	Over-dominant
4	*Xpsm*409.1 - *Xpsm*648	30.0	**14.6**	72.1	-28.2	-9.9	Over-dominant
Three-QTL model (acceptable score is 14.6 + 2.0 = 16.6)							
2	*Xpsm*708.1 - *Xpsmp*2237	36.1			-14.1	-7.6	Over-dominant
3	*Xpsm*18 - *Xpsmp*2070	1.7			10.0	-0.5	Additive, from ICMB 89111-P6
4	*Xpsm*409.1 - *Xpsm*648	29.4	**19.2**	83.2	-24.7	-9.4	Over-dominant

Table 14. QTL results for downy mildew resistance against a pathogen population from the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru (*Sg*153) using PlabQTL composite interval mapping and MapMaker/QTL interval mapping methods for single- and multiple-QTL Models

LG	Marker locus interval	Position (cM)	Likelihood ratio/LOD score	Variance explained (%)	Additive effect	Dominance effect	Inheritance
PlabQTL							
2	*Xpsm*708.1 - *Xpsmp*2237	0.0	3.2	9.1	-9.2	0.2	Additive
2	*Xpsmp*2237 - *Xpsmp*2072	8.0	5.9	14.6	-9.5	-10.8	Over-dominant
3	*Xpsm*18 - *Xpsmp*2070	4.0	3.7	9.6	9.9	-1.5	Partially recessive to additive, from ICMB 89111-P6
4	*Xpsm*409.1 - *Xpsm*648	26.0	3.2	9.5	-15.3	3.8	Recessive
MapMaker/QTL							
2	*Xpsm*708.1 - *Xpsmp*2237	28.1	10.4	66.5	-28.4	-22.5	Over-dominant
4	*Xpsm*409.1 - *Xpsm*648	37.3	**12.9**	70.5	-30.6	-22.8	Over-dominant
7	*Xpsmp*2203 - *Xpsm*160	27.3	8.8	67.1	-27.5	-30.5	Over-dominant
Two-QTL model (acceptable score is 12.9 + 2.0 = 14.9)							
2	*Xpsmp*2072 - *Xpsmp*2077	3.1			-7.2	-7.8	Over-dominant
4	*Xpsm*409.1 - *Xpsm*648	36.0	**19.4**	76.4	-28.2	-20.1	Over-dominant
Three-QTL model (acceptable score is 19.4 + 2.0 = 21.4)							
2	*Xpsm*708.1 - *Xpsmp*2237	26.7			-13.8	-6.7	Over-dominant
3	*Xpsm*18 - *Xpsmp*2070	1.4			4.7	-0.6	Additive, from ICMB 89111-P6
4	*Xpsm*409.1 - *Xpsm*648	36.1	**21.9**	86.8	-26.1	-20.5	Over-dominant
Four-QTL model (acceptable score is 21.9 + 2.0 = 23.9)							
2	*Xpsm*708.1 - *Xpsmp*2237	26.2			-12.5	-6.6	Over-dominant
2	*Xpsmp*2225 - *Xpsmp*2089	39.2			-6.2	-9.2	Over-dominant
3	*Xpsm*37 - *Xpsm*18	5.4			5.1	1.3	Dominant, from ICMB 89111-P6
4	*Xpsm*409.1 - *Xpsm*648	36.7	**25.1**	90.4	-25.3	-20.3	Over-dominant

was dominant to over-dominant, with the more resistant alleles being contributed by resistant parent ICMB 90111-P6 for all QTLs except that mapping to LG3, for which susceptible parent 89111-P6 contributed the resistance allele.

4.6.3. Downy mildew resistance QTLs for the New Delhi (*Sg*298) pathogen population

Composite interval mapping using PlabQTL detected three downy mildew resistance QTLs effective against the pathogen population from IARI, New Delhi; two QTLs on LG2 (at loci *Xpsm*708.1+0.0 cM and *Xpsm*2237+10 cM) and one on LG4 (at locus *Xpsm*648+8.0 cM). Single-QTL models using simple interval mapping implemented in MapMaker/QTL also detected three QTLs but these were distributed across on LG2, LG4 and LG7. The best single-QTL model had a LOD score of 10.5, and explained 63.6% of variation in DM reaction among 172 $F_{2:4}$ self-bulks of the mapping population progenies that were screened against this pathogen population (Table 15). The mode of inheritance for all of these DMR QTLs was over-dominance except that PlabQTL indicated additive to partially dominant inheritance of one QTL on LG2 and one QTL on LG4. In all cases resistance alleles for these QTLs were contributed by the resistant parental line ICMB 90111-P6.

The LOD score for the best two-QTL model (17.0) was significantly better than that for the best single-QTL model (10.5). The best two-QTL model included QTL on LG2 and LG4 that together explained 82.1% of observed phenotypic variation in disease reaction to this pathogen population with over-dominant resistance from ICMB 90111-P6 at both QTLs. The best three-QTL model (LOD = 18.8) was not significantly better than the best two-QTL model of the inheritance of downy mildew resistance effective against the New Delhi pathogen population.

4.6.4. Downy mildew resistance QTLs for the Jamnagar (*Sg*200) pathogen population

Single QTL-model in simple interval mapping with MapMaker/QTL identified three QTLs for DM resistance effective against this pathogen population, one each on LG2, LG4 and LG7 (Table 16). The first two QTLs on LG2 and LG4 were confirmed by MapMaker/QTL in a two-QTL-model with an acceptable LOD score of 19.8, which was greater than the critical value of 14.7 = 12.7+2.0. The portion of observed phenotypic

Table 15. QTL results for downy mildew resistance against a pathogen population from the Indian Agricultural Research Institute (IARI), New Delhi (*Sg*298) using PlabQTL composite interval mapping and MapMaker/QTL interval mapping methods for single- and multiple-QTL Models

LG	Marker locus interval	Position (cM)	Likelihood ratio/LOD score	Variance explained (%)	Additive effect	Dominance effect	Inheritance
PlabQTL							
2	*Xpsm*708.1 - *Xpsmp*2237	0.0	2.9	8.4	-5.8	-0.5	Additive to partially dominant
2	*Xpsmp*2237 - *Xpsmp*2072	10.0	4.7	11.8	-4.9	-6.5	Over-dominant
4	*Xpsm*648 - *Xpsm*344	8.0	2.9	7.5	-6.1	-0.9	Additive to partially dominant
MapMaker/QTL							
2	*Xpsm*708.1 - *Xpsmp*2237	29.9	5.6	56.2	-16.2	-14.4	Over-dominant
4	*Xpsm*409.1 - *Xpsm*648	39.0	**10.5**	63.6	-20.1	-12.7	Over-dominant
7	*Xpsmp*2203 - *Xpsm*160	29.9	3.5	54.6	-16.1	-17.2	Over-dominant
Two-QTL model (acceptable score is 10.5 + 2.0 = 12.5)							
2	*Xpsm*708.1 - *Xpsmp*2237	26.2			-9.4	-8.8	Over-dominant
4	*Xpsm*409.1 - *Xpsm*648	39.5	**17.0**	82.1	-19.5	-13.2	Over-dominant
Three-QTL model (acceptable score is 17.0 + 2.0 = 19.0)							
2	*Xpsm*708.1 - *Xpsmp*2237	27.0			-8.8	-7.8	Over-dominant
2	*Xpsmp*2225 - *Xpsmp*2089	44.1			-5.2	-5.6	Over-dominant
4	*Xpsm*409.1 - *Xpsm*648	44.0	**18.8**	89.6	-22.0	-15.9	Over-dominant

Table 16. QTL results for downy mildew resistance against a pathogen population from the Junagadh Agricultural university Millet Research Station (JAU MRS), Jamnagar (*Sg*200) using PlabQTL composite interval mapping and MapMaker/QTL interval mapping methods for single- and multiple-QTL Models

LG	Marker locus interval	Position (cM)	Likelihood ratio/LOD score	Variance explained (%)	Additive effect	Dominance effect	Inheritance
PlabQTL							
2	*Xpsm*708.1 - *Xpsmp*2237	0.0	3.8	10.8	-9.9	1.2	Additive to partially recessive
2	*Xpsmp*2237 - *Xpsmp*2072	8.0	3.8	9.6	-7.5	-8.3	Over-dominant
MapMaker/QTL							
2	*Xpsm*708.1 - *Xpsmp*2237	26.3	**12.7**	67.2	-27.5	-21.0	Over-dominant
4	*Xpsm*409.1 - *Xpsm*648	37.3	11.2	68.3	-29.2	-19.4	Over-dominant
7	*Xpsmp*2203 - *Xpsm*160	29.9	7.7	66.8	-23.5	-25.6	Over-dominant
Two-QTL model (acceptable score is 12.7 + 2.0 = 14.7)							
2	*Xpsm*708.1 - *Xpsmp*2237	25.1			-27.6	-19.5	Over-dominant
4	*Xpsm*409.1 - *Xpsm*648	40.1	**19.8**	88.8	-14.4	-7.4	Over-dominant
Three-QTL model (acceptable score is 19.8 + 2.0 = 21.8)							
2	*Xpsm*708.1 - *Xpsmp*2237	25.4			-28.0	-19.6	Over-dominant
4	*Xpsm*409.1 - *Xpsm*648	40.2			-14.4	-7.6	Over-dominant
7	*Xpsmp*2203 - *Xpsm*160	9.0	**20.2**	89.2	2.2	-0.2	Additive, from ICMB 89111-P6
Four-QTL model (acceptable score is 21.8 + 2.0 = 23.8)							
2	*Xpsm*708.1 - *Xpsmp*2237	25.8			-27.0	-20.2	Over-dominant
2	*Xpsmp*2201 - *Xpsmp*2232.2	17.1			-3.3	7.7	Recessive
4	*Xpsm*409.1 - *Xpsm*648	39.9			-12.8	-9.0	Over-dominant
7	*Xpsmp*2203 - *Xpsm*160	12.3	**23.6**	91.7	3.5	1.3	Dominant, from ICMB 89111-P6

variation explained by this two-QTL model was 71.2%, with over-dominant inheritance of resistance from resistant parent ICMB 90111-P6 for both QTLs.

Three- and four-QTL models were not significantly better than this. The composite interval mapping implemented in PlabQTL detected two DM resistance QTLs on LG2 that were effective against the Jamnagar pathogen population, one potentially recessive to additive in its mode of inheritance and the other over-dominant, with resistance alleles in both cases inherited from resistant parent ICMB 90111-P6.

4.6.5. Downy mildew resistance QTLs for the Durgapura (*Sg*151) pathogen population

MapMaker/QTL interval mapping method identified three DMR QTLs on LG2, LG4, and LG7. PlabQTL composite interval mapping detected four significant QTLs, two QTLs each on LG2 and LG4 located between two consecutive marker intervals. This best single-QTL model detected by interval mapping on LG4 (*Xpsm*409.1+39.1) (Table 17) recorded a high LOD score of 10.1 and explained 68.5% of recorded phenotypic variation in DM incidence among 172 $F_{2:4}$ self-bulk progenies against this pathogen population. The best two-QTL model explained 71.2% of this phenotypic variation with significantly better LOD score (>12.1 = 10.1 + 2.0) than the best single-QTL model. MapMaker/QTL interval mapping also identified a valid three-QTL model significantly better than the best two-QTL model with a LOD score of 15.0 (>14.1 = 12.1+2.0) and accounting for 82.3% of observed phenotypic variation. Dominant to over-dominant inheritance of resistance from resistant parent ICMB 90111-P6 was observed for the DM resistance QTLs identified by simple interval mapping of the data from screen against this pathogen population.

4.6.6. Downy mildew resistance QTLs for the Jalna (*Sg*150) pathogen population

For this pathogen population, three downy mildew resistance QTLs have been mapped on LG2, LG4, and LG7; with two DMR QTLs detected by each software package. One common QTL on LG4 was detected by both analyses (Table 18). A major QTL located on LG4 was detected by simple interval mapping with the single-QTL model for this having a LOD value of 10.2 and accounting for a large part of the observed phenotypic variation (62.9%) in the screen of the mapping population progenies against this pathogen population. The best two-QTL model proved to be significantly better than the best

Table 17. QTL results for downy mildew resistance against a pathogen population from the Rajasthan Agricultural University Agricultural Research Station (RAU ARS), Durgapura (*Sg*151) using PlabQTL composite interval mapping and MapMaker/QTL interval mapping methods for single- and multiple-QTL Models

LG	Marker locus interval	Position (cM)	Likelihood ratio/LOD score	Variance explained (%)	Additive effect	Dominance effect	Inheritance
PlabQTL							
2	*Xpsm*708.1 - *Xpsmp*2237	0.0	4.6	12.9	-9.7	2.8	Recessive
2	*Xpsmp*2237 - *Xpsmp*2072	5.0	2.6	6.8	-3.9	-8.8	Over-dominant
4	*Xpsm*409.1 - *Xpsm*648	34.0	3.5	10.3	-19.7	2.6	Additive to partially recessive
4	*Xpsm*648 - *Xpsm*344	6.0	3.7	9.4	-9.8	-0.7	Additive
MapMaker/QTL							
2	*Xpsm*708.1 - *Xpsmp*2237	27.3	7.0	58.2	-21.9	-17.7	Over-dominant
4	*Xpsm*409.1 - *Xpsm*648	39.1	**10.1**	68.5	-28.3	-14.5	Over-dominant
7	*Xpsm*160 - *Xpsm*190	34.5	3.0	14.3	-10.8	-5.4	Over-dominant
Two-QTL model (acceptable score is 10.1 + 2.0 = 12.1)							
2	*Xpsmp* 2201 - *Xpsmp* 2232.2	9.3			-5.4	2.2	Recessive
4	*Xpsm* 409.1 - Xpsm 648	38.2	**12.1**	71.2	-27.7	-14.9	Over-dominant
Three-QTL model (acceptable score is 12.1 + 2.0 = 14.1)							
2	*Xpsm*708.1 - *Xpsmp*2237	26.1			-21.8	-17.0	Over-dominant
2	*Xpsmp*2225 - *Xpsmp*2089	51.2			-3.3	-4.5	Over-dominant
4	*Xpsm*409.1 - *Xpsm*648	37.2	**15.0**	82.3	-14.8	-4.2	Dominant

Table 18. QTL results for downy mildew resistance against a pathogen population from the Maharashtra Seed Company (MAHYCO) Jalna (*Sg*150) using PlabQTL composite interval mapping and MapMaker/QTL interval mapping methods for single- and multiple-QTL Models

LG	Marker locus interval	Position (cM)	Likelihood ratio/LOD score	Variance explained (%)	Additive effect	Dominance effect	Inheritance
PlabQTL							
4	*Xpsm*409.1 - *Xpsm*648	42.0	3.7	10.7	-8.9	-0.3	Additive
7	*Xpsmp*2074 - *Xpsmp*2263	1.0	**4.6**	11.5	5.3	-2.8	Recessive, from ICMB 89111-P6
MapMaker/QTL							
2	*Xpsmp*2225 - *Xpsmp*2089	30.8	4.5	55.4	-12.2	-12.0	Over-dominant
4	*Xpsm*409.1 - *Xpsm*648	40.9	**10.2**	62.9	-13.2	-10.1	Over-dominant
Two-QTL model (acceptable score is 10.2 + 2.0 = 12.2)							
4	*Xpsm*409.1 - *Xpsm*648	40.7			-13.2	-10.1	Over-dominant
7	*Xpsmp*2224 - *Xpsmp*2074	5.0	**13.9**	66.8	3.0	-2.9	Recessive, from ICMB 89111-P6

single-QTL, model with a higher LOD value of 13.9 (>12.2 = 10.2+2.0) and explaining a larger portion (66.8%) of the observed phenotypic variation among progenies in this screen.

MapMaker/QTL single-QTL models indicated dominant to over-dominant resistance with governing the favorable alleles from the resistance parent ICMB 90111-P6. However, the best two-QTL model included a QTL governed by recessive resistance alleles coming from susceptible parental line ICMB 89111-P6. This recessively inherited QTL on LG7 was also detected by composite interval mapping implemented in PlabQTL, but its single-QTL model was not detected as significantly by simple interval mapping implemented in MapMaker/QTL.

4.6.7. Downy mildew resistance QTLs for the Maiduguri pathogen population (UWB screen 43)

MapMaker/QTL detected only one DMR QTL effective against this pathogen population on LG2 (*Xpsm*708.1+29.2 cM). But composite interval mapping implemented in PlabQTL identified three QTLs, two DMR QTLs on LG2 (*Xpsm*708.1+0 cM and *Xpsmp*2237+10 cM) and one on LG4 (*Xpsm*409.1+22.0 cM). The highest LOD score recorded was 16.2 by best single-QTL by interval mapping method which accounted for 69.5% of observed phenotypic variation for DM reaction with over dominant from ICMB 89111-P6 (Table 19). The best two-QTL model was not accepted, as its LOD score of 18.1 was slightly less than the required critical value of 16.2+2.0 = 18.2.

4.6.8. Downy mildew resistance QTLs for the Bamako pathogen population (UWB screen 45)

Four putative DMR QTLs effective against the pathogen population from Bamako, Mali identified by simple interval mapping as implemented in MapMaker/QTL, on LG1, LG2, LG4 and LG7. Composite interval mapping confirmed the position of two of these QTL on LG4 and LG7. The best single-QTL model for simple interval mapping was that for LG4 which had a LOD value 15.5, and accounted for 73.9% of observed phenotypic variation among 164 $F_{2:4}$ self-bilks mapping population progenies screened against this pathogen population (Table 20). The best two-QTL model recorded a LOD score of 17.2, which was slightly lower than the critical level (15.2+2.0 = 17.5); but the best three-QTL model was accepted with a LOD value of 19.6, which was significantly better than the

Table 19. QTL results for downy mildew resistance against a pathogen population from the Lake Chad Research Institute (LCRI), Maiduguri, Nigeria using PlabQTL composite interval mapping and MapMaker/QTL interval mapping methods for single- and multiple-QTL Models

LG	Marker locus interval	Position (cM)	Likelihood ratio/LOD score	Variance explained (%)	Additive effect	Dominance effect	Inheritance
PlabQTL							
2	*Xpsm*708.1 - *Xpsmp*2237	0.0	2.7	8.1	-6.3	1.1	Additive/Partially recessive
2	*Xpsmp*2237 - *Xpsmp*2072	10.0	6.1	15.7	-9.6	-7.6	Over-dominant
4	*Xpsm*409.1 - *Xpsm*648	22.0	5.9	17.3	-15.6	1.5	Additive
MapMaker/QTL							
2	*Xpsm*708.1 - *Xpsmp*2237	29.2	**16.2**	69.5	-24.6	-21.1	Over-dominant
Two-QTL model (acceptable score is 16.2 + 2.0 = 18.2)							
2	*Xpsmp*2072 - *Xpsmp*2077	0.3			-2.9	-3.3	Over-dominant
4	*Xpsm*409.1 - *Xpsm*648	36.8	**18.1**	71.9	-26.7	-21.3	Over-dominant

Table 20. QTL results for downy mildew resistance against a pathogen population from Bamako, Mali using PlabQTL composite interval mapping and MapMaker/QTL interval mapping methods for single- and multiple-QTL Model

LG	Marker locus interval	Position (cM)	Likelihood ratio/LOD score	Variance explained (%)	Additive effect	Dominance effect	Inheritance
PlabQTL							
4	*Xpsm*409.1 - *Xpsm*648	16.0	11.8	33.9	-27.6	9.7	Recessive
7	*Xpsm*160 - *Xpsm*190	41.0	3.0	8.8	-8.6	-2.7	Dominant
MapMaker/QTL							
1	*Xpsm*492 - *Xpsmp*2273	12.3	5.9	31.7	-20.3	-4.5	Dominant
2	*Xpsmp 2201* - *Xpsmp*2232.2	14.2	3.2	15.8	-10.0	13.0	Recessive
4	*Xpsm*409.1 - *Xpsm*648	20.8	**15.5**	73.9	-28.9	-4.4	Partially dominant
7	*Xpsm* 160 - *Xpsm*190	42.1	5.0	15.0	-13.3	-0.6	Additive
Two-QTL model (acceptable score is 15.5 + 2.0 = 17.5)							
4	*Xpsm*409.1 - *Xpsm*648	20.7			-28.9	-3.9	Partially dominant
7	*Xpsm*160 - *Xpsm*190	33.0	**17.2**	80.2	-7.0	-3.2	Over-dominant
Three-QTL model (acceptable score is 17.5+ 2.0 = 19.5)							
4	*Xpsm*409.1 - *Xpsm*648	19.8			-27.3	-5.2	Partially dominant
7	*Xpsm*160 - *Xpsm*190	35.7			-5.6	-3.6	Over-dominant
2	*Xpsmp*2201 - *Xpsmp*2232.2	20.5	**19.6**	80.3	-6.0	6.5	Recessive

best single-QTL and best two-QTL models (critical value = 19.2 = 15.2+2.0+2.0). This three-QTL model explained 80.3% of observed phenotypic variation among the 164 progenies of the mapping population when screened against this DM pathogen population. The mode of inheritance for the resistances detected by this three-QTL model ranged from recessive for the QTL on LG2, to partially dominant for that on LG4 and over-dominant for that on LG7, with resistant parent ICMB 90111-P6 contributing the more resistant allele in each case. However, composite interval mapping results suggest that the LG4 resistant QTL is recessive while that on LG7 is dominant.

4.7. AGRONOMIC PERFORMANCE OF HHB 94-LIKE HYBRIDS FROM MULTILOCATION TRIALS

4.7.1. Analysis of variance

Eleven entries including nine HHB 94-like hybrids and two hybrid controls (HHB 94-original and HHB 181) were sown in replicated trials in ten test environments (location × year combinations) in India during *kharif* seasons of 2002 and 2003. The test environments different from states were grouped into three major clusters *viz.* Andhra Pradesh and Haryana (trials conducted in four test environments each) and Rajasthan (trials conducted in two test environments). The data recorded from these 5-replication randomized complete block design trials for 15 important agronomic traits, including grain and stover yield and their components were subjected to analysis of variance (ANOVA) using Genstat version 6.0. Separate ANOVAs for each of the ten individual test environments, pooled data across each of the three state-wise multiple-test environment clusters and pooled data across all ten test environments were performed to study variation among the genotypes, the test environments and their genotype × environment (G × E) interactions for each of the traits under study.

ANOVA Table 21 presents estimates of the mean sums of squares for all among genotypes, among environments and G × E interactions for each of the traits from the replicated data from all the ten test environments. Mean sums of squares were highly significant for genotypes, environments and G × E interactions for all traits except in case of G × E interactions for effective plant stand. The test environments represented the major source of variation in this experiment followed by genotypes and G × E interactions. In order to better understand the nature of the G × E interactions, ANOVAs

Table 21. Analysis of variance showing mean sums of squares of grain and stover yield and their component traits for nine HHB 94-like hybrids and two control hybrids (HHB 94 and HHB 181) across ten test environments in India during *kharif* 2002 and 2003

Source of variation	df	Mean sums of squares														
		GY	FT	EPS	PH	PL	PD	FSY	DSY	SMC	ETN	PY	1000GM	PGN	TAGBY	HI
Replication	4	1140	9.46	11.41	210.23	3.89	3.82	101565	19893	29.22	30.67	4853	0.91	62454	39374	23.21
Genotype	10	18867**	125.12**	14.81**	1101.53**	61.10**	41.98**	480626**	121148**	89.44**	188.01**	25436**	4.02**	231135**	77450*	260.88**
Environment	9	586600**	1021.13**	94.84**	14550.64**	160.72**	160.14**	15650549**	2800428**	4598.70**	4253.80**	942833**	43.80**	5202883**	5645078**	3704.05**
G × E	90	5201**	6.27**	2.51	142.92**	2.90**	6.14**	117143**	33971**	21.97**	39.16**	8295**	0.95**	113542**	66655**	24.44**
Pooled error	436	3174	2.13	2.46	59.58	0.90	1.16	67139	15519	13.68	17.09	5283	0.45	74827	33265	14.38

Table 22. Analysis of variance showing mean sums of squares of grain and stover yield and their component traits for nine HHB 94-like hybrids and two control hybrids (HHB 94 and HHB 181) across four test environments in Andhra Pradesh during *kharif* 2002 and 2003

Source of variation	df	Mean sums of squares														
		GY	FT	EPS	PH	PL	PD	FSY	DSY	SMC	ETN	PY	1000GM	PGN	TAGBY	HI
Replication	4	2101	2.89	0.64	42.62	2.03	1.57	31316	8561	15.14	19.14	1789	0.37	28153	16743	7.65
Genotype	10	16087**	68.31**	2.74	1297.75**	11.46**	5.49**	324590**	50698**	55.52**	225.03**	18527**	5.57**	310210**	33518**	196.38**
Environment	3	79599**	1027.00**	157.96**	13889.82**	50.28**	1.69	19637649**	457520**	1183.99**	1114.43**	95275**	1.04*	1145394**	726410**	104.72**
G × E	30	2106	6.10**	2.11	79.54	1.42*	1.07	56580	6262	5.84	19.82	3766	0.67**	21355	14863	8.27
Pooled error	172	2044	0.86	1.76	55.06	0.81	0.90	37840	4301	6.77	23.72	2675	0.36	31754	10980	8.14

*, ** significant at the 0.05 and 0.01 levels of probability, respectively

GY - grain yield (g/m^2), FT - time to 50 % flowering, EPS - effective plant stand (plants/m^2), PH - plant height (cm), PL - panicle length (cm), PD - panicle diameter (mm), FSY - fresh straw yield (g/m^2), DSY - dry straw yield (g/m^2), SMC - straw moisture content (%), ETN - effective tiller number (tillers/m^2), PY - panicle yield (g/m^2), 1000GM - thousand grain mass (g), PGN - panicle grain number, TAGBY - total above-ground biomass yield (g/m^2), HI - harvest index (%); df - degree of freedom

were also performed for three state-wise clusters of the test environments and for each of the ten individual test environments.

Results from the state-wise pooled analyses for all the three multiple-test environment clusters are presented in the Tables 22-24.

ANOVA performed for the Andhra Pradesh test environment cluster (Table 22) revealed significant variation among genotypes for all traits except effective plant stand, among environments for all traits except panicle diameter. However, genotype × environment (G × E) interactions were significant only for time to 50% flowering, panicle length, and 1000-grain mass. As in case of pooled analysis across all ten test environments, the environmental variances were highest followed by genotypic variances and G × E interaction variances for all observed traits in Andhra Pradesh test environment cluster.

ANOVA performed for Haryana test environments cluster (Table 23) showed a slightly different pattern from Andhra Pradesh. For Haryana test environments significant differences were detected among genotypes for all traits except grain yield, panicle yield, 1000-grain mass and total above-ground biomass yield, among environments for all traits, and G × E interactions for all traits except effective plant stand, panicle grain number and harvest index. Environmental variances were observed to be highest for all traits, followed by genotypic variances (except for grain yield, effective tillers number, panicle yield, 1000-grain mass and total above-ground biomass yield where G × E interaction variances were larger than genotypic variances.

ANOVA performed for the Rajasthan test environment cluster trials conducted during *kharif* 2003 at only two locations (Table 24) revealed significant differences among genotypes for traits time to 50% flowering, plant height, panicle length and panicle diameter only, and among test environment variances for all traits except harvest index. Significant G × E interactions within this environment cluster were also obtained only for except grain yield, panicle length and harvest index. The pattern of distribution of observed variation across test environments, genotypes and genotype × environment interactions within this Rajasthan test environment cluster was similar to that for Haryana The variation contributed by G × E interactions was observed to be higher than that from genotypes only for grain yield and for harvest index. However, for time to 50%

Table 23. Analysis of variance showing mean sums of squares of grain and stover yield and their component traits for nine HHB 94-like hybrids and two control hybrids (HHB 94 and HHB 181) across four test environments in Haryana during *kharif* 2002 and 2003

Source of variation	df	Mean sums of squares														
		GY	FT	EPS	PH	PL	PD	FSY	DSY	SMC	ETN	PY	1000GM	PGN	TAGBY	HI
Replication	4	2763	13.83	15.39	249.75	1.28	0.93	147932	44121	41.83	22.42	11114	0.34	241812	87828	8.17
Genotype	10	7281	30.33**	14.67**	333.36**	40.31**	24.10**	261443**	106036**	75.93**	59.51**	12787	0.59	222041*	86918	79.46**
Environment	3	193717**	1042.55**	81.23**	7504.87**	26.15**	429.32**	1480235**	627920**	213.52**	501.37**	435917**	40.31**	11258010**	2068100**	236.89**
G × E	30	9515**	4.17**	2.96	93.17*	3.45**	11.88**	247291**	80681**	37.17**	61.61**	17226**	1.01**	153327	163826**	16.89
Pooled error	172	4570	2.01	3.25	53.53	0.74	0.96	106847	30364	19.38	16.02	8661	0.50	117480	62689	14.22

Table 24. Analysis of variance showing mean sums of squares of grain and stover yield and their component traits for nine HHB 94-like hybrids and two control hybrids (HHB 94 and HHB 181) across two test environments in Rajasthan during *kharif* 2003

Source of variation	df	Mean sums of squares														
		GY	FT	EPS	PH	PL	PD	FSY	DSY	SMC	ETN	PY	1000GM	PGN	TAGBY	HI
Replication	4	5839	12.38	6.34	319.81	3.55	2.98	160298	20868	50.81	14.54	7380	2.15	190314	45971	80.37
Genotype	10	2957	48.00**	3.33	187.49**	17.36**	25.84**	17281	4740	9.22	5.13	2768	0.74	85026	9638	48.72
Environment	1	469782**	1094.63**	19.49**	31728.83**	157.44**	131.56**	2929179**	1833349**	7032**	395.96**	539826**	45.98**	7063120**	4362759**	57.75
G × E	10	4490*	3.09	1.44	51.05	3.48**	3.00	19985	4579	17.45	6.49	3036	0.68	111688	11204	80.80**
Pooled error	84	2475	4.22	1.98	67.77	1.38	2.16	40884	7016	13.69	6.09	3472	0.49	63869	16521	25.38

*, ** significant at the 0.05 and 0.01 levels of probability, respectively

GY - grain yield (g/m^2), FT - time to 50 % flowering, EPS - effective plant stand (plants/m^2), PH - plant height (cm), PL - panicle length (cm), PD - panicle diameter (mm), FSY - fresh straw yield (g/m^2), DSY - dry straw yield (g/m^2), SMC - straw moisture content (%), ETN - effective tiller number (tillers/m^2), PY - panicle yield (g/m^2), 1000GM - thousand grain mass (g), PGN - panicle grain number, TAGBY - total above-ground biomass yield (g/m^2), HI - harvest index (%); df - degree of freedom

flowering, plant height, panicle length and diameter, genotypic variances were greater than G × E interaction variances within this test environment cluster.

4.7.2. Mean performance of HHB 94-like hybrids in multilocation trials

The mean performance of nine HHB 94-like hybrids and two hybrid control entries were tested and compared against grand mean for each trait pooled across each of the three state-wise test environment clusters and across all ten test environments. The nine experimental hybrids were developed by using as female parents three pairs of sub-selections of ICMA 89111, ICMB 89111 and ICMB 90111 (where the ICMB 89111 and ICMB 90111 have been used to generate pear millet mapping populations) and crossing these to a common pollinator (G73/107). In general, the comparisons made among HHB 94-like hybrids, revealed no significant differences in grain or stover yield performance for pooled data sets within any of state-wise test environment clusters or across all ten test environments (Tables 25-28).

4.7.2.1. Grain yield (g/m^2)

The mean performance among HHB 94-like hybrids pooled across the ten test environments revealed no significant differences in grain yield which ranged from a minimum of 279 g/m^2 (ICMH 02005) to a maximum of 351 g/m^2 (HHB 181). None of the experimental hybrid could significantly exceed the grain yield performance of the hybrid control entries HHB 94-original (334 g/m^2) and HHB 181 across these ten trials. Hybrids produced on sub-selections of ICMA 89111-P2, ICMA 89111-P5 and ICMA 89111-P6 produced numerically higher grain yields than those on the corresponding sub-selections of ICMB 89111 and ICMB 90111. Very low to low (0.00 to 0.25) operational heritabilities (calculated on plot basis) were observed for grain yield in for each of the test environment clusters as well as for the pooled analysis across all ten test environments but operational heritability values (calculated on mean entry basis) were very high for Andhra Pradesh and across all ten test environments (0.87 and 0.72, respectively).

Trial mean grain yield performance was highest for Andhra Pradesh test environment cluster (410 g/m2) followed by Haryana (291 g/m^2) and Rajasthan (184 g/m^2). In Andhra Pradesh and Haryana, HHB 94-like hybrids based on the sub-selections of ICMA 89111 recorded marginally higher grain yields than those based on sub-selections of ICMB 89111 and ICMB 90111, suggesting an effect of cytoplasm on this

trait. In contrast, in Rajasthan, HHB 94-like hybrids based on the sub-selections of ICMB 89111 were observed to have marginally higher grain yields than their ICMA 89111- and ICMB 90111- sub-selection-based counterparts. Among the nine experimental hybrids in this trial HHB 94-P5A produced the highest grain yield both in Andhra Pradesh (435 g/m^2) and Haryana (307 g/m^2), as well as across all test environments (378 g/m^2) while HHB 94-P2B had numerically highest grain yield (198 g/m^2) in Rajasthan. A larger proportion of G × E interactions for grain yield were due to poor performance of the HHB 94-original and HHB 95-P5A to larger extent and ICMH 02006, HHB 181 and HHB 94-P2B to smaller extent (Figure 38) in Rajasthan test environment cluster.

4.7.2.2. Time to 50% flowering

The mean flowering time of the 11 entries in these trials ranged from 45 days to 51 days, with a grand mean of 49 days pooled across ten test environment (Table 25). Experimental versions of HHB 94-based on sub-selections of ICMA 89111 recorded a uniform mean time of 49 days to reach 50% flowering followed by test hybrids based on ICMB 89111 (49-51 days) and ICMB 90111 (50-51 days). Relatively early flowering was observed for HHB 181 (45 days).

Significant differences in flowering time were noticed among HHB 94-like hybrids in Andhra Pradesh test environment cluster, where this ranged from 43 days (HHB 181) to 50 days (ICMH 02005 and ICMH 02006) with a grand mean of 47 days. In the Haryana and Rajasthan test environment clusters, control entry hybrid HHB 181 flowered significantly earlier than all other HHB 94-like hybrids included in this trial and test environment cluster grand mean flowering times ranged from 50 to 51 days. Moderate to high operational heritabilities (calculated on plot mean basis) were recorded within each test environment cluster varying from 0.35 (Haryana cluster) to 0.62 (Andhra Pradesh test environment cluster) as well as for the pooled analysis across all ten test environments in this trial (0.45). Five HHB 94-like hybrids (HHB 94-P5A, HHB 94-P2B, HHB 94-P5B, ICMH 02002 and ICMH 02006) in Haryana test environment cluster deviated from the mean values across all ten test environments, and thus were the major contributors to G × E interactions for time to 50% flowering (Figure 39).

Table 25. Comparisons of mean performances of HHB 94-like hybrids for grain and stover yield and their component characteristics (1-4) across three environment clusters and across all ten test environments during *kharif* 2002 and 2003

Genotypes	Grain yield (g/m^2)				Time to 50% flowering				Effective plant stand (plants $/m^2$)				Plant height (cm)			
	AP	HRY	RAJ	All	AP	HRY	RAJ	All	AP	HRY	RAJ	All	AP	HRY	RAJ	All
HHB 94-P2A	427.8	305.3	163.2	325.9	47	51	50	49	11.9	11.6	11.3	11.7	180	201	188	190
HHB 94-P5A	435.3	306.8	154.2	327.7	47	52	49	49	12.2	12.6	11.5	12.2	174	201	179	186
HHB 94-P6A	423.0	298.1	184.8	325.4	46	51	49	49	12.0	12.4	11.7	12.1	171	196	183	183
HHB 94-Original	426.0	309.2	198.4	333.7	46	51	49	49	12.2	12.7	11.4	12.3	175	197	176	184
ICMH 02002	397.3	277.2	171.3	304.0	48	51	52	50	12.0	13.2	11.2	12.3	191	198	187	193
ICMH 02005	357.5	248.5	182.6	278.9	50	52	52	51	11.8	10.8	9.6	11.0	191	199	185	193
ICMH 02006	369.4	282.7	180.4	296.9	50	51	53	51	12.3	12.5	11.2	12.2	193	199	183	194
HHB 94-P2B	415.7	285.8	197.7	320.1	47	52	50	49	12.4	12.5	11.3	12.3	181	198	183	188
HHB 94-P5B	402.7	294.1	194.2	317.6	47	52	50	50	12.2	13.1	10.9	12.3	175	198	186	186
HHB 94-P6B	400.8	281.2	182.8	309.4	46	51	49	49	12.4	12.9	10.8	12.3	174	194	180	183
HHB 181 (Control)	453.9	315.7	214.9	350.8	43	48	45	45	11.1	10.7	10.4	10.8	176	186	174	180
Grand mean	409.9	291.3	184.1	317.3	47	51	50	49	12.0	12.3	11.0	12.0	180	197	182	187
Minimum	357.5	248.5	154.2	278.9	43	48	45	45	11.1	10.7	9.6	10.8	171	186	174	180
Maximum	453.9	315.7	214.9	350.8	50	52	53	51	12.4	13.2	11.7	12.3	193	201	188	194
SE (+/-)	20.8	31.1	22.7	26.1	0.4	0.7	0.9	0.7	0.6	0.8	0.6	0.7	3.4	3.4	3.8	3.6
CV (%)	11.4	23.9	27.6	18.4	2.0	2.9	4.2	3.1	11.3	15.1	13.0	13.6	4.2	3.8	4.6	4.3
HSD	94.7	141.6	103.2	118.7	2.0	3.0	4.3	3.1	2.8	3.8	2.9	3.3	15.6	15.3	17.1	16.2
h^2 (plot basis)	0.25	0.00	0.01	0.07	0.62	0.35	0.53	0.45	0.02	0.16	0.09	0.09	0.50	0.16	0.17	0.20
h^2 (mean basis)	0.87	0.00	0.01	0.72	0.91	0.86	0.94	0.95	0.23	0.80	0.57	0.83	0.94	0.72	0.73	0.87

AP and HRY indicate mean performance across four test environments each in Andhra Pradesh and Haryana respectively; RAJ indicates mean performance across two test environments in Rajasthan; All indicates mean performance across all ten test environments in Andhra Pradesh, Haryana and Rajasthan; HSD – honestly significant difference

Figures 38-41. Rank line graphs based on mean performance of yield and yield components (1-4) of nine HHB 94-like hybrids and two hybrid controls in three state-wise test environment clusters during *kharif* 2002 and 2003. For each trait, 11 HHB 94-like hybrids are placed along x-axis and the ranks of their state-wise mean performance on y-axis.

Figure 38. Grain yield (g/m^2)

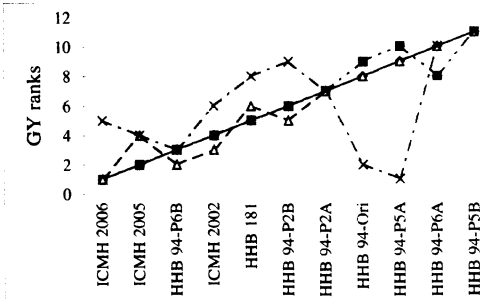


Figure 39. Time to 50% flowering (d)

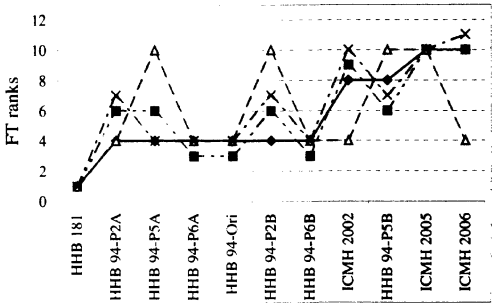


Figure 40. Effective plant stand (plants/m^2)

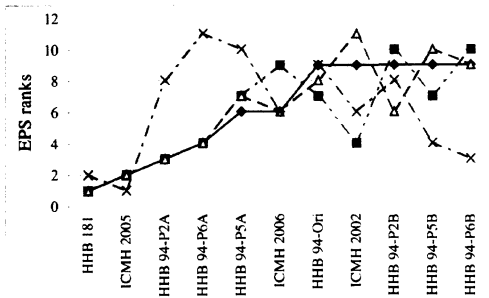
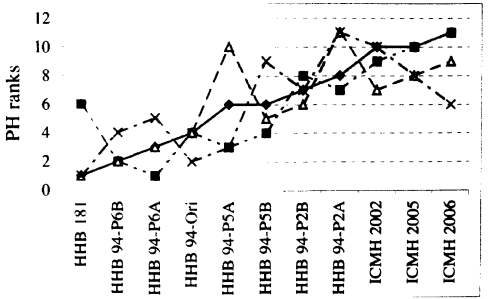


Figure 41. Plant height (cm)



4.7.2.3. Effective plant stand (plants/m^2)

Effective plant stands ranging from 10.8 plants/m^2 to 12.3 plants/m^2 with a grand mean of 11.9 plants/m^2 were observed across all ten test environments (Table 25). No significant differences were noticed among trial entries for effective plant stand based on pooled means across the ten test environments as well as each of the state-wise test environment clusters (Andhra Pradesh, Haryana and Rajasthan). HHB 94-like hybrids produced on sub-selections of ICMB 89111 demonstrated a uniform and slightly numerically higher numbers (plants/m^2) in the pooled analysis across all ten test environments as well as across the Andhra Pradesh test environment cluster. In contrast, HHB 94-like hybrids based on sub-selections of ICMA 89111 recorded slightly numerically better means for this trait than did other versions of this hybrid in Rajasthan. Effective plant stand was recorded with minimum and maximum values of 11.1 plants/m^2 and 12.4 plants/m^2 with a grand mean of 12.0 plants/m^2 for the Andhra Pradesh test environment cluster; 10.7 plants/m^2 and 13.2 plants/m^2 with a grand mean of 12.3 plants/m^2 for the Haryana test environment cluster; and 9.6 plants/m^2 and 11.7 plants/m^2 with a grand mean 11 plants/m^2 for the Rajasthan test environment cluster. Hence there were no obvious differences for this trait among the experimental entries in this trait in any of the state-wise subsets of test environments. High operational heritability (on an entry mean basis) were observed for the pooled analysis of effective plant stand across all ten test environments (0.83), across Haryana (0.80), and Rajasthan (0.57) test environment clusters, as well as moderate for the Andhra Pradesh cluster (0.23), while more narrowly calculated operational heritabilities (on a plot basis) were very low for all test environment clusters (0.02 to 0.16). Relatively uniform effective plant stand was recorded for most of the HHB 94-like hybrids across test environment clusters except HHB 94-P2A, HHB 94-P5A, HHB 94-P6A, HHB 94-P5B and HHB 94-P6B in Rajasthan test environment cluster and ICMH 02002 in Andhra Pradesh test environment cluster, and hence these hybrids largely contributed to G × E interactions (Figure 40).

4.7.2.4. Plant height (cm)

The mean performance of HHB 94-like hybrids for plant height across ten test environments is presented in the Table 25 and revealed no significant differences. Similarly, few differences for plant height were observed between trial entries across state-wise test environment clusters for Andhra Pradesh, Haryana and Rajasthan. Plant

height ranged from 180 cm to 194 cm with a grand mean of 187 cm from pooled data across ten test environments. Hybrids based on sub-selection of ICMB 90111 (ICMH 02002, ICMH 02005 and ICMH 02006) recorded numerically the highest mean plant heights across the Andhra Pradesh test environments. Across the Haryana test environment cluster, hybrids HHB 94-P2A and HHB 94-P5A recorded significantly greater mean plant heights (201 cm) than HHB 181 (184 cm) which was the shortest trial entry across these test environments. Control hybrid HHB 181 had the numerically shortest plant height across the Haryana and Rajasthan test environment clusters and across all ten test environments, but for the Andhra Pradesh test environment cluster HHB 94-P6A (171 cm) was numerically the shortest hybrid. Plant height recorded high operational heritability (calculated on plot basis) values at Andhra Pradesh test environment (0.50) but was low for other test environment clusters as well as for the pooled analysis across all ten test environments. The operational heritability (calculated on an entry mean basis) values were very high for this trait ranging from 0.72 to 0.94 across the three state-wise test environment clusters. The deviation in ranks based on mean values of most of test hybrids across test environments in three individual clusters from the ranks based on mean values across all ten test environments was much less for plant height except deviations of the test entry HHB 181 in Andhra Pradesh, HHB 94-P5A in Haryana and ICMH 02006 in Rajasthan test environment cluster which contributed much of the G × E interactions (Figure 41).

4.7.2.5. Panicle length (cm)

ICMH 02002, ICMH 02005, ICMH 02006 and HHB 181 produced shorter panicle length than other HHB 94-like hybrids as indicated by the pooled analysis across all ten test environments and across the state-wise clusters for Andhra Pradesh, Haryana, and Rajasthan (Table 26). The pooled means showed a range from 17.6 cm for ICMH 02006 to 20.5 cm for HHB 94-P2A, with an over all grand mean of 19.4 cm, and a minimum honestly significant difference between entry means of 2.0 cm. Panicles of HHB 94-like hybrids produced on sub-selections of ICMA 89111 were marginally longer than other test entries but these differences were only significant for the three experimental hybrids produced on sub-selections of ICMB 90111. HHB 94-original recorded marginally higher mean panicle lengths than the second control hybrid, HHB 181, for the over all grand mean across all ten test environments as well as for each of the state-wise test

environment clusters, but these differences were only significant in case of the Haryana test environment cluster. The highest entry mean panicle length of 22.4 cm was observed for HHB 94-P5B across the Haryana test environment cluster. Moderate to high operational heritability values calculated on plot basis ranging from 0.35 to 0.59, were observed for panicle length in this trial while the broader operational heritability values calculated on an entry mean basis ranged from 0.80 to 0.95 for this trait. Although Table 21 showed statistically significant but small and of little practical importance G × E interactions for panicle length, only one test hybrid (HHB 94-P5B) in Rajasthan test environment cluster performed poorly and the deviation in rank based on mean value of this entry across this cluster from across pooled data contributed to the observed G × E interactions (Figure 42).

4.7.2.6. Panicle diameter (mm)

The mean panicle diameter ranged from 23.2 mm to 26.2 mm with an observed grand mean of 24.3 mm and a minimum honestly significant difference between entries of 2.3 mm over all ten test environments (Table 26). Mean values of panicle diameter recorded in state-wise test environment clusters were marginally higher for Andhra Pradesh followed by the Rajasthan and Haryana clusters of test environments, with overall grand means of 24.5 mm, 24.2 mm and 24.1 mm, respectively. HHB 94-like hybrids based on sub-selections of ICMB 90111 produced panicles with marginally greater diameter than those sub-selections of based on ICMA 89111 and ICMB 89111 and several of these were had significantly greater panicle diameters than control entry HHB 181 in the pooled analysis across the Haryana and Rajasthan test environment clusters as well as in the pooled analysis across all ten test environments. High operational heritability (calculated on plot basis) was observed for this trait across the Rajasthan test environment cluster (0.50), with much lower values for the Andhra Pradesh (0.19) and Haryana (0.16) test environment clusters resulting in a moderate value for pooled analysis across all ten test locations. The observed operational heritability values calculated ranged from 0.51 for the Haryana cluster to 0.88 for the Rajasthan cluster. Two test hybrids (HHB 181 and HHB 94-P6A) showed variation in their ranks based on mean performance of panicle diameter across Andhra Pradesh test environment cluster and hence contributed to statistically significant but of relatively smaller portion of G × E interactions for this trait (Figure 43).

Table 26. Comparisons of mean performances of HHB 94-like hybrids for grain and stover yield and their component characteristics (5-8) across three environment clusters and across all ten test environments during *kharif* 2002 and 2003

Genotypes	Panicle length (cm)				Panicle diameter (mm)				Fresh straw yield (g/m^2)				Dry straw yield (g/m^2)			
	AP	HRY	RAJ	All	AP	HRY	RAJ	All	AP	HRY	RAJ	All	AP	HRY	RAJ	All
HHB 94-P2A	18.7	22.3	20.4	20.5	24.5	23.9	23.5	24.0	1751	1797	819	1583	482	777	317	567
HHB 94-P5A	18.6	21.9	20.0	20.2	24.0	23.9	23.6	23.9	1750	1829	884	1608	499	825	331	596
HHB 94-P6A	18.2	22.2	20.5	20.2	24.0	24.0	24.1	24.0	1663	1662	865	1503	455	756	325	549
HHB 94-Original	18.2	22.2	19.6	20.1	24.4	23.8	23.4	24.0	1684	1699	880	1529	470	768	365	569
ICMH 02002	16.9	19.5	18.1	18.2	25.5	26.3	27.1	26.2	1927	1901	828	1697	601	883	326	659
ICMH 02005	16.6	18.9	17.3	17.7	25.1	25.8	27.0	25.8	2042	2009	960	1812	565	925	376	671
ICMH 02006	16.7	18.9	16.8	17.6	24.5	24.6	25.3	24.7	1953	1876	880	1708	581	964	341	686
HHB 94-P2B	17.8	20.7	20.1	19.4	25.1	24.0	23.4	24.3	1846	1742	881	1611	525	800	342	598
HHB 94-P5B	18.5	22.4	19.9	20.3	24.0	23.8	23.3	23.8	1852	1819	935	1655	501	816	376	602
HHB 94-P6B	18.2	21.7	20.3	20.0	24.0	22.8	23.3	23.4	1761	1649	868	1537	453	728	332	539
HHB 181 (Control)	18.0	19.8	18.6	18.8	24.4	22.7	22.0	23.2	1654	1674	846	1500	504	823	320	595
Grand mean	17.9	20.9	19.2	19.4	24.5	24.1	24.2	24.3	1808	1787	877	1613	512	824	341	603
Minimum	16.6	18.9	16.8	17.6	24.0	22.7	22.0	23.2	1654	1649	819	1500	453	728	317	539
Maximum	18.7	22.4	20.5	20.5	25.5	26.3	27.1	26.2	2042	2009	960	1812	601	964	376	686
SE (+/-)	0.4	0.4	0.5	0.4	0.4	0.5	0.7	0.5	89.6	150.5	92.2	120.0	30.2	80.2	38.2	57.7
CV (%)	5.2	4.2	6.2	5.1	4.0	4.2	6.2	4.6	11.1	18.8	23.5	16.6	13.2	21.8	25.1	21.4
HSD	1.9	1.8	2.5	2.0	2.0	2.0	3.0	2.3	407.6	684.9	419.6	545.9	137.4	365.1	173.9	262.5
h^2 (plot basis)	0.35	0.59	0.44	0.47	0.19	0.16	0.50	0.25	0.24	0.01	0.00	0.09	0.32	0.03	0.00	0.08
h^2 (mean basis)	0.88	0.91	0.80	0.95	0.80	0.51	0.88	0.85	0.83	0.05	0.00	0.76	0.88	0.24	0.03	0.72

AP and HRY indicate mean performance across four test environments each in Andhra Pradesh and Haryana respectively; RAJ indicates mean performance across two test environments in Rajasthan; All indicates mean performance across all ten test environments in Andhra Pradesh, Haryana and Rajasthan; HSD – honestly significant difference

Figures 42-45. Rank line graphs based on mean performance of yield and yield components (5-8) of nine HHB 94-like hybrids and two hybrid controls in three state-wise test environment clusters during *kharif* 2002 and 2003. For each trait, 11 HHB 94-like hybrids are placed along x-axis and the ranks of their state-wise mean performance on y-axis.

Figure 42. Panicle length (cm)

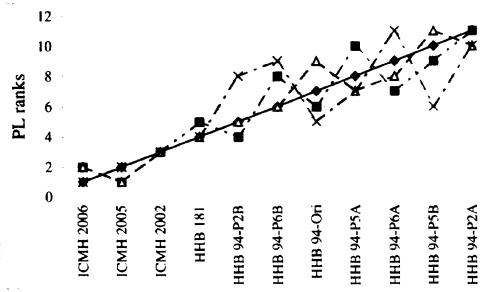


Figure 43. Panicle diameter (mm)

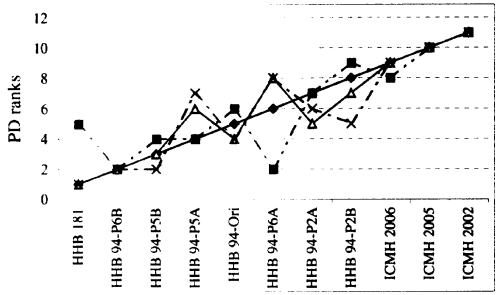


Figure 44. Fresh straw yield (g/m^2)

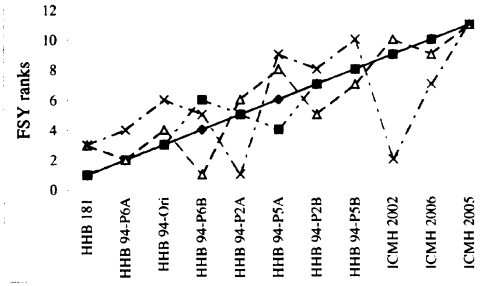
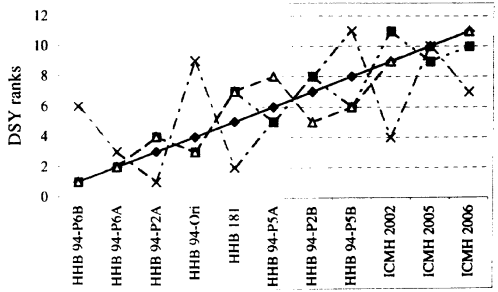


Figure 45. Dry straw yield (g/m^2)



4.7.2.7. Fresh straw yield (g/m^2)

HHB 94-like-hybrids did not differ significantly for mean fresh straw yield when compared against grand means of all ten test environments and the three state-wise test environment cluster. Fresh straw yield ranged from 1500 g/m^2 to 1812 g/m^2 with a grand mean of 1613 g/m^2 and a minimum honestly significant difference between entry means of 546 g/m^2 across test environments. Hybrid based on sub-selection of ICMB 90111 (particularly ICMH 02005) produced slightly greater fresh straw yields (Table 26) than those produced on the sub-selections of ICMA 89111 and ICMB 89111, but the pair-wise differences were not significant for the pooled analysis across all ten test environments. Similar results were observed individually across the Andhra Pradesh, Haryana and Rajasthan clusters of test environments. The highest grand mean of 1806 g/m^2 was recorded for Andhra Pradesh followed by Haryana (1787 g/m^2) and Rajasthan (877 g/m^2). Neither of the control hybrid entries could perform significantly better than any of the nine experimental hybrids in even one of the state-wise clusters of test environments. Operational heritabilities calculated on plot basis for fresh straw yield were low, ranging from 0.00 (Rajasthan) to 0.24 (Andhra Pradesh), but when calculated on an entry mean basis, operational heritability estimates were very high for Andhra Pradesh (0.83) and across all ten test environments (0.76), but very low (0.05) for trials conducted in Haryana and Rajasthan. The larger deviation in ranks based on mean for fresh straw yield of test hybrids (mainly HHB 94-P2A and ICMH 02002) in Rajasthan and HHB 94-P6B in Haryana test environment cluster largely contributed G × E interactions for this trait (Figure 44).

4.7.2.8. Dry straw yield (g/m^2)

Dry straw yield entry means ranged from 539 g/m^2 to 686 g/m^2 with an over all grand mean of 603 g/m^2 and a minimum honestly significant difference between entry means of 263 g/m^2 across all ten test environments (Table 26), so that none of the observed differences between entry means from this pooled analysis for dry straw yield were significant. Among the state-wise clusters of test environments Haryana (824 g/m^2) recorded the highest grand mean for dry straw yield followed by Andhra Pradesh (512 g/m^2) and Rajasthan (341 g/m^2). Hybrids produced on sub-selection of ICMB 90111 recorded numerically higher dry straw yields in pooled analysis across all ten test environments as well as across Andhra Pradesh and Haryana test environment clusters but

for the lowest yielding Rajasthan test environment these were at par with hybrids based on sub-selection of ICMB 89111 and only slightly numerically higher than hybrids of sub-selections of ICMA 89111. All hybrids tested exhibited higher mean dry straw yields across the Haryana test environment cluster than across either the Andhra Pradesh or Rajasthan clusters. The calculated operational heritabilities (on an entry mean basis) were greater from Andhra Pradesh (0.88) and across all ten test environments (0.72) than across the state-wise test environment clusters for Haryana (0.24) and Rajasthan (0.03) as shown in Table 26. The more narrowly calculated plot basis operational heritabilities indicated that only across the trials conducted in Andhra Pradesh was, if possible, to detect significant genetic variation between the 11 trial entries, and this is borne out by the significant dry stover yield superiority of entry ICMH 02002 over HHB 94-P6B, which was the only honestly significant difference detected for this trait. The inconsistency in mean performance of five HHB 94-like hybrids (HHB 94-P2B, ICMH 02002 and ICMH 02006) in Rajasthan test environment cluster resulting in deviation of their rank orders from ranks based on mean performance of these test hybrids across all ten test environment cluster largely contributed to G × E interactions for dry straw yield (Figure 45).

4.7.2.9. Straw moisture content (%)

A grand mean of 62.3%, with a range of 59.9% to 64.3% (Table 27), and a minimum honestly significant difference between entry means of 7.8% was recorded for the pooled analysis across all ten test environments for straw moisture content. Among the three state-wise test environment clusters, the grand mean was highest for Andhra Pradesh (70.6%) followed by Rajasthan (62.4%) and Haryana (54.0%) with minimum to maximum entry means ranging from 67.7% to 73.4%, 61.0% to 63.3% and 49.6% to 56.5%, respectively. These results suggest that stover samples for the Haryana and Rajasthan test environments, which were sun-dried, were not fully dry when dry straw yield measurements were made. Hence the dry stover yields for these test environments are likely to be systematically over-estimated and their stover moisture contents are likely to be systematically under-estimated in this study. Hybrid HHB 94-P6B recorded the numerically highest straw moisture content in the pooled analysis across all ten test environments, across the state-wise test environments cluster for Andhra Pradesh and Rajasthan, but only in case of Andhra Pradesh did this translate into a statistically

significant higher straw moisture content than any other entry in the trial (ICMH 02002 had a straw moisture content of only 67.7% for this test entry cluster). Straw moisture contents had the highest operational heritability (calculated broadly on an entry mean basis) for Andhra Pradesh (0.89), but were only moderate for Haryana (0.51) and zero for Rajasthan. The more narrowly calculated plot basis operational heritabilities for straw moisture content were moderate for Andhra Pradesh, low for Haryana and across the ten test environments, and zero for Rajasthan. HHB 94-like hybrids (ICMH 02006, HHB 181, HHB 94-original, HHB 94-P5B and HHB 94-P2B) showed larger deviation in their ranks based on hybrid mean performance in Rajasthan test environment cluster than the two test environment clusters in Andhra Pradesh and Haryana which collectively contributed to the observed significant G × E interactions for straw moisture content (Figure 46) in the present study.

4.7.2.10. Effective tiller number (tillers/m^2)

The mean effective tiller number, a major component of yield, ranges from 16 to 32 for the pooled analysis across all ten test environments with a grand mean of 29 and a minimum honestly significant difference be entries of 8.7 tillers/m^2 (Table 27). The mean performance of both the controls, HHB 94-original (32) and HHB 181 (32), were the highest observed across all ten test environments, but were not statistically significantly superior to any other trial entry. This trend was also followed in across test environment clusters for Andhra Pradesh, Rajasthan and Haryana except that for Andhra Pradesh, HHB 181 did have significantly more effective tillers (42) than did ICMH 02005 (31). HHB 94-like hybrids produced on sub-selections of ICMB 89111 had slightly more numbers of effective tillers than did those HHB 94-like hybrids produced on sub-selections of ICMA 89111 or ICMB 90111 (Table 27). The overall grand mean for effective tiller number was higher for the Andhra Pradesh test environment cluster (37) than for Haryana (29) and Rajasthan (17). The lowest operational heritabilities (on an entry mean basis) for effective tiller number were observed for the Rajasthan (0.02) and Haryana (0.00) test environment clusters, while these values were high for the Andhra Pradesh test environment (0.91). In case of the more narrowly estimated plot basis heritability values, only that for the Andhra Pradesh test environment was high enough (0.31) to suggest significant differences between test entry means, which was borne out by the observed significant difference in entry mean effective tiller number for HHB 181

Table 27. Comparisons of mean performances of HHB 94-like hybrids for grain and stover yield and their component characteristics (9-12) across three environment clusters and across all ten test environments during *kharif* 2002 and 2003

Genotypes	Straw moisture content (%)				Effective tillers number (tillers/m^2)				Panicle yield (g/m^2)				1000-grain mass (g)			
	AP	HRY	RAJ	All	AP	HRY	RAJ	All	AP	HRY	RAJ	All	AP	HRY	RAJ	All
HHB 94-P2A	71.3	56.5	61.7	63.5	37	29	15	29	548	497	219	462	8.93	7.18	7.28	7.90
HHB 94-P5A	70.6	54.9	63.5	62.9	38	28	15	29	548	482	220	456	9.08	7.38	7.44	8.07
HHB 94-P6A	71.5	54.7	63.9	63.3	39	29	16	30	544	479	245	458	9.36	7.04	7.73	8.11
HHB 94-Original	71.0	54.9	61.5	62.6	40	31	17	32	547	489	251	465	8.78	7.31	7.50	7.94
ICMH 02002	67.7	53.6	61.8	60.9	34	26	15	27	523	463	224	439	8.38	7.40	7.34	7.78
ICMH 02005	71.3	53.7	61.8	62.4	31	27	15	26	466	448	248	415	7.98	7.23	7.46	7.58
ICMH 02006	68.9	49.6	62.5	59.9	33	27	15	27	492	453	224	423	7.48	6.88	6.94	7.13
HHB 94-P2B	70.6	54.0	62.2	62.3	34	31	16	29	525	468	246	446	8.58	7.30	7.44	7.84
HHB 94-P5B	71.7	55.2	61.0	63.0	39	31	15	31	530	491	251	459	8.93	7.39	8.05	8.13
HHB 94-P6B	73.4	55.6	63.4	64.3	39	30	17	31	522	461	262	445	8.64	7.04	7.53	7.78
HHB 181 (Control)	68.3	51.5	63.3	60.6	42	30	17	32	578	538	265	499	8.44	7.35	7.44	7.80
Grand mean	70.6	54.0	62.4	62.3	37	29	16	29	529	479	241	452	8.60	7.23	7.47	7.82
Minimum	67.7	49.6	61.0	59.9	31	26	15	26	466	448	219	415	7.48	6.88	6.94	7.13
Maximum	73.4	56.5	63.9	64.3	42	31	17	32	578	538	265	499	9.36	7.40	8.05	8.13
SE (+/-)	1.2	2.0	1.7	1.7	2.2	1.8	1.1	1.9	23.8	42.9	26.9	33.7	0.28	0.32	0.32	0.31
CV (%)	3.8	8.4	6.1	6.1	13.6	14.2	16.1	14.5	10.1	20.0	24.9	16.7	7.2	10.0	9.5	8.9
HSD	5.5	9.2	7.7	7.8	10.2	8.4	5.1	8.7	108.4	195.0	122.3	153.2	1.27	1.46	1.46	1.41
h^2 (plot basis)	0.27	0.08	0.00	0.08	0.31	0.00	0.00	0.12	0.20	0.00	0.00	0.06	0.37	0.00	0.01	0.10
h^2 (mean basis)	0.89	0.51	0.00	0.75	0.91	0.00	0.00	0.79	0.80	0.00	0.00	0.67	0.88	0.00	0.08	0.76

AP and HRY indicate mean performance across four test environments each in Andhra Pradesh and Haryana respectively; RAJ indicates mean performance across two test environments in Rajasthan; All indicates mean performance across all ten test environments in Andhra Pradesh, Haryana and Rajasthan; HSD – honestly significant difference

Figures 46-49. Rank line graph based on mean performance of yield and yield components (9-12) of nine HHB 94-like hybrids and two hybrid controls in three state-wise test environment clusters during *kharif* 2002 and 2003. For each trait, 11 HHB 94-like hybrids are placed along x-axis and the ranks of their state-wise mean performance on y-axis.

Figure 46. Straw moisture content (%)

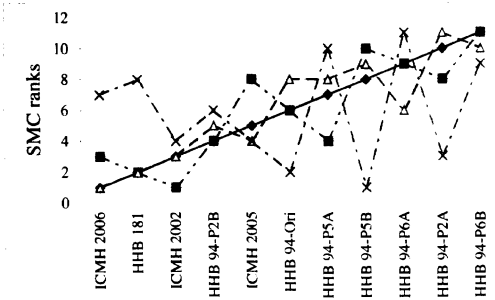


Figure 47. Effective tiller number (tillers/m^2)

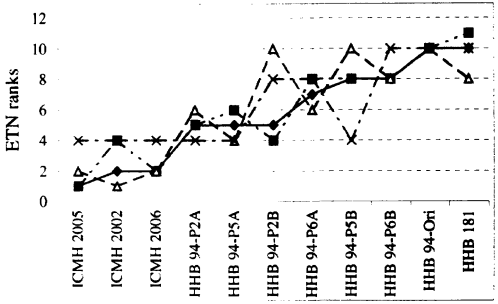


Figure 48. Panicle yield (g/m^2)

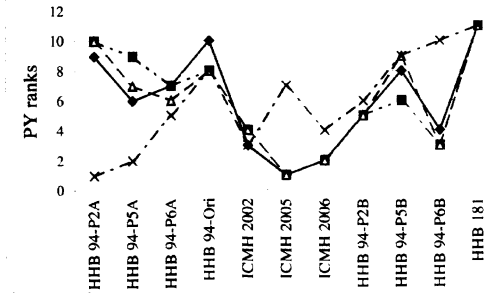
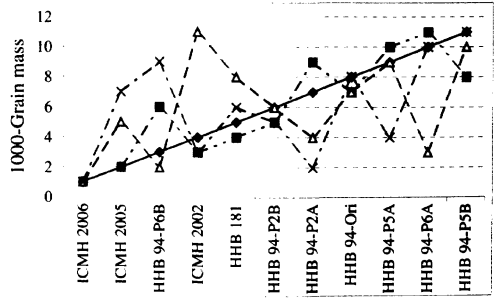


Figure 49. 1000-Grain mass (g)



(42) and ICMH 02005 (31) in this sub-set of test environment. Effective tiller number recorded relatively consistent rank orders for most of HHB 94-like hybrids across all test environment clusters except ICMH 02005 and HHB 94-P5B in Rajasthan and HHB 94-P2B in Haryana test environment cluster, that altogether significantly contributed to G × E interactions for this trait (Figure 47).

4.7.2.11. Panicle yield (g/m^2)

No significant differences were observed among the eleven trial entries for panicle yield, which ranged from 415 g/m^2 to 499 g/m^2 with grand mean of 452 g/m^2 and a minimum honestly significant difference between entry means of 153 g/m^2 in the pooled analysis across all ten test environments (Table 27). HHB 181 (499 g/m^2) recorded the numerically highest mean panicle yield followed by HHB 94-original (465 g/m^2) and HHB 94-P2A (462 g/m^2). Similar trends were observed for Andhra Pradesh, Haryana and Rajasthan. In general, HHB 94-like hybrids based on sub-selections of ICMA 89111 performed marginally better than others in all test environment clusters except Rajasthan where hybrids based on ICMB 89111 had numerically longer panicle yields. None of the multiple-test environment clusters showed any significant differences for panicle yield among genotypes, except that HHB 181 had marginally significantly greater panicle yield than ICMH 02005 across the Andhra Pradesh test environments. Lower trial mean values for panicle yield were recorded across the Rajasthan test environments than for those in Andhra Pradesh and Haryana. High operational heritability (on entry mean basis) was observed for this trait across the four Andhra Pradesh test environments (0.80), but operational heritabilities for it dropped to zero for the Haryana and Rajasthan test environment clusters. The inconsistent performance of ICMH 02005, HHB 94-P6B, HHB 94-P5A and HHB 94-P2A in Rajasthan test environment cluster largely contributed to significant G × E interactions for panicle yield (Figure 48).

4.7.2.12. 1000-grain mass (g)

Entry means 1000-grain mass ranged from 7.13 g to 8.13 g with an overall grand mean of 7.82 g and a minimum honestly significant difference of 1.41 g across ten locations (Table 27). HHB 94-P5B recorded the numerically highest mean value followed by HHB 94-P6A (8.00 g) for this trait, while ICMH 02006 (7.13 g) recorded the lowest, but these across-environment entry mean values were not significantly different. Similar trends

were observed for the pooled analyses across the four Haryana test environments and across the two Rajasthan test environments, with no significant differences being in entry mean performance for 1000-grain mass. However, across the four Andhra Pradesh test environments, HHB 94-P6A (9.36 g), HHB 94-P5A (9.08 g), HHB 94-P2A (8.93 g) and HHB 94-P5B (8.93 g) recorded entry mean 1000-grain mass values that were significantly greater than that of ICMH 02006 (7.48 g). Very high operational heritabilities (calculated on entry mean basis) were obtained across the ten test environments (0.76) and for Andhra Pradesh (0.88) while very low values were obtained for Haryana (0.00) and Rajasthan (0.08). Most of the HHB 94-like hybrids showed larger deviations in rank orders based on their mean performance of 1000-grain mass in Rajasthan test environment cluster (Figure 49) from their rank orders of mean pooled data across all ten test environments which contributed to major portion of G × E interactions.

4.7.2.13. Panicle grain number (grains/panicle)

Panicle grain numbers did not significantly differ among the eleven hybrids evaluated in this experiment (Table 28). Panicle grain number ranged from 1309 to 1553 with a grand mean of 1410 and minimum honestly significant difference of 576 in the pooled analysis across ten test environments. ICMH 02006 (1553) produced highest average number of grains per panicle across these ten test environments followed by HHB 181 (1464), and HHB 94-P2A (1456) while HHB 94-P6B (1309) recorded lowest mean value for this trait, but these differences were not statistically significant.

Across the Andhra Pradesh test environment cluster, only ICMH 02006 (1552) had a significantly larger mean panicle grain number than HHB 94-P5B (1173), which had the lowest entry mean for this trait. Across both the Haryana and Rajasthan test environment cluster, no significant differences in entry mean panicle grain number were detected in this experiment. Moderately low to very high (0.31-0.93) broad-sense operational heritabilities (calculated on an entry mean basis) were obtained for this trait from all test environment clusters except Rajasthan (0.00). Andhra Pradesh test environment also had moderate heritability estimated (0.33) for the more narrowly calculated plot basis operational, while those for the other test environment clusters were near zero for panicle grain number (Table 28). This variable performance of majority of the HHB 94-like hybrids for panicle grain number across test environment clusters, especially in Haryana and Rajasthan resulted in deviation in rank orders from the ranks

based on mean entry across all ten test environment leading to significantly large G × E interactions (Figure 50).

4.7.2.14. Total above-ground biomass yield (g/m^2)

The HHB 94-like hybrids in this experiment exhibited no significant differences for total above grained biomass yield in the pooled analysis across all ten test environments and any of them state-wise test environment clusters. With a grand mean of 1054 g/m^2, biomass yield ranged from 984 g/m^2 to 1109 g/m^2 with a minimum honestly significant difference of 384 g/m^2 across all test environments (Table 28). Grand means across individual test environment clusters were observed to be higher for Andhra Pradesh (1042 g/m^2) and Haryana (1302 g/m^2) than Rajasthan (582 g/m^2), but none of the pair-wise differences between trial entry means across a cluster of test environments was statistically significant.

In general, HHB 94-like hybrids produced on sub-selection ICMB 90111 gave higher biomass yields than those produced on sub-selections of either ICMA 89111 or ICMB 89111. HHB 94-P6B (984 g/m^2) recorded the numerically lowest mean biomass yield across the ten test environments. HHB 94-like hybrids produced significantly less total above-ground biomass yield in the Rajasthan test environment than in those of Andhra Pradesh and Haryana. Extremely low plot-basis operational heritabilities for biomass yield were obtained for each of the three state-wise test environment clusters (0.00 to 0.07), indicating the lack of any heritable variation for this trait among the test entries in this experiment. The large variation in rank order of HHB 94-like hybrids for total above-ground biomass yield in Rajasthan along with small variation in rank order of hybrids planted in Andhra Pradesh and Haryana test environment cluster together contributed to larger G × E interactions for this trait (Figure 51).

4.7.15. Harvest index (%)

Across the ten test environments in this experiment, entry mean harvest index ranged from 26.7% to 33.5%, with a grand mean of 31.1% and a minimum honestly significant difference of 8.0%. Thus no significant pair-wise differences in entry mean harvest index values could be identified from this pooled analysis. Similar patterns were observed for pooled analysis across the test environment cluster for harvest index of Haryana and Rajasthan. However, in case of the Andhra Pradesh test environment cluster, the three

Table 28. Comparisons of mean performances of HHB 94-like hybrids for grain and stover yield and their component characteristics (13-15) across three environment clusters and across all ten test environments during *kharif* 2002 and 2003

Genotypes	Panicle grain number				Total above ground biomass yield (g/m^2)				Harvest index (%)			
	AP	HRY	RAJ	All	AP	HRY	RAJ	All	AP	HRY	RAJ	All
HHB 94-P2A	1335	1567	1474	1456	1031	1274	537	1029	41.5	23.7	30.9	32.3
HHB 94-P5A	1287	1567	1348	1411	1048	1307	551	1052	41.7	23.1	29.2	31.8
HHB 94-P6A	1195	1497	1473	1371	999	1236	570	1008	42.3	23.8	33.0	33.1
HHB 94-Original	1256	1392	1498	1359	1018	1258	616	1033	42.0	24.5	32.3	33.1
ICMH 02002	1413	1451	1481	1442	1124	1346	550	1098	35.5	20.6	30.4	28.5
ICMH 02005	1453	1295	1494	1398	1030	1363	624	1082	34.5	17.9	28.9	26.7
ICMH 02006	1552	1537	1588	1553	1072	1417	565	1109	34.6	20.2	30.0	27.9
HHB 94-P2B	1464	1267	1594	1411	1050	1268	588	1045	39.8	23.0	32.8	31.7
HHB 94-P5B	1173	1405	1531	1337	1031	1306	627	1061	39.3	22.9	30.6	31.0
HHB 94-P6B	1210	1379	1368	1309	975	1189	594	984	41.4	23.4	30.1	32.0
HHB 181 (Control)	1299	1531	1659	1464	1082	1360	585	1094	42.1	23.5	36.6	33.5
Grand mean	1331	1444	1501	1410	1042	1302	582	1054	39.5	22.4	31.3	31.1
Minimum	1173	1267	1348	1309	975	1189	537	984	34.5	17.9	28.9	26.7
Maximum	1552	1567	1659	1553	1124	1417	627	1109	42.3	24.5	36.6	33.5
SE (+/-)	82.1	157.8	115.3	126.7	48.3	115.3	58.6	84.5	1.3	1.7	2.3	1.8
CV (%)	13.8	24.4	17.2	20.1	10.4	19.8	22.5	17.9	7.4	17.3	16.4	12.7
HSD	373.4	718.2	524.5	576.3	219.5	524.6	266.8	384.3	6.0	7.9	10.5	8.0
h^2 (plot basis)	0.33	0.03	0.00	0.03	0.07	0.00	0.00	0.01	0.54	0.17	0.00	0.22
h^2 (mean basis)	0.93	0.31	0.00	0.51	0.56	0.00	0.00	0.14	0.96	0.79	0.00	0.91

AP and HRY indicate mean performance across four test environments each in Andhra Pradesh and Haryana respectively; RAJ indicates mean performance across two test environments in Rajasthan; All indicates mean performance across all ten test environments in Andhra Pradesh, Haryana and Rajasthan; HSD – honestly significant difference

Figures 50-52. Rank line graphs based on mean performance of yield and yield components (13-15) of nine HHB 94-like hybrids and two hybrid controls in three state-wise test environment clusters during *kharif* 2002 and 2003. For each trait, 11 HHB 94-like hybrids are placed along x-axis and the ranks of their state-wise mean performance on y-axis.

Figure 50. Panicle grain number

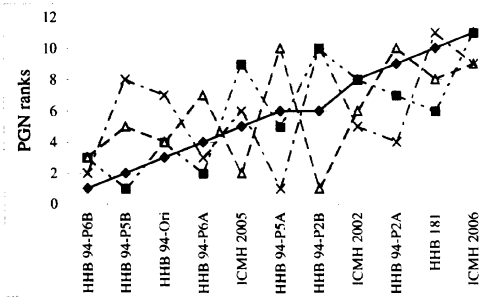


Figure 51. Total above ground biomass yield (g/m^2)

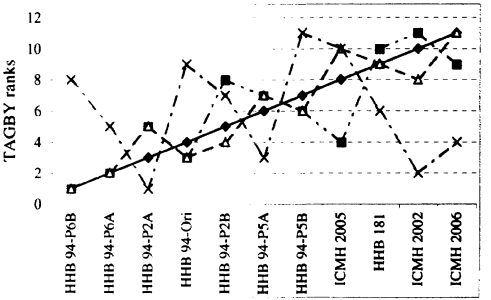
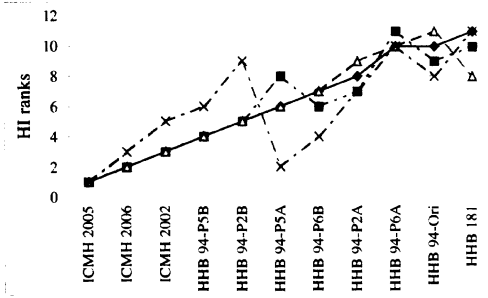
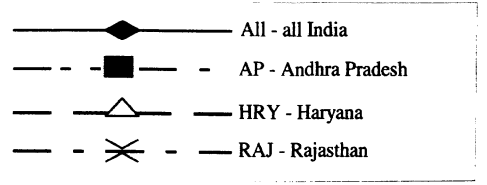


Figure 52. Harvest index (%)



Legends



HHB 94-like hybrids produced on sub-selections of ICMB 90111 had significantly lower harvest index values than any of the five A_1 cytoplasm hybrids (HHB 94-P2A, HHB 94-P5A, HHB 94-P6A, HHB 94-original and HHB 181). A grand mean harvest index of 39.5% was recorded across the four test environments in Andhra Pradesh which was higher than the test environment cluster means for Rajasthan (31.3%) and Haryana (22.4%). The two hybrid control entries (HHB 94-original and HHB 181) recorded mean harvest index values at par with each other across all three of the state-wise test environment clusters. Across the Andhra Pradesh and the Haryana test environment clusters high operational heritabilities (on an entry mean basis) for harvest index were observed (0.96 and 0.79, respectively), but harvest index was not heritable across the Rajasthan test environment (0.00). The more narrowly operational heritability (calculated on entry mean basis) estimate for harvest index was also high across the Andhra Pradesh test environments (0.54) and moderate across all ten test environments (0.22) and across the four Haryana test environments (0.17), but zero for the Rajasthan test environments (Table 28). No major variation was observed in ranks based on mean values of HHB 94-like hybrids for harvest index across all test environment clusters except for three hybrids (HHB 94-P2B, HHB 94-P5A and HHB 94-P6B) from ranks based on mean entry across all ten test environment which resulted in statistically significant G × E interactions (Figure 52).

4.8. MEAN PERFORMANCE FROM INDIVIDUAL TEST ENVIRONMENTS

4.8.1. Grain yield (g/m^2)

Among all ten individual test environment trials, that conducted in field RCE 24 at ICRISAT-Patancheru during the 2002 *kharif* season (RCE 24 K02) produced the highest trial mean grain yield (465 g/m^2), with entry means ranging between 397 g/m to 520 g/m^2 (Table 29). At the same time, the trial conducted at Nagaur during the 2003 *kharif* season (NGR K03) produced the lowest trials mean grain yield (119 g/m^2) with entry means ranging from 89 g/m^2 to 142 g/m^2. All other test environments produced trials with moderate to good trial mean grain yield levels (233 to 406 g/m^2). Entry mean-based operational heritability for grain yield in these trials was highest (0.73) for trials in RP 9A K02 (conducted at ICRISAT-Patancheru, Andhra Pradesh during *kharif* 2002) and lowest (zero) in the low-yielding *kharif* 2003 in Nagaur test environment (Rajasthan) (Table 29). The observed F values for trials conducted at all individual test environments revealed

significant differences in grain yield among genotypes except at Nagaur and Bawal during the *kharif* season of 2003. HHB 94-like hybrids based on sub-selections of ICMB 90111 produced higher grain yields and had lower rank orders than other entries in this trial in most test environments except Bawal and Hisar in 2002 and Durgapura in 2003. Control entries HHB 181 and HHB 94-original had among the lowest mean grain yields except for HHB 181, which performed very well at Bawal in both 2002 and 2003, and Hisar in 2003 in most test environments (mean data of each location is given in Appendix 3).

4.8.2. Time to 50% flowering

HHB 94-like hybrids in this experiment recorded an early trial mean time to complete 50% flowering (41 days) in the RP 6B K03 test environment, with entry means ranging from 37 to 47 days. Trials conducted at RCE 24 K02, Hisar K03, Nagaur K03 and Bawal K03 took time 51-55 days to reach 50% flowering, where as at other test environments less time (<50 days) was required. The entry mean-based operational heritability values for this trait ranged from 0.62 (Hisar K03) to 0.99 (RP 6B K03) across the ten test environments, with high values (>90%) for HHB 94-like hybrid versions in the RP 9A K02, RCE 24C K03 and Hisar K02 test environments (Table 30). HHB 181 completed 50% flowering much earlier than any other hybrid in this trial, and was the earliest flowering entry in each of the ten test environments, and was followed by HHB 94-orignial, HHB 94-P6A and HHB 94-P6B in most of these environments. ICMH 02005 was consistently one of the latest flowering entries in each of the ten test environments (Appendix 3). These consistence performances contributed to the relatively high operational heritabilities observed for this trait (Table 30).

4.8.3. Effective plant stand (plant/m^2)

The individual test environment trial means for effective plant stand were greater than 10.5 plant/m^2 in all test environments except RP 9A K02 (9.5 plant/m^2) (Table 31). The highest entry mean plant stands (15 plants/m^2) were recorded in the RCE 24C K03 and Hisar K03 test environments, with the minimum entry mean value (8.0 plants /m^2) being observed in the RP 9A K02 test environment. The Bawal K02 trial had the highest entry mean-based operational heritability (0.76) for this trait, suggesting difficulties in stand establishment for one or more trial entries in this particular test environment. In trial test

Table 29. ANOVA summary for grain yield (g/m^2) from HHB 94-like hybrids trial conducted in ten individual test environments

Trait	Grain yield (g/m^2)									
Test environment	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	BWL K02	BWL K03	HSR K02	HSR K03	DGR K03	NGR K03
Mean	464.8	386.0	382.6	406.3	319.0	252.4	361.1	232.8	249.4	118.7
Minimum	397.4	315.8	321.6	358.8	263.3	206.0	294.7	178.9	178.4	89.3
Maximum	519.7	445.7	420.6	439.8	367.2	303.3	480.7	278.3	295.2	141.6
SE (+/-)	22.8	20.9	16.4	16.0	19.4	26.9	33.8	22.2	23.6	16.3
CV (%)	11.0	12.1	9.6	8.8	13.6	23.8	21.0	21.4	21.1	30.7
F ratio	3.29**	2.62*	3.75**	2.40*	3.54**	1.54	3.16**	2.21*	2.22*	0.96
h^2 (on plot basis)	0.31	0.25	0.35	0.22	0.34	0.10	0.30	0.20	0.20	0.00
h^2 (on mean basis)	0.70	0.62	0.73	0.58	0.72	0.35	0.68	0.55	0.55	0.00

Table 30. ANOVA summary for time to 50% flowering (d) from HHB 94-like hybrids trial conducted in ten individual test environments

Trait	Time to 50 % flowering (d)									
Test environment	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	BWL K02	BWL K03	HSR K02	HSR K03	DGR K03	NGR K03
Mean	51	47	49	41	46	55	49	54	47	53
Minimum	48	43	45	37	44	50	46	51	42	47
Maximum	52	50	52	47	47	58	51	56	49	57
SE (+/-)	0.4	0.4	0.4	0.3	0.3	0.9	0.3	0.7	0.7	1.0
CV (%)	1.8	1.8	2.0	1.6	1.5	3.5	1.3	2.9	3.3	4.2
F ratio	5.89**	26.21**	15.09**	107.77**	8.27**	6.42**	22.10**	2.61*	6.97**	7.13**
h^2 (on plot basis)	0.50	0.83	0.74	0.96	0.59	0.52	0.81	0.24	0.54	0.55
h^2 (on mean basis)	0.83	0.96	0.93	0.99	0.88	0.84	0.96	0.62	0.86	0.86

Table 31. ANOVA summary for effective plant stand from HHB 94-like hybrids trial conducted in ten individual test environments

Trait	Effective plant stand ($plants/m^2$)									
Test environment	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	BWL K02	BWL K03	HSR K02	HSR K03	DGR K03	NGR K03
Mean	12.0	13.6	9.5	12.8	10.5	12.9	13.2	12.3	10.5	11.5
Minimum	12.0	11.0	8.0	12.0	8.5	12.0	10.7	10.0	9.0	10.0
Maximum	12.0	15.0	11.0	13.0	11.9	14.0	14.5	15.0	11.0	13.0
SE (+/-)	0.5	0.6	0.8	0.4	0.5	0.8	0.7	1.0	0.6	0.5
CV (%)	8.8	9.9	18.7	6.2	10.5	13.9	12.0	17.4	12.5	10.3
F ratio	0.36	2.99**	0.89	1.04	4.16**	0.47	2.26*	2.38*	1.64	1.37
h^2 (plot basis)	0.00	0.29	0.00	0.01	0.39	0.00	0.20	0.22	0.11	0.07
h^2 (mean basis)	0.00	0.67	0.00	0.04	0.76	0.00	0.56	0.58	0.39	0.27

* significant at 0.05 and 0.01 levels of probability, respectively

Note: RCE 24, RCE 24, RP 6B, and RP 9A are field locations at ICRISAT-Patancheru (Andhra Pradesh); K02 and K03 indicate *kharif* 2002 and *kharif* 2003, respectively. BWL and HSR indicate field locations at CCS HAU RRS, Bawal and CCS HAU Hisar, respectively (Haryana); and DGR and NGR indicate field locations at RAU RRS Durgapura and RAU RRS Nagaur, respectively (Rajasthan)

environments RP 6B K03, PR 9A K02, RCE 24 K02 and Bawal K03, operational heritabilities less than 0.04 for effective plant stand indicate that management of the trials during stand establishment was very good. The calculated F ratio were observed to be significant only for the Hisar K03, Hisar K02 and Bawal K02 test environments where trial management during stand establishment of these over-sown trials appears to have been less than optimal (Table 31). ICMH 02005 achieved low ranks for entry mean effective plant stand in most of the test environments and was often closely followed by HHB 181.

4.8.4. Plant height (cm)

Trials in the Patancheru RP 9A K02, Patancheru RCE 24 K02, Hisar K03, Hisar K02, Bawal K02 and Durgapura K03 test environments had tall entries having a maximum height equal to or greater than 200 cm. Nagaur K03 and RCE 24C K03 recorded a minimum entry mean plant heights of 157 cm and 150 cm, respectively. All four individual test environments at Patancheru as well as the Hisar K03 trial had high entry mean-basis operational heritability estimates >0.85 for this trait, while in other test environments these values were moderately high, with a minimum of 0.39 in the Durgapura K03 test environment (Table 32). Significant values for F ratios were recorded for plant height in all test environments except Bawal K02 and Durgapura K03. Lower plant height was observed among HHB 94-like hybrids produced on sub-selections of ICMB 90111 at most of the test environments and for hybrid HHB 94-P5A in the Bawal K02 and Bawal K03 test environments (Appendix 3).

4.8.5. Panicle length (cm)

HHB 94-like hybrids produced larger panicles at Bawal K02, Bawal K03, Hisar K02, Hisar K03, and Durgapura K03 with trial mean panicle lengths more than 20 cm while RP 9A K02 recorded the lowest trial mean panicle length of 16.8 cm and the lowest operational heritability (0.56) for this trait. Higher operational heritability estimates (>0.86) were recorded for this trait at all individual test environments in Haryana, at Durgapura K03 and at all individual test environments at Patancheru (A.P.) except RP 9A K02 (0.56). Table 33 revealed significant differences as indicated by for F ratios of all ten individual test environments. The three hybrids of sub-selections of ICMB 90111 registered the greatest panicle lengths in most of these test environments. HHB 94-P2B

Table 32. ANOVA summary for plant height (cm) from HHB 94-like hybrids trial conducted in ten individual test environments

Trait	Plant height (cm)									
Test environment	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	BWL K02	BWL K03	HSR K02	HSR K03	DGR K03	NGR K03
Mean	195	164	192	170	194	182	208	204	199	165
Minimum	188	150	182	160	186	172	201	184	190	157
Maximum	207	177	207	190	200	188	214	212	207	174
SE (+/-)	2.7	3.1	2.6	3.4	3.6	3.2	2.5	2.7	3.4	3.0
CV (%)	3.1	4.2	3.1	4.5	4.2	3.9	2.7	3.0	3.8	4.1
F ratio	7.74**	6.47**	11.99**	8.94**	1.31	2.27*	2.84**	8.85**	1.88	2.85**
h^2 (on plot basis)	0.57	0.52	0.69	0.61	0.06	0.20	0.27	0.61	0.15	0.27
h^2 (on mean basis)	0.87	0.85	0.92	0.89	0.23	0.56	0.65	0.89	0.47	0.65

Table 33. ANOVA summary for panicle length (cm) from HHB 94-like hybrids trial conducted in ten individual test environments

Trait	Panicle length (cm)									
Test environment	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	BWL K02	BWL K03	HSR K02	HSR K03	DGR K03	NGR K03
Mean	17.2	18.6	16.8	18.7	21.3	20.8	20.1	21.7	20.4	18.0
Minimum	15.6	17.3	15.9	17.0	18.6	18.1	18.1	19.0	17.5	16.0
Maximum	18.5	19.6	17.7	19.8	23.9	22.6	21.4	23.2	22.0	19.5
SE (+/-)	0.4	0.3	0.4	0.3	0.5	0.4	0.3	0.4	0.3	0.6
CV (%)	5.5	3.1	4.9	4.2	4.8	4.1	3.1	4.0	3.7	7.7
F ratio	6.91**	10.11**	2.26*	7.77**	16.66**	20.15**	20.60**	14.33**	26.87**	2.76**
h^2 (on plot basis)	0.54	0.65	0.20	0.58	0.76	0.79	0.80	0.73	0.84	0.26
h^2 (on mean basis)	0.86	0.90	0.56	0.87	0.94	0.95	0.95	0.93	0.96	0.64

Table 34. ANOVA summary for panicle diameter (mm) from HHB 94-like hybrids trial conducted in ten individual test environments

Trait	Panicle diameter (mm)									
Test environment	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	BWL K02	BWL K03	HSR K02	HSR K03	DGR K03	NGR K03
Mean	24.3	24.7	24.4	24.6	24.5	25.9	20.1	26.1	25.3	23.1
Minimum	23.3	24.0	23.5	24.0	21.8	24.4	18.1	23.6	23.9	20.2
Maximum	25.9	25.6	25.6	25.8	28.0	29.7	21.4	29.5	27.3	26.9
SE (+/-)	0.4	0.3	0.5	0.3	0.6	0.5	0.3	0.3	0.5	0.8
CV (%)	3.9	3.1	4.4	2.4	5.1	4.5	3.1	2.8	4.4	7.5
F ratio	2.75**	2.65*	2.11*	6.66**	12.61**	11.74**	20.60**	31.73**	4.84**	7.56**
h^2 (on plot basis)	0.26	0.25	0.18	0.53	0.70	0.68	0.80	0.86	0.43	0.57
h^2 (on mean basis)	0.64	0.62	0.53	0.85	0.92	0.92	0.95	0.97	0.79	0.87

* ** significant at 0.05 and 0.01 levels of probability, respectively

Note: RCE 24, RCE 24, RP 6B, and RP 9A are field locations at ICRISAT-Patancheru (Andhra Pradesh); K02 and K03 indicate *kharif* 2002 and *kharif* 2003, respectively. BWL and HSR indicate field locations at CCS HAU RRS, Bawal and CCS HAU Hisar, respectively (Haryana); and DGR and NGR indicate field locations at RAU RRS Durgapura and RAU RRS Nagaur, respectively (Rajasthan)

and HHB 181 had the largest entry-mean panicle lengths in the RP 9A K02 and Hisar K03 test environments, respectively (Appendix 3).

4.8.6. Panicle diameter (mm)

The mean panicle diameter of hybrids in this trial ranged from 20.1 mm in the trial conducted at Hisar in *kharif* 2002 to 26.1 mm in the trial conducted at this same location in *kharif* 2003. The four individual test environments in Haryana recorded higher operational heritability values for this trait (0.92 to 0.95). Moderate operational heritability values calculated most broadly on an entry-mean basis ranging from 0.85 and 0.87 were observed at RP 6B K03 and Nagaur K03, respectively, while the minimum value of 0.53 was observed for the PR 9A K02 test environment. F values were significant for this trial in all ten test environments (Table 34). HHB 94-like hybrids based on ICMA 89111, including both controls, were ranked higher for panicle diameter than these based on ICMB 90111 across individual test environments (Appendix 3) except for Hisar K02.

4.8.7. Fresh straw yield (g/m^2)

In the most of the test environments in Andhra Pradesh and Haryana fresh straw yields were high with trial means $\geq$1502 g/m^2. However, the two Rajasthan test environments exhibited much lower values for this trait, with Nagaur K03 having the minimum trial mean value (714 g/m^2). HHB 94-like hybrids recorded the highest entry-mean-based operational heritability value at RP 6B K03 (0.90) for this trait. RP 9A K02, Nagaur K03 and Durgapura K03 had operational heritability values of zero for this trait (Table 35), so no differences in entry-means were detectable in these test environments. For the other test environments, moderate operational heritability values were observed for this trait. F ratios were non-significant for fresh straw yield at RP 9A K02, Nagaur K03, Bawal K03 and Durgapura K03. Entry mean data (Appendix 3) shows lower ranks for fresh straw yield for HHB 94-P6A and the two control entries at a majority of test environments. Hybrids based on ICMB 90111 (ICMH 02002, ICMH 02005 and ICMH 02006) produced lower fresh straw yields and so had higher ranks.

4.8.8. Dry straw yield (g/m^2)

Variation in methods used to dry straw samples prior to estimation of dry straw yields resulted in dramatically different estimates for the four test environments at ICRISAT-Patancheru (where over-drying of sub-samples was used to determine straw moisture contents, which was then used to calculate dry stover yields from fresh stover yields) and the remaining six test environments where stover samples were weighed after several weeks of sun-drying in the field in order to estimate dry stover yields (Table 36). These procedures resulted in low trial mean dry stover yields at Patancheru, with moderate operational heritability estimates for this trait. The remaining six test environments could be divided into two groups–those in Haryana, which had very high trial means and moderate operational heritability estimates, and those in Rajasthan, which had low trial means, operational heritabilities of zero, and no significant differences between entries. Across test environments, the version of HHB 94 produced on ICMA 89111-P6 was consistently among the best in this trial for dry straw yield while experimental hybrids produced on sub-selections of ICMB 90111 were often among the worst (Appendix 3).

4.8.9. Moisture content (%)

For reasons described in the previous paragraph trial mean straw moisture content values were higher for all the four individual test environments at Patancheru all >70% (except RCE 24C K03, 60.5%) than for most of the other six test environments (all except Nagaur K03, 70%). Lower mean values were observed for this trait in all Haryana test environments and for Durgapura K03 where it ranged between 52% and 56% due to relative ineffective sun-drying. Entry mean based operational heritability values were highest for the trial conducted at Hisar K02 (0.81) and lowest for Durgapura K02 (0.00). Moderately high operational heritability was recorded for this trait in the rest of the test environments (Table 37). Three test environments namely Hisar K03, Bawal K03 (both from Haryana test environment cluster) and Durgapura K03 (Rajasthan) had non-significant F ratios for this trait and there are the lowest operational heritabilities and among the least trial mean values for this trait. Hybrid ICMH 02006 was consistent among the entries with the highest straw moisture content in most test environments for this trait followed by ICMH 02002 and HHB 181 for this trait. HHB 94-P6B had poorer ranking orders for straw moisture content in most test environments (Appendix 3).

Table 35. ANOVA summary for fresh straw yield (g/m^2) from HHB 94-like hybrids trial conducted in ten individual test environments

Trait	Fresh straw yield (g/m^2)									
Test environment	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	BWL K02	BWL K03	HSR K02	HSR K03	DGR K03	NGR K03
Mean	2647	1286	1795	1502	1695	1717	2033	1703	1040	714
Minimum	2425	1118	1648	1253	1472	1361	1747	1072	908	632
Maximum	2919	1424	1962	2000	1917	2196	2564	2088	1129	793
SE (+/-)	106.4	63.5	94.8	69.2	80.6	156.8	144.8	115.9	96.7	68.2
CV (%)	9.0	11.0	11.8	10.3	10.6	20.4	15.9	15.2	20.8	21.4
F ratio	2.75**	2.19*	0.99	10.45**	2.24*	1.99	3.01**	5.51**	0.50	0.61
h^2 (on plot basis)	0.26	0.19	0.00	0.65	0.20	0.17	0.29	0.47	0.00	0.00
h^2 (on mean basis)	0.64	0.54	0.00	0.90	0.55	0.50	0.67	0.82	0.00	0.00

Table 36. ANOVA summary for dry straw yield (g/m^2) from HHB 94-like hybrids trial conducted in ten individual test environments

Trait	Dry straw yield (g/m^2)									
Test environment	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	BWL K02	BWL K03	HSR K02	HSR K03	DGR K03	NGR K03
Mean	641	456	514	439	803	740	980	774	470	212
Minimum	547	390	457	349	642	544	784	533	419	182
Maximum	805	547	592	566	947	959	1357	915	549	240
SE (+/-)	36.7	27.1	26.5	22.9	49.9	64.3	91.9	61.0	46.0	22.9
CV (%)	12.8	13.3	11.5	11.7	13.9	19.4	21.0	17.6	21.9	24.1
F ratio	3.73*	3.10**	2.38*	9.37**	3.33**	2.71**	4.49**	3.26**	0.75	0.51
h^2 (on plot basis)	0.35	0.30	0.22	0.63	0.32	0.26	0.41	0.31	0.00	0.00
h^2 (on mean basis)	0.73	0.68	0.58	0.89	0.70	0.63	0.78	0.69	0.00	0.00

Table 37. ANOVA summary for moisture content (%) from HHB 94-like hybrids trial conducted in ten individual test environments

Trait	Straw moisture content (%)									
Test environment	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	BWL K02	BWL K03	HSR K02	HSR K03	DGR K03	NGR K03
Mean	75.8	64.5	71.3	70.7	52.8	56.7	52.3	54.3	54.4	70.4
Minimum	72.4	60.5	68.9	66.5	49.6	53.7	44.1	49.6	51.8	68.3
Maximum	78.6	67.1	72.8	75.3	56.7	61.0	57.9	58.3	57.4	71.3
SE (+/-)	1.1	1.4	0.8	1.2	1.5	2.2	1.7	2.2	2.2	0.6
CV (%)	3.2	4.8	2.6	3.9	6.5	8.9	7.4	9.0	9.1	1.9
F ratio	2.44*	2.62*	2.20*	3.45**	3.17**	1.14	5.37**	1.73	0.92	2.42*
h^2 (on plot basis)	0.22	0.25	0.19	0.33	0.30	0.03	0.47	0.13	0.00	0.22
h^2 (on mean basis)	0.59	0.62	0.55	0.71	0.68	0.12	0.81	0.42	0.00	0.59

* ** significant at 0.05 and 0.01 levels of probability, respectively

Note: RCE 24, RCE 24, RP 6B, and RP 9A are field locations at ICRISAT-Patancheru (Andhra Pradesh); K02 and K03 indicate *kharif* 2002 and *kharif* 2003, respectively. BWL and HSR indicate field locations at CCS HAU RRS, Bawal and CCS HAU Hisar, respectively (Haryana); and DGR and NGR indicate field locations at RAU RRS Durgapura and RAU RRS Nagaur, respectively (Rajasthan)

4.8.10. Effective tiller number (tillers/m^2)

Highest numbers of effective tillers were obtained from HHB 94-like hybrid trial conducted at RP 6B K03 Patancheru (A.P.), which had entry mean ranging from 36 to 49 tillers/m^2 and a trial mean of 43.2 tillers/m^2. Hybrids in the Nagaur K03 test environment produced smallest effective numbers of tillers with a trial mean of 13.7 tillers/m^2. Moderate numbers of effective tillers, with a trial mean from 27.5 tillers/m^2 to 36.4 tillers/m^2 were observed. Operational heritability (on plot basis) values were high (0.62) in Hisar K02 test environment and zero at Durgapura K03. At other locations, moderate operational heritability values were observed for this trait. F-tests revealed significant differences among genotypes for all test environments except Bawal K02, Nagaur K03 and Durgapura K03 (Table 38). Appendix 3 shows higher rank orders for this traits across many test environments among hybrids produced on ICMA 89111 (including controls) or ICMB 89111 and lower ranks for this trait for hybrids produced on ICMB 90111, which suggests that ICMB 90111 could be used to improve the tillering ability of the ICMA/B 89111 and its hybrids.

4.8.11. Panicle yield (g/m^2)

Highest trial mean panicle yield was obtained from the HHB 94-like hybrid trial conducted at Hisar K02 (595 g/m^2) where entry means ranged from 516 g/m^2 to 839 g/m^2. The trial conducted at Nagaur recorded the minimum mean panicle yield of 171 g/m^2, with a range among entries of 140 g/m^2 to 190 g/m^2. Mean panicle yield ranged from 409 g/m^2 to 595 g/m^2 for individual test environments both in Haryana and Andhra Pradesh, but were lower in the two Rajasthan test environments (Table 39). The highest entry-mean basis operational heritability (0.82) was observed for this trait in a trial conducted at Patancheru (RCE 24 K02), and the lowest (0.00) from trial conducted in Rajasthan (Nagaur K03). Operational heritability calculated on plot basis also followed this pattern. Non-significant F-test values were obtained for panicle yield from trials conducted at RP 6B K03, Hisar K03, Nagaur K03, Bawal K03 and Durgapura K03. In most trial data sets, better ranks for panicle yield were observed for experimental hybrids ICMH 02002, ICMH 02005, ICMH 02006 (HHB 94-like hybrids based on sub-selection ICMB 90111) and for HHB 94-P2B, while hybrids based on ICMA 89111 displayed poorer ranks for this trait (Appendix 3). Again this suggests that ICMB 90111 could be used to improve the panicle yields of hybrids produced on ICMA/B 89111 with pollinator G 73/107.

Table 38. ANOVA summary for effective tiller number (tillers/m^2) from HHB 94-like hybrids trial conducted in ten individual test environments

Trait	Effective tiller number (tillers/m^2)									
Test environment	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	BWL K02	BWL K03	HSR K02	HSR K03	DGR K03	NGR K03
Mean	36.4	34.9	32.9	43.2	25.6	27.5	30.3	32.6	17.5	13.7
Minimum	32.0	27.1	28.6	36.4	24.0	21.6	26.0	28.0	15.2	12.0
Maximum	40.8	44.2	37.4	49.0	29.0	33.4	42.0	39.5	19.0	15.0
SE (+/-)	1.9	2.2	2.1	2.5	1.8	1.6	1.4	1.7	1.3	0.8
CV (%)	11.7	14.2	14.0	12.8	15.8	13.3	10.3	11.6	16.0	13.0
F ratio	2.57*	4.12**	2.37*	2.84**	1.22	6.61**	8.98**	3.43**	0.87	1.50
h^2 (on plot basis)	0.24	0.38	0.22	0.27	0.04	0.53	0.62	0.33	0.00	0.09
h^2 (on mean basis)	0.61	0.76	0.58	0.65	0.18	0.85	0.89	0.71	0.00	0.33

Table 39. ANOVA summary for panicle yield (g/m^2) from HHB 94-like hybrids trial conducted in ten individual test environments

Trait	Panicle yield (g/m^2)									
Test environment	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	BWL K02	BWL K03	HSR K02	HSR K03	DGR K03	NGR K03
Mean	574	520	476	548	503	409	595	409	311	171
Minimum	488	448	405	514	448	341	516	356	260	140
Maximum	633	593	524	581	567	462	839	498	355	190
SE (+/-)	21.2	26.8	17.9	19.4	25.7	35.4	42.1	31.5	22.9	23.6
CV (%)	8.3	11.5	8.4	7.9	11.4	19.3	15.8	17.3	16.4	30.7
F ratio	5.56**	2.25*	4.15**	1.40	2.84**	1.20	4.36**	1.79	1.84	0.36
h^2 (on plot basis)	0.48	0.20	0.39	0.07	0.27	0.04	0.40	0.14	0.14	0.00
h^2 (on mean basis)	0.82	0.56	0.76	0.28	0.65	0.17	0.77	0.44	0.46	0.00

Table 40. ANOVA summary for 1000-grain mass (g) from HHB 94-like hybrids trial conducted in ten individual test environments

Trait	1000- Grain mass (g)									
Test environment	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	BWL K02	BWL K03	HSR K02	HSR K03	DGR K03	NGR K03
Mean	8.74	8.48	8.70	8.48	6.26	8.14	7.71	6.80	8.11	6.82
Minimum	7.27	7.76	7.72	7.16	5.42	6.95	7.17	6.30	7.44	5.77
Maximum	9.44	9.29	9.54	9.47	6.85	8.78	8.40	7.50	8.56	7.54
SE (+/-)	0.24	0.30	0.30	0.23	0.35	0.33	0.25	0.26	0.28	0.34
CV (%)	6.07	7.83	7.61	5.97	12.37	9.09	7.18	8.59	7.78	11.28
F ratio	7.78**	2.48*	3.57**	10.63**	2.01*	2.27*	1.85	1.70	1.21	1.61
h^2 (on plot basis)	0.58	0.23	0.34	0.66	0.17	0.20	0.15	0.12	0.04	0.11
h^2 (on mean basis)	0.87	0.60	0.72	0.91	0.50	0.56	0.46	0.41	0.18	0.38

* ** significant at 0.05 and 0.01 levels of probability, respectively

Note: RCE 24, RCE 24, RP 6B, and RP 9A are field locations at ICRISAT-Patancheru (Andhra Pradesh); K02 and K03 indicate *kharif* 2002 and *kharif* 2003, respectively. BWL and HSR indicate field locations at CCS HAU RRS, Bawal and CCS HAU Hisar, respectively (Haryana); and DGR and NGR indicate field locations at RAU RRS Durgapura and RAU RRS Nagaur, respectively (Rajasthan)

4.8.12. 1000-grain mass (g)

All test environments in the Patancheru cluster, along with Bawal K03 and Durgapura K03 recorded mean 1000-grain mass (TGM) between 8.11 g to 8.74 g. However, Bawal K02, Hisar K03 and Nagaur K03 trials had mean TGM values of 6.26 g, 6.80 g and 6.82 g respectively, with a slightly higher value for the Hisar K02 (7.71 g) trial. High mean-basis operational heritability values were obtained for trials conducted at Patancheru (RP 6B K03 was highest, 0.91) for TGM, and these were higher than those obtained in Haryana (moderate, 0.40-0.50) and Rajasthan (low 0.18 to 0.38), as presented in Table 40. Significant differences among entries were recorded in the most test environments except Hisar K02, Hisar K03, Nagaur K03 and Durgapura K03. The hybrid produced on sub-selections of ICMB 90111 (ICMH 02002, ICMH 02005 and ICMH 02006) ranked among the best for TGM in most test environments while HHB 94-P5A and HHB 94-P5B ranked consistently poor (Appendix 3).

4.8.13. Panicle grain number (grains/panicle)

The highest trial mean panicle grain number (2046) was observed in the Bawal K02 test environment, but this trait was not heritable in that particular test environment. All individual test environments in the Patancheru test environment cluster recorded trial mean values between 1138 and 1486, while Nagaur K03 and Durgapura K03 had trial mean panicle grain numbers of 1247 and 1754, respectively (Table 41). Operational heritability values for panicle grain number were higher for Patancheru (A.P.) test environments than Haryana and Rajasthan. F-test indicated significant differences among trial entries across all locations except Bawal K02, Hisar K03 and Nagaur K03, where operational heritability for panicle grain number was very low. In many of the individual test environments HHB 94-P6A, HHB 94-P6B, and the original HHB 94 ranked best for panicle grain number, while in most test environments ICMH 02006 ranked worst for this trait (Appendix 3).

4.8.14. Total above-ground biomass yield (g/m^2)

Trial means for total above-ground biomass yield (TAGBY) ranged from 383 g/m^2 in the Nagaur K03 test environment to 1575 g/m^2 in the Hisar K02 test environment, but this trait was not heritable in three of the ten trials and poorly heritable in another two, so significant differences between entry means were detected for this trait in only half of the

Table 41. ANOVA summary for panicle grain number from HHB 94-like hybrids trial conducted in ten individual test environments

Trait	Panicle grain number									
Test environment	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	BWL K02	BWL K03	HSR K02	HSR K03	DGR K03	NGR K03
Mean	1486	1332	1367	1138	2046	1145	1534	1052	1754	1247
Minimum	1291	1160	1182	926	1726	879	1227	933	1474	1049
Maximum	1729	1603	1579	1437	2469	1380	1930	1209	2006	1398
SE (+/-)	87.6	78.9	76.5	69.7	241.8	94.5	133.1	85.0	119.1	93.6
CV (%)	13.2	13.2	12.5	13.7	26.4	18.5	19.4	18.1	15.2	16.8
F ratio	2.62*	2.79*	2.58*	4.59**	1.09	2.95**	2.32*	0.76	2.02*	1.22
h^2 (on plot basis)	0.24	0.26	0.24	0.42	0.02	0.28	0.21	0.00	0.17	0.04
h^2 (on mean basis)	0.62	0.64	0.61	0.78	0.08	0.66	0.57	0.00	0.50	0.18

Table 42. ANOVA summary for total above ground biomass (g/m^2) yield from HHB 94-like hybrids trial conducted in ten individual test environments

Trait	Total-above-ground-biomass yield (g/m^2)									
Test environment	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	BWL K02	BWL K03	HSR K02	HSR K03	DGR K03	NGR K03
Mean	1214	976	990	987	1305	1146	1575	1182	782	383
Minimum	1082	885	922	880	1102	933	1325	888	684	349
Maximum	1369	1083	1064	1108	1426	1332	2195	1362	884	425
SE (+/-)	49.4	49.6	40.9	37.8	69.4	86.2	126.9	86.5	60.7	45.2
CV (%)	9.1	11.4	9.2	8.6	11.9	16.8	18.0	16.4	17.4	26.4
F ratio	2.33*	1.78	1.00	2.73**	2.21*	1.67	4.56**	2.56*	1.02	0.20
h^2 (on plot basis)	0.21	0.14	0.00	0.26	0.20	0.12	0.42	0.24	0.00	0.00
h^2 (on mean basis)	0.57	0.44	0.00	0.63	0.55	0.40	0.78	0.61	0.02	0.00

Table 43. ANOVA summary for harvest index (%) from HHB 94-like hybrids trial conducted in ten individual test environments

Trait	Harvest index (%)									
Test environment	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	BWL K02	BWL K03	HSR K02	HSR K03	DGR K03	NGR K03
Mean	38.4	39.6	38.7	41.5	24.6	22.0	23.4	19.7	32.1	30.6
Minimum	33.7	33.0	33.1	33.7	19.8	15.5	18.8	16.0	25.0	24.4
Maximum	42.0	42.3	41.5	45.1	28.8	25.4	26.5	22.2	36.7	36.4
SE (+/-)	1.4	1.3	1.1	1.3	1.4	1.6	1.9	1.4	2.6	1.6
CV (%)	8.3	7.5	6.6	6.8	12.9	16.6	18.6	16.4	17.9	11.9
F ratio	5.10**	5.30**	5.72**	10.74**	3.85**	3.27**	1.56	1.72	1.67	5.65**
h^2 (on plot basis)	0.45	0.46	0.49	0.66	0.36	0.31	0.10	0.13	0.12	0.48
h^2 (on mean basis)	0.80	0.81	0.83	0.91	0.74	0.69	0.36	0.42	0.40	0.82

* ** significant at 0.05 and 0.01 levels of probability, respectively

Note: RCE 24, RCE 24, RP 6B, and RP 9A are field locations at ICRISAT-Patancheru (Andhra Pradesh); K02 and K03 indicate *kharif* 2002 and *kharif* 2003, respectively. BWL and HSR indicate field locations at CCS HAU RRS, Bawal and CCS HAU Hisar, respectively (Haryana); and DGR and NGR indicate field locations at RAU RRS Durgapura and RAU RRS Nagaur, respectively (Rajasthan)

test environments (Table 42). No consistent trends in relative performances of the trial entries could be detected across the ten test environments (Appendix 3).

4.8.15. Harvest index (%)

Trial mean harvest index values ranged between 38.4% and 41.5% in the Andhra Pradesh test environments, which were moderately higher than those observed in the two Rajasthan test environments (30.6% and 32.1%) and markedly higher than those observed for the four Haryana test environments (19.7% to 24.6%). All individual test environments at Patancheru recorded high operational heritabilities for harvest index ranging between 0.80-0.90, followed closely by Nagaur K03 (0.82) and Bawal (0.74 in K02 and 0.69 in K03). Hisar K02 (0.36) had the lowest operational heritability for this trait. Harvest index recorded significant differences among genotypes in all test environments except Hisar K03, Hisar K02 and Durgapura K03 (Table 43). Hybrid ICMH 02005, ICMH 02006 and ICMH 02002 had the best ranks for mean HI in most of the individual test environments. In contrast to this, the ICMA/B 89111-based hybrids, including the two checks, had poorer ranks in most of these trials (Appendix 3). This suggests that it should be possible to use ICMB 90111 as a donor parent to improve the harvest index of ICMB 89111-based hybrids.

4.9. CHARACTER ASSOCIATIONS

4.9.1. Grain yield

Correlation studies among quantitative traits provides knowledge of the intensity of linkage and pleiotropic effects of genes controlling these traits, and thus help the pearl millet breeder in assessing the feasibility of joint selection for two or more than two traits and evaluating the effect of selection for secondary trait(s) on genetic gain in the primary trait. The replicated mean data from ten locations across three regions of the country (Andhra Pradesh, Haryana and Rajasthan) was computed and Spearman rank correlations were calculated using Genstat version 6.0. Grain yield was taken as the dependent variable and all other traits were correlated with it. Fresh straw yield, straw moisture content, effective tillers number, panicle yield, 1000-grain mass, panicle grain number, total above-ground biomass yield, and harvest index had significant positive correlations with grain yield. Among these traits, panicle yield (0.885) followed by effective tiller number (0.778), harvest index and 1000-grain mass recorded very strong and highly

significant associations with grain yield (Table 44). At the same time, traits like time to 50% flowering, plant height, panicle length and panicle diameter were negatively correlated with grain yield. Among these traits time to 50% flowering (–0.486) registered the highest significant negative correlation followed by panicle length (–0.306) and panicle diameter (–0.221). Effective plant stand and dry straw yield were found to have positive but non-significant relationships with grain yield.

4.9.2. Dry straw yield

Time to 50% flowering, plant height, panicle length, fresh straw yield, effective tiller number, panicle yield, 1000-grian mass and total above-ground biomass yield exhibited positive correlations with dry straw yield. Amongst all positively correlated traits, total above-ground biomass yield (0.826) followed by fresh straw yield (0.758) and plant height (0.715) revealed highly significant association with dry straw yield (Table 44). On the other hand, only harvest index showed a significant negative correlation (<0.05 probability level), and other traits like grain yield, effective plant stand, panicle diameter, straw moisture content and panicle grain number displayed a positive but non significant associations with dry straw yield.

4.9.3. Harvest index

Grain yield (0.651), straw moisture content (0.751), effective tiller number (0.472), panicle yield (0.352), and 1000-grain mass (0.680) not only had positive correlations but some of them also had highly significant associations with harvest index (Table 44). In contrast to this, time to 50% flowering (–0.546), plant height (–0.524), panicle diameter (–0.182) panicle length (–0.490), dry straw yield (–0.678) and total above-ground biomass yield (–0.446) were found to have significant negative relationships with harvest index. Non-significant correlations were observed for effective plant stand and panicle grain number with harvest index.

4.9.4. Time to 50% flowering

Significantly positive associations were observed for plant height, panicle diameter, fresh and dry straw yield with time to 50% flowering (Table 44). Effective plant stand, straw moisture content and total above-ground biomass yield showed positive yet non-significant correlations with time to 50% flowering. Very highly to moderately significant

Table 44. Spearman rank correlations for grain and stover yield and their component traits computed from ranks of across-location entry means among eleven HHB 94-like hybrids

	GY	FT	EPS	PH	PL	PD	FSY	DSY	SMC	ETN	PY	1000-GM	PGN	TAGBY	HI
GY	1														
FT	0.486**	1													
EPS	0.148	0.104	1												
PH	-0.035	0.208**	-0.155	1											
PL	-0.306**	-0.014	0.170*	0.216**	1										
PD	-0.221**	0.210**	-0.023	0.064	-0.020	1									
FSY	0.517**	0.206*	0.125	0.560**	-0.106	-0.031	1								
DSY	0.080	0.249**	0.137	0.715**	0.345**	0.071	0.758**	1							
SMC	0.411**	0.024	-0.038	-0.445**	-0.617**	-0.115	0.065	-0.561**	1						
ETN	0.778**	-0.273**	0.348**	-0.127	-0.113	-0.035	0.445**	0.061	0.381**	1					
PY	0.885**	-0.421**	0.287**	0.097	-0.063	-0.343**	0.605**	0.344**	0.154	0.719**	1				
1000-GM	0.639**	-0.224**	0.146	-0.263**	-0.339**	-0.038	0.172	-0.275**	0.511**	0.528**	0.396**	1			
PGN	0.175*	-0.360**	-0.332**	0.400**	-0.061	-0.125	0.130	0.223**	-0.229**	-0.377**	0.159	-0.144	1		
TAGBY	0.348**	0.097	0.246**	0.577**	0.269**	-0.072	0.826**	0.930**	-0.371**	0.293**	0.622**	-0.139	0.198*	1	
HI	0.651**	-0.546**	-0.030	-0.524**	-0.490**	-0.182*	-0.182*	-0.678**	0.717**	0.472**	0.352**	0.680**	0.031	-0.446**	1

* and **, significant at 0.05 and 0.01 probability levels, respectively

GY - grain yield (g/m^2), FT - time to 50 % flowering, EPS - effective plant stand (plants/m^2), PH - plant height (cm), PL - panicle length (cm), PD - panicle diameter (mm), FSY - fresh straw yield (g/m^2), DSY - dry straw yield (g/m^2), SMC - straw moisture content (%), ETN - effective tiller number (tillers/m^2), PY - panicle yield (g/m^2), 1000-GM - thousand grain mass (g), PGN - panicle grain number, TAGBY - total above-ground biomass yield (g/m^2), HI - harvest index (%)

negative associations of time to 50% flowering was registered with harvest index (–0.546), grain yield (–0.486), panicle yield (–0.421), 1000-grain mass (–0.224), panicle grain number (–0.360) and effective tiller number (–0.273).

4.10. YIELD QTL POTENTIAL FROM MULTILOCATION TRIALS

Eleven HHB 94–like hybrids and control entries, developed by pollination of three different types sub-selections (ICMA 89111, ICMB 90111 and ICMB 89111) with a common pollinator G 73/107, were subjected to various tests based on the replicated mean data for grain yield from multilocation trials depending upon the genetic background of female parents as presented in Table 45.

4.10.1. Pair wise comparisons between HHB 94-like hybrids based on their mean performance

4.10.1.1. Test of cytoplasmic effects on grain yield within the HHB 94 background

The ANOVA to test the significant of cytoplasmic effects on grain yield in HHB 94 background demonstrated little significance differences in grain yield due to use of the A_1 male-sterility inducing cytoplasm of the A-lines compared with the normal male-fertile cytoplasm of the B-lines. Majority of locations and their state-wise multiple-test environment clusters did not reveal any significant differences in mean grain yield between sub-selections of two cytoplasms based on ICMA 89111 and ICMB 89111 except from a trial conducted at Bawal K02 and Durgapura K03. Markedly significant differences in grain yield were observed between the hybrid versions HHB 94-P6A (ICMA 89111-P6) and HHB 94-P6B (ICMB 89111-P6) at Bawal K02 (Table 45-46). Other HHB 94-like hybrids, HHB 94-P2A and HHB 94-P5A differed significantly from HHB 94-P2B and HHB 94-P5B in a trial conducted at Durgapura during *kharif* 2003, indicating the variation in the grain yield performance among these hybrids based on these two different cytoplasm sources.

4.10.1.2. Tests of effects of selection for downy mildew reaction within ICMA/B 89111 on HHB 94 grain yield performance

HHB 94-like hybrids trials conducted at Durgapura during *kharif* 2003 indicated significant variation in mean grain yield between HHB 94-like hybrids developed from various sub-selections of male sterile line ICMA 89111 and HHB 94-original, using a

Table 45. Mean performance of HHB 94-like hybrids for grain yield (GY, g/m^2)) at individual test environments as well as across state-wise clusters of test environments, and across all ten test environments during *kharif* 2002 and 2003

Tr. No.	Entry	BWL K02	BWL K03	HSR K02	HSR K03	HRY	DGR K03	NGR K03	RAJ	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	AP	All
1	HHB 94-P2A	320.6	247.5	383.8	269.5	305.3	200.8	125.6	163.2	413.0	413.0	370.1	439.8	427.8	325.9
2	HHB 94-P5A	330.0	303.3	315.6	278.3	306.8	178.4	130.0	154.2	403.2	403.2	420.4	423.1	435.3	327.7
3	HHB 94-P6A	365.6	247.7	357.8	221.4	298.1	228.0	141.6	184.8	376.9	376.9	403.4	423.6	423.0	325.4
4	HHB 94-Original	361.7	296.7	329.7	248.6	309.2	278.8	118.1	198.4	382.3	382.3	396.6	417.2	426.0	333.7
5	ICMH 02002	328.3	213.4	324.1	242.8	277.2	240.6	102.0	171.3	388.1	388.1	379.3	358.8	397.3	304.0
6	ICMH 02005	283.9	213.9	297.8	198.4	248.5	257.1	108.1	182.6	315.8	315.8	321.6	395.2	357.5	278.9
7	ICMH 02006	263.3	206.0	453.1	208.5	282.7	271.6	89.3	180.4	371.4	371.4	334.4	372.8	369.4	296.9
8	HHB 94-P2B	308.3	266.5	294.7	273.6	285.8	276.2	119.2	197.7	391.3	391.3	398.7	414.2	415.7	320.1
9	HHB 94-P5B	308.9	267.2	365.6	234.8	294.1	256.7	131.7	194.2	351.5	351.5	387.4	403.5	402.7	317.6
10	HHB 94-P6B	271.1	278.6	368.8	206.4	281.2	260.1	105.5	182.8	407.2	407.2	376.1	392.3	400.8	309.4
11	HHB 181(Control)	367.2	235.8	480.7	178.9	315.7	295.2	134.6	214.9	445.7	445.7	420.6	429.4	453.9	350.8

Note: RCE 24, RCE 24, RP 6B, and RP 9A are field locations at ICRISAT-Patancheru (Andhra Pradesh); K02 and K03 indicate *kharif* 2002 and *kharif* 2003, respectively. BWL and HSR indicate field locations at CCS HAU RRS, Bawal and CCS HAU Hisar, respectively (Haryana); and DGR and NGR indicate field locations at RAU RRS Durgapura and RAU RRS Nagaur, respectively (Rajasthan); AP and HRY indicate mean performance across four test environments each in Andhra Pradesh and Haryana respectively; RAJ indicates mean performance across two test environments in Rajasthan; All indicates mean performance across all ten test environments in Andhra Pradesh, Haryana and Rajasthan; HSD – honestly significant difference

common pollinator G73/107. Significant differences were also noticed between average of three hybrids based on sub-selections (-P2A, -P5A and -P6A) and HHB 94 original at Durgapura, confirming the effectiveness of selection with in genetic background of ICMA/B 89111 (Table 46).

4.10.1.3. Comparison between HHB 94 and HHB 181 (control entries)

Two hybrid control entries (HHB-94 and HHB 181) produced with a common male-sterile line (ICMA 89111) and different pollinators (G 73/107 and H 77/833-2) were compared for mean grain yield across ten test environments. Significant differences were recorded for mean grain for these two hybrids from trials conducted at Bawal K02 (Haryana test environment cluster), Durgapura K03 (Rajasthan test environment cluster), RP 9A K02, (Andhra Pradesh test environment cluster), and pooled entry means across all ten test environment cluster (Table 46).

4.10.1.4. Potential of ICMB 90111 to improve grain yield of HHB 94 (tested in normal fertile cytoplasm)

A test of grain yield QTL potential in cytoplasmic background of ICMB 90111 was conducted by subtracting the average grain yield of all three HHB 94-like hybrids based on ICMB 89111 from average grain yield of all three hybrids based on ICM 90111. Strikingly high differences were observed from multilocation trials at Bawal K03, Durgapura K03, Patancheru (Andhra Pradesh test environment cluster) and pooled across all ten test environments. The marked difference obtained is because of cytoplasm indicating possibility of potential yield QTLS in ICMB 90111 to improve HHB-94 (Table 46).

4.10.2. Pair-wise comparisons between HHB 94-like hybrids based on ANOVA

Analysis of variance was performed based on replicated yield data for comparing pairs of HHB 94-like hybrids based on different sub-selections of ICMA 89111, ICMB 89111 and ICMB 90111 used a female lines, at single degree of freedom contrasts using Genstat version 6.0 (Table 47). The results of comparison of mean performance of grain yield (from multilocation trials) were confirmed by tests of contrast at single degree of freedom.

Table 46. Comparisons as single degree of freedom contrasts based on mean performance of grain yield for individual test environments as well as across state-wise test environment clusters, and across all ten test environments in Haryana, Rajasthan and Andhra Pradesh during *kharif* 2002 and 2003

Test of cytoplasmic effect on GY within HHB 94 background

Comparisons of HHB 94-like hybrids	BWL K02	BWL K03	HSR K02	HSR K03	HRY	DGR K03	NGR K03	RAJ	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	AP	All
TR1 – TR8	12.2	-19.0	89.1	-4.1	19.6	-75.4*	6.4	-34.5	21.7	21.7	-28.6	25.5	12.1	5.8
TR2 – TR9	21.1	36.1	-50.0	43.5	12.7	-78.3*	-1.7	-40.0	51.7	51.7	33.0	19.6	32.7	10.1
TR3 – TR10	94.4*	-30.9	-11.0	15.0	16.9	-32.1	36.1	2.0	-30.3	-30.3	27.3	31.3	22.2	16.0
Mean (1,2,3) – Mean (8,9,10)	42.6	-4.6	9.4	18.1	16.4	-61.9	13.6	-24.2	14.3	14.3	10.6	25.5	22.3	10.6

Test effect of selection within ICMA 89111 for downy mildew disease resistance on grain yield performance

TR1 – TR4	-41.1	-49.2	54.1	20.9	-3.8	-78.0*	7.5	-35.3	30.7	30.7	-26.5	22.6	1.8	-7.8
TR2 – TR4	-31.7	6.6	-14.1	29.7	-2.3	-100.4*	11.9	-44.3	20.9	20.9	23.8	5.9	9.4	-6.0
TR3 – TR4	3.9	-48.9	28.1	-27.2	-11.0	-50.8	23.5	-13.6	-5.4	-5.4	6.8	6.4	-2.9	-8.3
Mean (1,2,3) –TR (4)	-22.9	-30.5	22.7	7.8	-5.7	-76.4*	14.3	-31.1	15.4	15.4	1.4	11.6	2.8	-7.4

Comparison between HHB 94 and HHB 181

TR11 – TR4	5.6	-60.9	151.0*	-69.7*	6.5	16.4	16.6	16.5	63.4*	63.4	24.0	12.3	27.9	17.1

Yield potential in ICMB 90111 to improve HHB 94 (tested in the normal fertile cytoplasm)

Mean (5,6,7) – Mean (8,9,10)	-3.4	-142.2*	10.6	-36.2	-42.8	-204.7*	-31.4	-118.1*	-20.2	-39.8	-55.6*	-33.8	-37.4*	-55.7*
SE (+/-)	19.43	26.90	43.70	22.23	20.61	23.56	16.31	27.58	20.93	22.83	16.37	15.98	12.62	16.59
LSD	55.53	76.90	126.20	63.55	57.50	67.34	46.62	77.43	59.82	65.27	46.80	45.69	35.20	46.10

Note: RCE 24, RCE 24, RP 6B, and RP 9A are field locations at ICRISAT-Patancheru (Andhra Pradesh); K02 and K03 indicate *kharif* 2002 and *kharif* 2003, respectively. BWL and HSR indicate field locations at CCS HAU RRS, Bawal and CCS HAU Hisar, respectively (Haryana); and DGR and NGR indicate field locations at RAU RRS Durgapura and RAU RRS Nagaur, respectively (Rajasthan); All indicates mean performance across all ten test environments in Andhra Pradesh, Haryana and Rajasthan

4.10.2.1. Test of significance of cytoplasmic effect on grain yield within HHB 94 background

ANOVA for test of significance of cytoplasmic effect on grain yield within HHB 94 background demonstrated no significance differences in grain yield in most of the test environments except Bawal, Durgapura K03, and the cluster at Patancheru (Andhra Pradesh), confirming that there was no cytoplasm effect on grain yield. The comparison made between two HHB 94-like hybrids based on sub-selections, ICMB 89111-P6 and ICMB 90111-P6 exhibited remarkably significant differences in grain yield at Bawal K03 (at $p>0.01$, level of significance). Even further to this, significance differences were also recorded for grain yield comparison made between average performances of the three hybrids, HHB 94 -P2, -P5 and -P6 and hybrid based on sub-selections of ICMA 89111 and ICMB 89111 backgrounds at Bawal K02, Durgapura K03, and Multiple-test environment cluster Patancheru. HHB 94-P2A and HHB 94-P5A also differed from HHB 94-P2B and HHB 94-P5B respectively at Durgapura K03 exhibiting variation in grain yield due to cytoplasmic differences.

4.10.2.2. Test of selections within ICMB 89111

Analysis of variance for test of selections within ICMB 89111 at single degree of freedom between HHB 94-like hybrids based on sub-selections within ICMA 89111 background showed no significant differences in grain yields at any of the locations except at Durgapura. Not only this, the average performances of hybrids from three sub-selections (HHB 94-P2A, -P5A and -P6A) when tested against the original version of HHB 94-like hybrids reveled no remarkable differences at any of the individual or group of test environments except Durgapura K03.

4.10.2.3. Comparison between the two control entries hybrids, HHB 94 and HHB 181

ANOVA of the pair-wise comparison between two control hybrids HHB 94 and HHB 181, developed by pollinating male-sterile line ICMA 89111 with different restorer lines (G 73/107 and H 77/833-2, respectively), revealed significant differences only in trials conducted in three test environments: Hisar K03, RP 9A K02, and RP 6B K03 (Table 47).

Table 47. Analysis of variance of pair-wise comparisons based on replicated yield data at single degree of freedom contrasts for individual test environments as well as across state-wise test environment clusters, and across all ten test environments

Source of variation	Df	BWL K02	BWL K03	HSR K02	HSR K03	HRY	DGR K03	NGR K03	RAJ	RCE 24 K02	RCE 24 K03	RP 9A K02	RP 6B K03	AP	All
Replication	4	24238		17555	9252	2193	12059	4697	5842	4928	1996	8193	714	2102	2707
Treatment	10	6682**	5565	14486	5467*	6868	6171*	1275	2957	5746*	8574**	5020**	3066*	16086**	18513
Test effect of selection within ICMA 89111 for downy mildew disease resistance on grain yield performance															
TR1-TR8	1	373	904	15864	41	2399	14196*	103	5940	1174	2231	2045	1630	1469	407
TR2-TR9	1	1114	3254	5000	4720	2419	15327*	8	8010	6695	1728	2728	954	10662	3154
TR3-TR10	1	22299**	2387	239	562	3204	2573	3248	20	2300	9138	1872	2449	4927	6739
Mean (1,2,3) - Mean (8,9,10)}	1	13606*	160	527	2464	7985	28748**	1381	8764	1547	11334*	843	4860	14949*	8366
Test effect of selections within 89111															
TR1-TR4	1	4225	6046	5846	1094	449	15221*	142	6210	2351	938	1756	1277	34	2030
TR2-TR4	1	2507	109	396	2209	29	25226**	355	9798	1094	425	1416	87	882	845
TR3-TR4	1	38	5984	1582	1849	1627	6451	1386	929	73	947	117	102	85	2004
Mean (1,2,3) - TR (4)	1	1977	3488	1547	229	745	21903**	770	7229	888	1121	7	508	116	2356
Comparison between HHB 94 and HHB 181															
TR11-TR4	1	136	9257	1407	12131*	11	674	2707	1362	4651	7554	13399**	5792*	7795	5038
Yield potential in ICMB 90111 to improve HHB 94 (tested in the normal fertile cytoplasm)															
Mean (5,6,7) - Mean (8,9,10)	1	77	26710**	45602*	3533	10631	467	688	2712	10041*	360	1444	376	30097**	39428
Error		1887	3619	7631	2472	8074	2775	1330	7606	2190	2607	1340	1277	3188	13494

Note: RCE 24, RCE 24, RP 6B, and RP 9A are field locations at ICRISAT-Patancheru (Andhra Pradesh); K02 and K03 indicate *kharif* 2002 and *kharif* 2003, respectively. BWL and HSR indicate field locations at CCS HAU RRS, Bawal and CCS HAU Hisar, respectively (Haryana); and DGR and NGR indicate field locations at RAU RRS Durgapura and RAU RRS Nagaur, respectively (Rajasthan); All indicates mean performance across all ten test environments in Andhra Pradesh, Haryana and Rajasthan; df - degree of freedom

4.10.2.4. Potential of ICMB 90111 to improve grain yield of HHB 94 (tested in normal fertile cytoplasm)

The average grain yield performance of three HHB 94-like hybrids (HHB 94-P2B, HHB 94-P5B and HHB 94-P6B) produced by crossing pollinator G 73/107 on sub-selections of ICMB 89111 was tested against the average of three HHB 94-like hybrids onto sub-selections of ICMB 90111 (ICMH 02002, ICMH 02005, ICMH 02006) (Table 46 and 47). ANOVA for these single-degree of freedom comparisons showed significant differences only at Bawal K03, Hisar K02, and RCE 24C K02, and for Andhra Pradesh test environment cluster. Results were inconsistent but in general the versions of HHB 94 tended to be marginally out-yield their counterpart produced on sub-selections of ICMB 90111 (Table 47).

CHAPTER 5

DISCUSSION

5. DISCUSSION

5.1. DOWNY MILDEW SCREENING

5.1.1. Downy mildew inheritance studies

Downy mildew is a menace to pearl millet production in SAT regions of the world. It causes devastating losses and last century has witnessed pearl millet downy mildew epidemics many times in India. The parental lines ICMB 89111-P6 (susceptible to downy mildew) and ICMB 90111-P6 (resistant to downy mildew) and their $F_{2:4}$ mapping population progenies were screened against six Indian and two African pathogen populations of *S. graminicola* under greenhouse conditions in the present study. Parental line ICMB 90111-P6 was found to be highly resistant and exhibited no symptoms of infection against pathogen populations from Patancheru, Maiduguri and Bamako but it showed moderate susceptibility to those from Jamnagar, New Delhi, Durgapura, Jalna and Jodhpur. At the same time, parental line ICMB 89111-P6 recorded very high downy mildew incidence (DMI) in screens against pathogen populations from Durgapura, Jamnagar and Patancheru. Moderately high downy mildew disease incidence was observed on this parental line when screened against pathogen populations from Bamako, Maiduguri, Jodhpur and New Delhi.

Downy mildew susceptible control entry (7042S) exhibited a high degree of susceptibility across all of the pathogen populations used in this study ranging between 75% and 100% DMI. On the other hand, resistant control entry P1449-2 showed high levels of resistance against all six Indian pathogen populations used in this study. For rest the rest of the control entries (resistant and susceptible), variable downy mildew disease reactions were observed in screens across the various pathogen populations. This differential reaction of PT 732B is a clear case of pathogen population-specific host-plant resistance as described by van der Plank (1963, 1968), Day (1974) and many others.

The Mendelian segregation for downy mildew disease reaction among the 172 $F_{2:4}$ mapping population progenies when screened against pathogen populations from Patancheru, New Delhi, Durgapura (India) and Maiduguri and Bamako (Africa) gave good fits to 3 resistant : 1 susceptible (single dominant resistance gene) at natural DMI

breakpoints of 30%, 25%, 40%, 20% and 50% respectively, indicating role of single major dominant gene controlling a major portion of resistance to each of these pathogen populations from Jodhpur. But interestingly, the mapping population under study also segregated in a Mendelian segregation ratio of 1 resistant : 3 susceptible in the screen against the pathogen population from Jodhpur at breakpoint of 15% DMI (Table 9). In a previous study, Azhaguvel (2001) observed a resistant : susceptible segregation ratio 3:1 (monogenic dominant resistance) against a pathogen population from Jodhpur, and a 1:3 (monogenic recessive resistance) ratio against pathogen populations from Jamnagar (India) and Sadore, Niger (Africa). Appadurai *et al.* (1975) indicated the role of a single resistance dominant gene, which was supported by a non-significant χ^2 test for goodness of fit from F_2 segregating data to the 3:1 ratio. Furthermore, Dass *et al.* (1984), Thakur *et al.* (1992) and Singh (1995) also reported resistance to be dominant over susceptibility and largely controlled by one or a few major genes. Except in one case where resistance was reported to be recessive (Singh *et al.*, 1978), pearl millet downy mildew resistance has generally been observed to be dominant and variation in segregating populations is typically continuous (Singh *et al.*, 1993a).

Mendelian segregation among the 172 $F_{2:4}$ self-bulks for downy mildew disease reaction against the pathogen population from Jodhpur showed a best fit to the digenic ratio of 13:3 at the natural breakpoint of 55% DMI, indicating the presence of two dominant genes (one basic gene and one inhibitor gene). Similar segregation ratios were also obtained in DM screens against pathogen populations from Patancheru, Durgapura, Jamnagar and Bamako, Mali at natural breakpoints 40%, 50%, 50% and 60% DMI, respectively in the present study.

The 172 $F_{2:4}$ mapping population progenies screened against four Indian pathogen populations from (ICRISAT Patancheru; MAHYCO Jalna; JAU MRS Jamnagar and IARI New Delhi) and two African pathogen populations (from Maiduguri, Nigeria and Bamako, Mali) exhibited segregation patterns that fit digenic ratios of 15:1 (resistant : susceptible) at DMI natural breakpoints ranging between 30% and 85%, indicating the involvement of duplicate dominant genes governing resistance to each of these downy mildew pathogen populations from India and Africa. In previous study, Deswal and Govila (1994) reported that host plant resistance to downy mildew was controlled by complementary gene action for the pathogen from New Delhi (9:7) and duplicate for the

pathogen from Tamil Nadu (15:1). Such variable response of the host to pathogen populations from different production environments was also reported earlier by Joshi and Ugale (2002) who observed differential digenic Mendelian ratios (13:3 and 15:1) in two different screening environments for a single segregating host population. Duplicate dominant factors controlling pearl millet host-plant resistance to downy mildew have been reported by Singh (1974), Shinde *et al.* (1984) and Kataria *et al.* (1993).

Mendelian segregation among these 172 $F_{2:4}$ self-bulk also recorded good fits to a 55:9 ratio at a DMI breakpoint of 65%, and 9:55 ratio at a break point of 5% DMI, in screens against pathogen populations from Jodhpur, India and Bamako, Mali, respectively, indicating that resistance to these pathogen populations is not simply inherited in this segregating host population and suggesting the role of epistatic interactions of two or three major genes in control of disease reaction in this cross. This is in complete agreement with an earlier report (Azhaguvel, 2001), based on progeny of cross IP 18293 × Tift 238D1 screened against a pathogen population from Bamako, Mali. However, it is not clear from this analysis whether the segregating resistance genes conferring disease reaction to these pathogen populations were necessarily the same. A 9:55 ratio indicates the presence of either a dominant inhibitor of resistance conferred by two duplicate dominant genes, or the presence of a recessively inherited resistance modified by two additional duplicate genes. An alternate description of this genetic architecture is that the population is segregating for a failed major dominant resistance gene that now confers sufficient susceptibility to the particular pathogen population so that two dominant resistance genes of similar effects are only expressed when the recessive susceptibility allele is homozygous at the failed resistance locus. A possible parallel for this was observed by Wells and Hanna (1988), who reported four independent genes including duplicate dominant resistance genes and a recessive inhibitor of resistance controlling reaction to pearl millet leaf spot disease caused by *Bipolaris setariae*. Segregation of progenies from the $F_{2:4}$ mapping population in the current study was slightly skewed toward the resistant parent, ICMB 90111-P6, against DM screens against both of these two pathogen populations.

The population of pearl millet downy mildew from MAHYCO, Jalna found to be much less virulent than the other seven pathogen populations used in screening the mapping population in this study. This pathogen population displayed extreme

skewedness of segregating mapping population progenies towards the disease reaction of resistance parent ICMB 90111-P6. In contrast, pathogen population of *S. graminicola* from Bamako, Mali was observed to be the most virulent included in this study. Other pathogen populations for which the mapping population progenies exhibited segregating pattern highly skewed towards the resistant parent included those from Maiduguri (Nigeria), and Patancheru, Jamnagar, and New Delhi (India), where in each case the majority of 172 $F_{2:4}$ mapping population progenies showed DMI values less than 20%. In contrast, screen of this set of mapping population progenies against two highly virulent pathogen populations of *S. graminicola* (one from Jodhpur, India and other from Bamako, Mali), exhibited more nearly normal segregation patterns suggesting that inheritance of resistance to these two pathogen population is more complex. Pathotype specificity of host resistance is considered a common feature of obligate biotrophs (Sidhu, 1986) and previously has been described for pearl millet downy mildew (Thakur *et al.*, 1992).

The mapping population of F_2:F_4 self-bulks derived from the cross of susceptible and resistance parental lines of ICMB 89111-P6 and ICMB 90111-P6 also gave good fits to 63:1 ratios (triplicate dominant resistance genes) at DMI breakpoint levels of 40%, 60%, 75%, 90% and 95% when screened against pathogen populations from Jalna, New Delhi, Maiduguri, Durgapura and Jodhpur, respectively. Four gene interaction with Mendelian segregation ratios of 255:1 (resistant : susceptible) were also suggested from screens against Indian pathogen populations from Jalna and New Delhi at natural breakpoints of 55% and 85% DMI, respectively. However, two dominant and duplicate homozygous allele played important role in favoring resistance against these pathogen population from India. Such trigenic (55:9) and tetragenic (229:27) control of the inheritance of pearl millet downy mildew resistance has also been reported by Joshi and Ugale (2002). Wells and Hanna (1988) also reported four independent genes including duplicate resistance genes and a recessive resistance inhibitor of resistance controlling reaction to a pearl millet leaf spot disease caused by *Bipolaris setariae*. Similarly, Basavaraju (1978) and Basavaraju *et al.* (1980) concluded that resistance to pearl millet downy mildew is not simply inherited, but is due to a complex series of non-allelic interactions. Many authors like Tyagi and Iqbal Singh (1989), Deswal and Govila (1994), and Kataria *et al.* (1994) have concluded that such non-additive gene action is responsible for much of the heritable variability for host plant reactions of downy mildew, agreeing with simpler studies that show resistance to often be dominant or partially dominant.

In the present study, many of the host plant resistances detected were found to be dominant. Most previous genetic studies of downy mildew resistance in pearl millet have found dominance to be important (Appadurai *et al.*, 1975; Gill *et al.*, 1975; Pethani *et al.*, 1980, Basavaraju *et al.*, 1981b; Mehta and Dang, 1987). The QTLs detected for resistance to pearl millet downy mildew in the present study were found to be over-dominant. Over-dominance has also been detected in previous studies (Singh *et al.*, 1978; Basavaraju *et al.*, 1981b; Dass *et al.*, 1984; Jones *et al.*, 1995) and could be explained in terms of buffering effects of heterozygosity. Several previous studies have concluded that downy mildew resistance in pearl millet is quantitative in nature (Singh *et al.*, 1978; Singh *et al.*, 1980; Kenneth, 1981; Basavaraju *et al.*, 1981a), which simply implies that several genes of small individual effect are segregating and the trait is essentially polygenic in its inheritance.

5.1.2. Spearman rank correlation

Spearman rank correlations based on ranks of entry mean disease reactions of the 172 F_2-derived F_4 families screened against each of the eight pathogen populations from India and Africa (Table 10 and 11) showed very strong similarities between pathogen populations from Patancheru and New Delhi, as well as those from Patancheru and Maiduguri. A general trend of stronger correlation was observed amongst the six pathogen populations from India than between the two African pathogen populations. Pathogen populations from northern India (Jodhpur, New Delhi, Durgapura and Jamnagar) were more highly correlated among themselves than were the two pathogen populations from southern India (Patancheru and Jalna). Similar results have been reported by Azhaguvel (2001) in DM screens of mapping population progenies derived from cross IP 18293 × Tift 238D1 against the same six Indian pathogen populations.

5.1.3. Cluster analysis

The dendrograms (Figures 14a and 14b) based on Spearman rank correlation coefficients depicted essentially the same relationships as the correlations themselves (Table 10 and 11) and hence gave similar inferences. Two highly virulent pathogen populations (Jodhpur and Bamako) showing relatively poor correlation were clustered into one group but far away from each other. The less virulent pathogen population from Jalna formed a separate group, relatively more dissimilar from other pathogen populations in all

dendrograms (Figures 13 and 14) and also exhibited very poor correlation with those from the rest of Indian and African pathogen populations (Tables 10 and 11). Among the Indian pathogen populations, those from Jamnagar and Patancheru were found to be closely associated to each other than to the rest of pathogen populations. This could possibly be because of greater genetic similarity of virulency among these two pathogen populations in spite of the large physical distance between the research stations on-which they were originally collected. Other possible reasons could be the allogamous nature of pathogen populations (Thakur *et al.*, 1992) and host-pathogen specificity breaking such geographical barriers in terms of virulency/pathogenocity. The observed strong correlation between Indian and African pathogen populations also supports the same hypothesis. Significant differences have been noticed between the two types of dendrograms constructed in this study. Among Indian pathogen populations, those from Patancheru and New Delhi, and from Jodhpur and Durgapura, have displayed close relationships when entry mean DMI values of the 172 $F_{2:4}$ mapping population progenies (Figure 13a) were used as the basis of the comparisons. Among all eight pathogen populations from India and Africa, Jamnagar has shown very close similarity with Durgapura and Patancheru has shown with New Delhi when comparisons were based on entry mean DMI values (Figure 13b). These correlation studies and cluster analyses have provided useful groupings of the downy mildew pathogen populations of *S. graminicola* that could be used by plant breeders and plant pathologists in chalking out future downy mildew resistance gene deployment strategies for pearl millet in India.

5.1.4. Host plant-pathogen population variability

The analyses of variance for each of the eight individual screens against pathogen populations used in this study have indicated significant variability in downy mildew reaction among the 172 $F_{2:4}$ self-bulks. The pathogen population from Jodhpur (India) was observed to be most virulent (37.3% trial mean DMI), followed by that from Bamako (Mali, West Africa) (34.9% trial mean DMI). Previous studies have indicated that West African host genotypes were potentially more susceptible to Indian than West African pathogen populations and conversely some Indian hosts were more vulnerable to pearl millet downy mildew pathogen populations of West African than to those from India (Ball *et al.*, 1986). The pathogen populations from Jalna and Maiduguri were found to be the least virulent among the eight included in this study. Pathogen populations of Indian

origin exhibited higher virulence than pathogen populations from Africa in present study. Similar observations on host plant and pathogen variability in pearl millet downy mildew screening studies have been earlier discussed by Ball (1983), who reported that West African isolates of *S. graminicola* were generally more pathogenic than Indian isolates. All screens against individual pathogen populations in current study have exhibited very high operational heritabilities on entry-mean basis [ranging between 0.80 (Jalna) and 0.94 (Maiduguri)] and relatively lower operational heritabilities on plot basis [ranging between 0.58 (Jalna) and 0.64 (Jodhpur)], as expected from theory.

5.1.5. Genotype × pathogen population interactions

Downy mildew caused by *S. graminicola* is a serious disease of F_1 hybrid cultivars of pearl millet. The pooled ANOVA from replicated data of 172 $F_{2:4}$ mapping population progenies screened against six pathogen populations from India and two pathogen populations from Africa, revealed no significant variability in downy mildew incidence (DMI, %), arcsin-transformed values of DMI (DMA, radians) and downy mildew disease plant count (DMC) among pathogen populations from the two continents. However, significant variability in DMI, DMA, TPC, and DMC, was detected between pathogen populations from Asia and between pathogen populations from Africa (except TPC) (Table 7). Intercontinental variation among pathogen populations of *S. graminicola* has been discussed by Ball and Pike (1984). The major reason for this variation in DM disease reaction of the mapping population progenies in screen against different pathogen populations from India and Africa is considered is the existence of physiological specialization within *S. graminicola* (ICRISAT, 1989; Thakur and Rao, 1997) and differences in the geographical distribution and differentiation of pearl millet downy mildew pathogen populations. Significant effects of entries and entries × pathogen populations (across both continents, and within Asia and Africa individually) were observed for downy mildew incidence (DMI, DMA, TPC and DMC) except for Africa pathogen population for total plant count (TPC). Similar results have been reported by Thakur *et al.* (2001) from a screen of pearl millet A-lines against different pearl millet downy mildew populations from India in greenhouse conditions as well as in field conditions.

The variance component analysis (Table 8) has also revealed significant variability among 172 $F_{2:4}$ mapping population progenies in DMI, DMA, TPC and DMC

pooled across all eight Indian and African pathogen populations. At the same time, non-significant variability was recorded in DMI entries pooled across pathogen populations from within India compared to entries pooled across pathogen populations from Africa. Pathogen variability of pearl millet downy mildew populations measured in terms of their host cultivar reactions to different pathogen populations has previously been studied by Ball (1983), Ball and Pike (1984) and Thakur *et al.* (1997). Genotype × pathogen population interactions were significant for DMI, DMA, TPC (but not across the six India pathogen populations) and DMC across all pathogen populations from India. The breeding behaviours of both the pathogen and the host are allogamous, so both are highly variable (Thakur *et al.*, 1992), which consequently has hampered studies of the inheritance of downy mildew disease resistance and ultimately breeding of DM resistant pearl millet cultivars. Variation within *S. graminicola* has also bees reported on the basis of differences in size of the asexual structures, number of nuclei, seed borne nature and soluble proteins in the pathogen (Shetty *et al.*, 1980). Among the sources of variation in current study (genotype, pathogen population and genotype × pathogen population), the largest proportion of variability in downy mildew incidence was accounted for largely by mapping population progeny genotypes followed by genotype × pathogen population interactions and least by variation between the pathogen populations themselves. Similar observations have also been reported by Thakur *et al.* (2001) from screens of pearl millet A-lines against pathogen populations from Patancheru, Mysore, Durgapura and Jalna. A few earlier studies (Sastry *et al.*, 1995; Thakur *et al.*, 1999) have shown that these four pathotypes are quite diverse for their virulence and genetic makeup, and that they are representative of such variation prevalent in major pearl millet growing areas of India.

Very high entry-mean basis operational heritability (>0.94) has been recorded for all four variables (DMI, DMA, TPC and DMC) from screens against the six Indian pathogen populations, which were conducted at ICRISAT-Patancheru. But screens against pathogen populations from Africa (which were conducted at the University of Wales, Bangor) recorded comparatively low entry-mean basis operational heritabilities for DMI (>0.69). All eight pathogen populations altogether showed very high broad-sense heritability (>0.93) for all four variables under study. In previous studies, Jones *et al.* (2002) also recorded high operational heritabilities ranging from 0.75 to 0.90 and significantly high variation between F_4 family means for each screen. Greenhouse evaluation of 172 $F_{2:4}$ self-bulks against most of the eight pathogen populations provided

results skewed more towards the resistant parent (ICMB 90111-P6) and recorded high coefficients of variation (CV). However, heritability estimates and F ratios for genotypes, and for genotypes and pathogen interaction variances were highly significant for the various measures of disease reactions, despite the high CV values for these traits. An earlier study of DM screening of $F_{2:4}$ mapping population progenies against these same pathogen populations, carried out by Azhaguvel (2001), also showed skewedness of mapping population progeny values towards resistant parent.

5.2. PARENTAL POLYMORPHISM

The parental lines ICMB 89111-P6 and ICMB 90111-P6 when screened for marker polymorphism against a set of SSR primer pairs and RFLP marker probe-enzyme combinations. Although they exhibited a high level of polymorphism (>55%) as expected, only about 40% of these were very clear and scorable polymorphisms could be used in this study to genotype the 172 F_2 mapping population progenies across 46 marker loci including 26 SSRs and 20 RFLPs. Cross pollinated species tend to have high levels of DNA polymorphism and virtually all crosses which don't involve closely related individuals will give sufficient maker polymorphism for linkage map development (Helentjaris, 1987). In general the level of polymorphism found in inbreeding species is lower than in the out-crossing species such as pearl millet (Miller and Tanksley, 1990). This is the expected level of polymorphism from the SSR and RFLP markers assessed was higher than that normally found in predominantly self-pollinating cereal crops like rice (McCouch *et al.*, 1988) and wheat (Chao *et al.*, 1989; Devos *et al.*, 1992). The amount of observed RFLP polymorphism in barley was less than 28% (Graner *et al.*, 1991; Heun *et al.*, 1991).

In pearl millet, Liu *et al.* (1994) reported 56% average pair-wise polymorphism for RFLP probe-enzyme combinations among elite parental hybrid lines. In the present study, the percentage of polymorphism varied with type of markers. The amount of RFLP polymorphism also differed with the four different restriction enzymes used. The highest level of polymorphism was recorded with restriction enzymes *Hind*III followed by *EcoR*V, *EcoR*I and *Dra*I. Azhaguvel (2001) obtained a slightly different order of *Hind*III>*Dra*I>*EcoR*V>*EcoR*I for high level of polymorphism for a different pearl millet cross. In fact *Dra*I exhibited polymorphism for number of probe-parental line combinations but because of high-distorted segregation or unclear scoring they were

excluded from the present study. Simple sequence repeats SSRs are abundant in eukaryotic genomes and provide a co-dominant, usually highly polymorphic markers system (Tautz and Ranz, 1984; Bryan *et al.*, 1997). Out of 80 pearl millet SSR primer pairs screened against the parent lines of the cross under study, only 26 SSR (33%) revealed good scorable polymorphisms.

Microsatellites have proven informative to study genetic relationship among closely related plant species as well as among sub-populations of single species (Bowcock *et al.*, 1994) because of their exceptionally high level of polymorphism. In addition, microsatellites exhibit co-dominant inheritance and their detection can be automated (Hernandez *et al.*, 2002). These factors are essential for effective discrimination between closely related lines (Akkaya *et al.*, 1992). Plant SSRs are reported to exhibited high levels of polymorphism with as many as 37 alleles at individual loci in barley (*Hordeum vulgare* L.) (Sanghai-Maroof *et al.*, 1994) and 26 alleles in soybean (Rongwen *et al.*, 1995). In plants, the level of SSR polymorphism has been shown to be 10 times higher than with RFLP markers (Akkaya *et al.*, 1992; Senior and Heun, 1993; Bell and Ecker, 1994). Although relationships between degree of polymorphism and number of repeats have been reported in some species (Sanghai-Maroof *et al.*, 1994; Fisher *et al.*, 1998), theoretically number of repeats is in correlation with mutation rate not with degree of polymorphism (Brink-Mann *et al.*, 1998; Xu *et al.*, 2000). The degree of polymorphism detected by recently developed pearl millet SSR primer pairs did not correlate with the number of repeats (Budak *et al.*, 2003).

5.2.1. Segregation of marker loci and their distortion

Distorted segregation of molecular marker loci appears to be a common phenomenon in many crop species. It has been reported in a number of previous studies [Cloutier *et al.* (1991); Bentolila *et al.* (1992); Cloutier and Landry (1994), Rivard *et al.* (1996) and Yamagishi *et al.* (1996)] but no universally accepted satisfactory explanation has been offered for this phenomenon. In present study, about 40% of the marker loci (including both SSRs and RFLPs) segregated as per the expected ratio of 1:2:1 among the 172 $F_{2:4}$ self-bulks. The remaining 60% of loci exhibited significant segregation distortion in this mapping population. Segregation distortion can be consequences of genetic elements that exhibit the phenomenon of meiotic drive that is a mechanism of the meiotic division cause one member of a pair of heterozygous alleles or heteromorphic chromosomes to be

transmitted to the progeny in excess of the expected Mendelian ratio of 50% (Sandler and Novitski, 1957; Sandler and Golic, 1985; Lyttle, 1991). Segregation distortion can occur due to gamete selection (especially among male gametes of selfed F_1 plants that contributed to F_2 seed production or through selective influence of the gynoecium including genetic incompatibility), environmental effects both biotic and abiotic, and differential competitive ability of genetically variable pollen (Lyttle, 1991; Xu, 1997). Segregation distortion have been reported in wide range of organisms, including plants in which species or strains hybrids have exhibited preferential dysfunction of gametes carrying one chromosomal class. This can occur in either microspores (Cameron and Moav, 1957; Endo, 1982; Tsujimoto and Tsunewaki, 1985), or megaspores (Scoles and Kibirge-Sebunya, 1983), or both (Rick, 1966). Segregation distortion is most commonly observed in interspecific crosses; however, pervious studies showed distortion phenomenon also occur in intraspecific pearl millet crosses (Liu *et al.*, 1994; Busso *et al.*, 1995). As an extreme example, a rice intraspecific recombinant inbred population (CO39/ Mori berekan) was reported to have 98.8% of marker loci skewed towards the indica parent (Wang *et al.*, 1994). Variation in the timings of stigma emergence and anthesis under the selfing bag among F_2 plants in protogynous pearl millet provide additional factors that can contribute to the distorted segregation patterns observed in this population.

The phenomenon of segregation distortion is one of the limitations in the present map constructed from 172 selfed individuals F_2. This is because of the fact that it may affect both the establishment of linkage groups and estimation of recombination frequencies. Calculations of linkage distance usually assume no segregation distortion, which could cause over-estimation of recombination frequency between linked markers (Paran *et al.*, 1995). Segregation results among 172 F_2 progenies based on cross ICMB 89111-P6 × ICMB 90111-P6 are presented in Table 12. Out of total 46 polymorphic marker loci screened for this mapping population, 28 marker loci showed segregation distortion. The significant χ^2 values ranges from 4.40 (*Xpsmp*2089 on LG2 at 5% significance level) to 20.81 (*Xpsm*588 on LG6 at 1% significance level). The degree of segregation distortion varied among marker types (SSRs and RFLPs) and segregation of SSR markers was generally more distorted than that of RFLPs in the present study. Analysis of allelic distribution at the marker loci exhibiting significant segregation distortion revealed some clear tendencies for abundance of one or another of parental

allele homozygote or of heterozygote alleles. Interestingly, significant segregation distortion favoring male parent (ICMB 90111-P6) alleles over those of female parent (ICMB 89111-P6) were observed for eight marker loci (*Xpsmp*2273, *Xpsm*17, *Xpsmp*2080, *Xpsmp*2030, *Xpsmp*2089, *Xpsm*588, *Xpsmp*2018 and *Xpsm*696) and vice versa for only four loci (*Xpsmp*2072, *Xpsmp*2077, *Xpsmp*2201 and *Xpsm*409.1) when tested with χ^2 against expected Mendelian ratio of 1:1 between male and female parent alleles. This pattern of segregation distortion favoring the allele from a male parent has previously been reported in pearl millet by Liu *et al.* (1994), Azhaguvel (2001) and Kolesnikova-Allen (2001).

In the present study, six marker loci each on LG1 and LG2 (highest in number on any linkage group), and three marker loci on LG4 (least in number) exhibited distorted segregation among the 172 F_2 mapping population progenies. Surprisingly, all the markers located on LG6 exhibited highly distorted segregation skewed towards alleles from male parent ICMB 90111-P6 (Table 12). LG5 and LG7 accommodated five and four marker loci, respectively exhibiting segregation distortion in this mapping population. It has been reported that such segregation distortion is highly possible in pearl millet because of its protogynous nature (Liu *et al.*, 1994). Unexpectedly high abundance of heterozygotes was also observed for 22 segregation distorted marker loci on LG1, LG2, LG4, LG5, LG6 and LG7. Such segregation distortion can be caused by a number of reasons. One possible reason could be that markers with distorted segregation are tightly linked with genes that inhibit plant development and cause lower than expected frequencies of certain genotypes in the experimental population due to lower numbers of surviving individuals of those genotypes (Tai *et al.*, 2000). Further more, distortion favoring heterozygotes could be due to natural selection (O'Donoughue *et al.*, 1992) so that the sample of F_2 plants used to provide tissues for DNA isolation might not be a true representative sample of the individual F_2 plant from which they were derived and/or certain genotypes were present in lower than expected frequencies among the F_2 plants themselves. In the present study, it is liked that ICMB 89111 has alleles at one or more loci that contribute to poor germination and/or stand establishment in the F_2 generation, this would result in segregation distortion patterns similar to those observed in this study.

Another reason that could be contributed to segregation distortion in this study is mainly a mechanical or operational one due to difficulties in scoring page gels and

autoradiographs. In the process of Southern hybridization for RFLP data generation, multiple use of limited numbers of filters with multiple stripping of the used filters for each set of plants, as used in this study, caused some DNA to be removed from the filter, consequently hybridized bands get weaker with each use and can ultimately give blank spots on the autoradiograph leading to some data points scored as C or D or gaps. The different electric currents and texture of PAGE gel affect movement of PCR-amplified DNA during electrophoresis and may also lead to similar problems of scoring of bands in case of genotyping the mapping population with SSR markers. Large numbers of such gaps were scored in the current study either because of very faint bands difficult to score, non-digestion of DNA samples for RFLP, or poor amplification of DNA samples of particular plants in the population. All these activities might have lead to segregation distortion for some of the marker loci. Such events were also experienced by Kolesnikova-Allen (2001) and Azhaguvel (2001) in pearl millet.

5.2.2. Selection of Marker loci

The polymorphic RFLP and SSR markers used in this study were primarily selected based on maintaining a minimum inter-marker linkage distance of 15-20 cM in the consensus map of Qi *et al.* (2004). This is because most of the SSR and RFLP marker available at present have been mapped in this consensus map. But final selection of markers used for genotyping the mapping population in the present study was for clear and scorable polymorphism between the two parents and their F_1. The reason to select the markers with linkage distance roughly of 15-20 cM, is that what is detected as a QTL is a segment of chromosome of this length that may contain several loci affecting the trait not necessarily in the same direction (Tanksley, 1993), and QTL detection is not greatly enhanced by spacing more closely than 15-20 cM for which marker data generation is much more costly. However, in the present study, the marker analysis could not confirm the expected linkage relationships for some of the marker loci. This may be because of the relatively small population size used, genome lack of polymorphic markers in some genomic regions, use of marker loci present at distal ends leading larger map distances and limited genome coverage by marker used, and multiple-copies of some of marker loci (especially RFLPs) in the pearl millet genome.

5.3. GENETIC LINKAGE MAP FOR CROSS ICMB 89111-P6 × ICMB 90111-P6

A linkage map of 747.9 cM (Haldane) was constructed using 46 marker loci, which include both SSR and RFLP markers in the present study. The base map of pearl millet (Figure 53) has a genome length of 287.7 cM (Kosambi) produced from an F_2 mapping population from cross LGD 1-B-10 × ICMP 85410 (Liu *et al.*, 1994) based on RFLP markers only. Azhaguvel (2001) constructed a linkage map of 561.8 cM (Haldane) from 33 well-distributed RFLP markers, having comparatively smaller mapped genome length for his mapping population than the present mapping population study. Studying pearl millet cross W 504-1-1 × P310-17-B, Kolesnikova-Allen (2001) constructed an even smaller linkage map of 421 cM (Haldane) from a population of 175 F_2 individuals using 38 RFLP markers. Despite substantial increases over the base map, these three studies still continue to confirm that pearl millet has a short map in terms of genome length compared to all major cereals. There are number of examples showing higher genome lengths than pearl millet viz. RFLP-based genetic linkage map of rice constructed with more than 800 probes, had a length of 1491 cM (Causse *et al.*, 1994) and with addition of new markers has later reached a length of 1680 cM (Price *et al.*, 2000). In cowpea, the genetic map consists of 11 linkage groups spanning a total of 2670 cM, with an average distance of 6.43 cM between markers (Ouedraogo *et al.*, 2002). In barley a 1453 cM linkage map has been reported (Graner *et al.*, 1991; Heun *et al.*, 1991). O'Donoughue *et al.* (1992) constructed an oat (*Avena sativa*) linkage map using 194 RFLP probes with a length of 614 cM. An RFLP-based linkage map of sorghum of 1530 cM length has been constructed using maize and sorghum genomic probes (Pereira *et al.*, 1994; Subudhi and Nguyen, 2000). An RFLP-based genetic linkage map of rye (*Secale cereals* L.), a cross pollinated diploid species like pearl millet, has recently been extended to 1140 cM by addition of RAPD and isozyme markers (Masjoe *et al.*, 2001). In the current study skeleton map of less than 50 probe-enzyme combinations and primer pairs polymorphic between parental lines has been constructed with an average map of <20 cM inter-markers distance in pearl millet as per the suggestion of Liu *et al.* (1994), to locate QTLs of our interest.

The inclusion of polymorphic RFLP and SSR markers located on both, upper and lower distal ends of several linkage groups, quite far from putative centromeric regions has resulted in the increase in total map distance of the cross ICMB 89111-P6 × ICMB

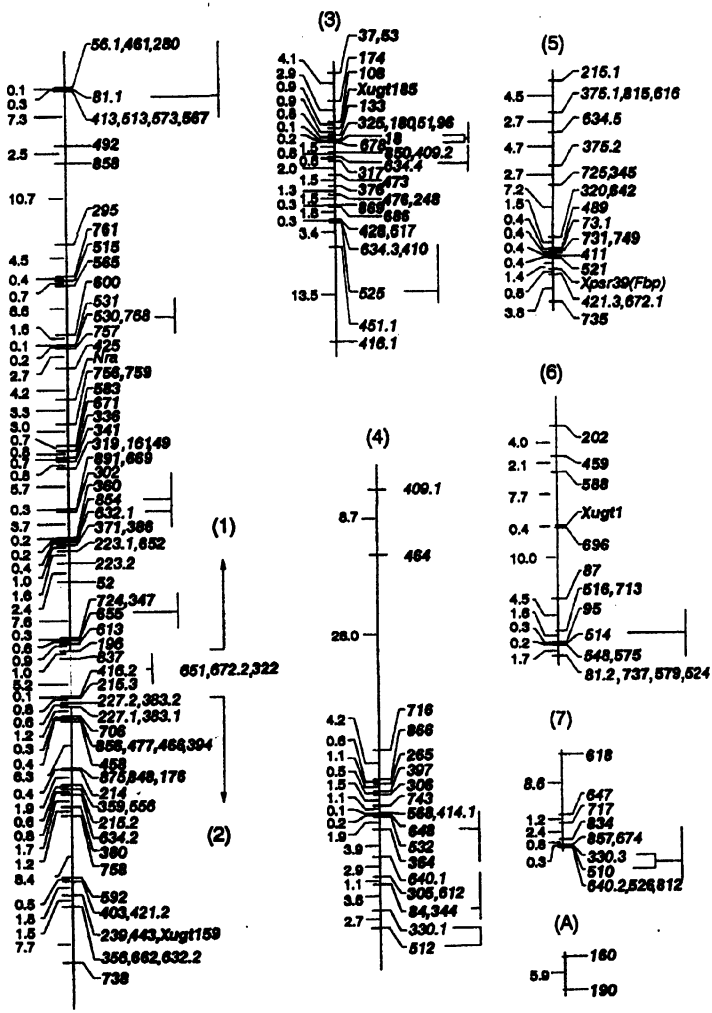
90111-P6, under study. Such marker loci have been mapped in this course of study on top of LG4, and on both ends of LG2 and LG7 as shown in Figures 26 and 27. Large inter-marker distances (>50 cM Haldane) have been recorded for several of these distally located maker loci leading to enhancement of total map length. Liu *et al.* (1994) (Figure 53) expected such increases in the length of the pearl millet linkage map. This expectation has been strengthened by subsequent mapping studies in pearl millet using different parental combinations. This increase in map length is because of adding new RFLP markers from pearl millet and other cereal crops (Devos *et al.*, 2000) along with AFLP and SSR markers. Qi *et al.* (2004) from John Innes Centre, UK has recently reported an update consensus map of pearl millet. The most recent version is readily accessible through the website http://jii05.jic.bbsrc.ac.uk.8000/cgi-bin/webace?db=millet.

Seven linkage groups (LGs) were obtained from this mapping population under study. The marker order and map length are essentially as expected based on comparison with the published pearl millet base map of Liu *et al.* (1994) and pearl millet consensus map (Qi *et al.*, 2004). Along with this, other recently constructed pearl millet RFLP-marker-based genetic linkage maps based on different populations (Table 48) have been compared with the map obtained from this study. The total genome length of those previously constructed maps ranged from 287.7 cM (LGD 1-B-10 × ICMP 85410) to 695.7 cM (81B-P6 × ICMP 451-P6) with an average map length of 546.0 cM, which is moderately less than the newly constructed map in this study.

5.3.1. Mapping of linkage group 1

Linkage group 1 (LG1) in the present study has a clear-cut tendency of increasing its map length with addition of flanking markers to both distal regions. The order of markers located on LG1 is the same as mentioned in pearl millet consensus map and across all previously studied populations. The cross ICMB 89111-P6 × ICMB 90111-P6 under study has a comparatively larger map length, 139.6 cM (Figure 26), for LG1 than all previously studied populations except PT 732B × P1449-2 (172.6 cM) (Nepolean, 2002). The possible explanation for this could be use of both SSR and RFLP markers from centromeric as well as distal regions of the linkage group in the present study. The shortest map length for LG1 was recorded for population IP 18293 × Tift 238D1 (Azhaguvel, 2001) possibly because of use of only RFLP probes in his study. Other alternative reasons could be the sizes of the various mapping populations, genetic

Figure 53. First published RFLP-based genetic linkage map of pearl millet based on cross LGD 1-B-10 × ICMP 85410 (Liu *et al.*, 1994). On left side of each linkage group are the map distances in cM (Kosambi) between marker loci and on the right side are the abbreviated locus names (the prefix *Xpsm* has been dropped for RFLP loci based on pearl millet probes from John Inn Centre).



constitution of their parental lines, and number and polymorphism of marker loci obtained for these parental lines. A total of seven marker loci including SSR and RFLP markers, with an average distance of 19.9 cM between markers, are found in LG1 of the newly developed map. The occurrence of highly significantly distorted segregation encountered for marker loci mapped on this LG could be another probable reason for its increased map length. The markers are fairly well distributed across this linkage group except near the upper end. In the original base map (Liu *et al.*, 1994) psendo-linkage of LG1 and LG2 was observed, but the present study witnessed a clear separation of LG1 and LG2.

5.3.2. Mapping of linkage group 2

This linkage group also has shown an increasing tendency of map length ranging from 31.6 cM (W 504-1-1 × P310-17-B) to 179 cM (841B × 863B) in previous studies. The observed map length for LG2 in the present study is the highest (192.3) yet reported. This increase in map length of LG2 is due to the use of RFLP marker *Xpsm*708.1, and microsatellite SSR marker *Xpsmp*2089 for genotyping and mapping the F_2 population. These two loci map on upper and lower distal ends, respectively, on this LG with larger inter-marker distances between these distal markers and the centromeric region of LG2. These two distally located marker loci were not genotyped and mapped on LG2 in most of the previous studies, and hence the map length for this linkage group has been extended in this study. A map length of 36.2 cM (Kosambi) for LG2 have been reported for the base map of pearl millet (Liu *et al.*, 1994) from a cross LGD 1-B-10 × ICMP 85410. Two loci (*Xpsm*708.1 and *Xpsm*708.2) together have added over 160 cM (Haldane) to the length of LG2 in a cross 841B × 863B. Basically the marker order recorded in present study is in perfect agreement with the consensus integrated linkage map of pearl millet (Qi *et al.*, 2004). Without addition of these two markers in the present study, the map distance becomes almost equal to the LG2 in a population (PT 732 × P 1449.2) studied by Nepolean (2002).

5.3.3. Mapping of linkage group 3

This linkage group has been recorded as the shortest among all seven linkage pearl millet groups in this study, which is in complete agreement with previous studies in crosses 841B × 863B (Yadav *et al.*, 2004), 81B-P6 × ICMP 451-P6 (Devos *et al.*, 2000; Qi *et al.*,

2004), W 504-1-1 × P310-17-B (Kolesnikova-Allen, 2001) and PT 732B × P14492 (Nepolean, 2002). No differences have been detected in order of marker loci on this linkage group as compared to the consensus integrated linkage map of pearl millet and other previously mapped populations. One good thing for this LG3 was that there was no observed segregation distortion across all marker loci included in this study.

5.3.4. Mapping of linkage group 4

The total map length of LG4 recorded from the present study is 98.3 cM (Haldane), which is relatively larger than the length of LG4 of the base map from population (LGD 1-B-10 × ICMB 85410) on one hand, but shorter than that from other recently mapped pearl millet populations. All the marker loci are well dispersed across length of LG4 except one marker locus which is located on far upper distal end of this linkage group at a map distance of 65.0 cM. Comparatively larger map distances between *Xpsm*409.1 and *Xpsm*648 have been reported by Azhaguvel (2001) and Kolesnikova-Allen (2001) for this linkage group (Table 48). But the base map has exhibited shorter map distance between the two markers as 46.0 cM. This can be explained, at least partly by the presence of segregation distortion for the two marker loci in present study, presence of putative double crossovers, and the different genetic constitution of the population under study. Marker order is also same as that of the base map LGD 1-B-10 × ICMP 85410 except that one multiple-copy marker locus, *Xpsm*416.3, is mapped to LG4 in present study. The marker loci, *Xpsm*416.3 was placed at the distal end of LG4 for this cross ICMB 89111-P6 × ICMB 90111-P6 under study because of very tight linkage of this marker locus to other marker loci mapped on LG4. The recombination level in this region is so low that there is no chance of separation of these markers more distinctly without dramatically increasing the size of the mapping population. The mapping population size in cross LGD 1-B-10 × ICMP 85410 was 133 individuals compared to 172 individuals in present mapping population, so it is expected that the present mapping population may give slightly more accurate estimation of marker locus order, genetic distances, and recombination frequencies. The other reason for the big gap between *Xpsm*409.1 and *Xpsm*648 is limited genome coverage in this mapping population by markers on LG4 and lack of marker polymorphism of any type in this region. The other reasonable explanation to this could be done to the use of the Kosambi mapping function in earlier studies, and use of the Haldane function in present study, but this is not sufficient to explain the

Table 48. Total mapped genome and linkage group lengths for seven pearl millet mapping populations. The map distances are shown in Haldane centimorgans (cM) except for mapping population 1 for which the Kosambi mapping function was used. The data for the published map of LGD 1-B-10 x ICMP 85410 is shown in **bold red font**, while that of presently studied cross ICMB 89111-P6 x ICMB 90111-P6 is shown in **bold green font**

Mapping populations	1	2	3	4	5	6	7
Pedigree LG	**LGD 1-B-10 x ICMP 85410**	81B-P6 x ICMP 451-P8	W 504-1-1 x P310-17-B	IP 18293 x Tift 238D1	PT 732B-P2 x P1449-2-P1	ICMB 841-P3 x ICMB 863-P2	**ICMB 89111-P6 x ICMB 90111-P6**
1	**73.4**	77.3	113.8	58.9	172.6	104.9	**139.6**
2	**36.2**	175.8	31.6	136.4	87.9	179.0	**192.3**
3	**38.2**	52.2	24.2	62.9	27.6	15.4	**30.2**
4	**63.2**	132.4	116.6	110.9	100.0	64.3	**98.3**
5	**30.9**	102.8	37.5	51.9	30.2	26.9	**50.1**
6	**32.5**	58.3	57.6	67.8	83.1	113.1	**42.2**
7	**13.3**	96.9	39.7	24.7	37.6	113.8	**195.2**
Total mapped genome length	**287.7**	695.7	421.0	513.5	539.0	617.4	**747.9**
Mapped (F_2) population size	**133**	184	175	142	136	147	**172**
Reference	**Liu *et al.*, 1994**	Devos *et al.*, 2000	Kolesnikova-Allen, 2001	Azhaguvel, 2001	Nepolean, 2003	Yadav *et al.*, 2004	**This study (Gulia, 2004)**

increased map length for LG4 in the present study.

5.3.5. Mapping of linkage group 5

LG5 is one of the shortest, with a total map length of 50.1 cM (Haldane) in the newly developed map. This is in complete agreement with the pearl millet integrated consensus map (Qi *et al.,* 2004) constructed using SSR and RFLP and other markers, which is based on four different pearl millet mapping populations. In the present study LG5 contains only SSR markers, which are very well dispersed across its entire length, including a newly mapped SSR marker (*Xpsmp*2261) that is located on the lower distal end of LG5 and has added 20.5 cM to the length of this linkage group. This marker locus displayed very good polymorphism between parental lines and tight linkage to other marker loci present on LG5 in present study. In addition to *Xpsmp*2261, three other SSR markers *Xpsmp*2208, *Xpsmp*2276 and *Xpsmp*2277 have been mapped with inter-marker distances of 4.7 cM and 1.3 cM, suggesting very close and tight linkage among them. The observed average distance between any two markers is equal to 6.3 cM, which is ideal inter-marker distance to detect QTL and initiate MAS. A previously mapped population (841B × 863B) had a map length of 102.8 cM for LG5, largest among all pearl millet populations mapped to date (Yadav *et al.*, 2004). The base map (Liu *et al.*, 1994) had a map length of 30.9 cM, which is the shortest amongst all populations except that based on cross PT 732B × P14492 (30.2 cM).

5.3.6. Mapping of linkage group 6

A slight increase of map length has been observed in present study (42.2 cM, Haldane), as compared to the published base map of 32.5 cM for LG6 (Liu *et al.*, 1994). The length of LG6 has kept increasing in newly mapped populations and reached upto 113.1 cM in 841B × 863B because of the newly linked marker *Xpsm*870, for which linkage to *Xpsm*713 was only detectable following the addition of intervening SSR marker *Xpsmp*2002. A majority of marker loci on LG6 are mapped in the centromeric region and the marker order remains intact as shown in the integrated consensus map (Qi *et al.*, 2004). There is shortage of markers to be mapped in both the upper and lower distal regions away from the centromere of this linkage group.

5.3.7. Mapping of linkage group 7

This was the largest linkage group (195.1 cM, Haldane) among all seven with eight marker loci for the population under study. There is a clear indication of increase in the map length of LG7 since the pearl millet base map was first published because more markers have been added to this linkage group in later studies compared to the originally published map (13.3 cM) of Liu *et al.* (1994). Surprisingly a more than 14-fold increase in map length of LG7 has been noticed in the present study compared to the pearl millet base map. In cross 841B × 863B, RFLP marker loci *Xpsm*269, *Xpsm*718 and SSR locus *Xpsmp*2203 added 100 cM to LG7 (Yadav *et al.*, 2004). Further to this, linking of two RFLP markers (*Xpsm*160 and *Xpsm*190) to the distal end of LG7 of pearl millet linkage map from cross based on ICMB 89111-P6 × ICMB 90111-P6 under study has subsequently provided a further increase of 102.1 cM in the total map length of LG7. In earlier study by Liu *et al.* (1994), these two linked RFLP markers have been mapped separately forming a sub-linkage group that segregated independently from the seven expected major linkage groups in pearl millet. The additional fragment, having these two marker loci, was mapped with 5.9 cM as intermarker distance between them.

5.4. QTL MAPPING FOR DOWNY MILDEW RESISTANCE

The phrase QTL mapping is no longer a new terminology in the 21st century, as QTL mapping has been established as an extremely powerful technique for detecting and locating specific regions of chromosomes that contribute to the control of host plant disease resistance or other quantitative traits of interest. It has enabled the independent segregation of resistance to different pathogen populations of *S. graminicola* to be demonstrated in pearl millet (Jones *et al.*, 1995, 2002; Kolesnikova-Allen, 2001; Azhaguvel, 2001; Breese *et al.*, 2002 and Nepolean, 2002). Pearl millet downy mildew has conventionally been considered as a quantitative trait having a significant effect of screening environment (both biotic and abiotic) on its expression. The QTL mapping approach can be used by plant breeders to predict quantitative effects of genomic regions controlling a trait having continuous variation, for specific genotypes by analysis of their DNA marker profiles. Continuous variation among the $F_{2:4}$ mapping population progenies has been found for host plant resistance against downy mildew in the present study (Figures 5-12). This has also been reported in various previous studies on the genetics of pearl millet downy mildew resistance (Singh *et al.*, 1980; Basavaraju *et al.*, 1981a & b;

Dass *et al.*, 1984; Shinde *et al.*, 1984). However, this does not necessary imply that its inheritance is complex and that many resistance genes are segregating. To obtain accurate and unbiased data points for this type of quantitative trait is usually very difficult and cumbersome. But generating accurate phenotypic data minimizing the influence of the abiotic environment is an indispensable prerequisite for precise host plant resistance QTL mapping (Paterson *et al.*, 1991a). The larger the environmental and genotype × environment interaction effects on a character (i.e. lower heritability), the less likely it is that statistically significant QTLs will be detected. Proper randomization, suitable number of replications and controlling other environmental variation are important factors for precise and accurate estimates of phenotypic values for use in QTL mapping. So, in the present study an improved downy mildew inoculation method was used with three replications of progenies and control entries screened in time (at Patancheru) or in space (at Bangor) in order to reduce environmental or mechanical effects. The segregation of even two host plant resistance genes can result in a continuous distribution of disease incidence among progenies in a mapping population, even with fairly high heritability. The progenies of the mapping population screened against pathogen populations in this study were $F_{2:4}$ self-bulks in which segregation of heterozygotes would result in less distinct classes than if the F_2 population itself have been screened under conditions of perfect heritability (but no replication) or fully homozygous random inbred lines derived from the F_2 population had been screened as reported by Jones *et al.* (1994).

The largest number of single QTLs has been detected using data from the screen against the pathogen population from Jodhpur. QTLs for host-plant resistance to this pathogen population were detected on LG1, LG2, LG3, LG4 and LG7. One QTL detected on LG4 was considered as the best single-QTL model with a LOD score of 11.0 for this pathogen population. Approximately 65% of observed phenotypic variation in this particular screen was found to be under the control of this major QTL (*Xpsm*409.1 + 31.0 cM) on LG4. To assess the effect of a specific locus on a particular trait, the percentage of phenotypic variation under control of each locus is estimated. According to the classification of major and minor gene traits in rice by Mackill and Junjian (2001), QTL controlling 25-50% of variation would be classified as major QTLs. In the present study, QTLs controlling downy mildew resistance explaining more than 50% observed phenotypic variation in a particular screen and having comparatively high LOD score values are considered as major resistance QTLs. One major QTL has been detected

against each of the six Indian pathogen populations and one of the two African pathogen populations (Bamako, Mali only, not Maiduguri, Nigeria) on LG4 of ICMB 90111-P6 near the vicinity of marker loci *Xpsm*409.1. All the detected QTLs are placed at distance ranging from 20 cM to 40 cM from *Xpsm*409.1 with significantly high LOD scores ranging for 10.1 to 15.5. High proportion of observed phenotypic variance (>65%) explained by these QTLs established them to be QTLs of major effect for downy mildew resistance (Table 13). The best two-QTL model was accepted with a higher LOD score, as qualified by the formula mentioned in Chapter 4 (Experimental Results), which explained observed phenotypic variance of 72.1%. The proportion of observed phenotypic variation explained by the best three-QTL model (83.2%) was substantially higher than that for the best two-QTL model for downy mildew disease reaction among 172 $F_{2:4}$ progenies, indicating detection of host plant resistance QTLs on LG2, LG3, and LG4. In earlier QTL mapping studies in pearl millet, Kolesnikova-Allen (2001) and Azhaguvel (2001) have also separately identified downy mildew resistance QTLs with major effects using similar multiple-QTL models.

QTL mapping analysis of phenotypic data sets from DM screens against pathogen populations from Durgapura, Patancheru, Jamnagar and New Delhi detected three QTLs mapped on LG2, LG4 and LG7 (Table 49). The marker locus *Xpsm*708.1 has been observed as a common flanking marker where QTLs for downy mildew resistance against these pathogen populations have been detected and located at distances varying from 27.3 cM distance to 29.9 cM. These QTLs have been identified as major host plant resistance QTLs with varying LOD scores (5.6 to 12.7). They control high proportions of the observed phenotypic variation (from 56.2% to 67.2%) for disease reaction among progenies of the mapping population under study. The results obtained from QTL mapping suggested no marked differences in genetic constitution of virulence in these three pathogen populations of different origins. But detection of QTLs on different linkage groups, on different positions of same linkage groups against some other pathogen populations of *S. graminicola* showed significant differences in the genetic structure of pathogenicity and virulence in pathogen populations from India and Africa. This fact has been supported by previous studies by Ball and Pike (1984), Azhaguvel (2001) and Kolesnikova-Allen (2001), where the differences between pathogen populations from India and Africa were found. There differences were attributed to the separate centres of diversity, established by divergent co-evolution of the host and the

Table 49. Summary of significant QTLS for pearl millet downy mildew resistance against Indian and African Pathogen populations of *S. graminicola* from results of simple interval mapping as implemented in mapmaker/QTL

Pathogen populations	Best single-QTL models							Highest qualified multiple-QTL models						
	LG[a]	Position[b]	LOD[c]	Var[d]	Add[e]	Dom[f]	Inher[g]	LG[a]	Position[b]	LOD[c]	Var[d]	Add[e]	Dom[f]	Inher[g]
Indian														
Jodhpur	1	*Xpsm*280 +36.2	3.3	9.7	-10.6	3.4	Rec							
	2	*Xpsmp*2201+17.7	3.9	16.7	-7.3	16.0	???							
	3	*Xpsm*37+16.3	3.0	9.1	10.1	-0.6	R/A	2	*Xpsm*708.1+36.1			-14.1	-7.6	OD
	4	*Xpsm*409.1+31.0	**11.0**	65.3	-27.2	-10.0	OD	3	*Xpsm*18+1.7			10.0	-0.5	Add
	7	*Xpsm*160+40.2	4.2	12.2	-10.9	5.5	Rec	4	*Xpsm*409.1+29.4	**19.2**	83.2	-24.7	-9.4	OD
Jalna	2	*Xpsmp*2225+30.8	4.5	55.4	-12.2	-12.0	OD	4	*Xpsm*409.1+40.7			-13.2	-10.1	OD
	4	*Xpsm*409.1+40.9	**10.2**	62.9	-13.2	-10.1	OD	7	*Xpsmp*2224+5.0	**13.9**	66.8	3.0	-2.9	Rec
Durgapura	2	*Xpsm*708.1+27.3	7.0	58.2	-21.9	-17.7	OD	2	*Xpsm*708.1+26.1			-21.8	-17.0	OD
	4	*Xpsm*409.1+39.1	**10.1**	68.5	-28.3	-14.5	OD	2	*Xpsmp*2225+51.2			-3.3	-4.5	OD
	7	*Xpsm*160+34.5	3.0	14.3	-10.8	-5.4	OD	4	*Xpsm*409.1+37.2	**15.0**	82.3	-14.8	-4.2	Dom
Patancheru	2	*Xpsm*708.1+28.1	10.4	66.5	-28.4	-22.5	OD	2	*Xpsm*708.1+26.2			-12.5	-6.6	OD
	4	*Xpsm*409.1+37.3	**12.9**	70.5	-30.6	-22.8	OD	2	*Xpsmp*2225+39.2			-6.2	-9.2	OD
	7	*Xpsmp*2203+27.3	8.8	67.1	-27.5	-30.5	OD	3	*Xpsm*37+5.4			5.1	1.3	Dom
								4	*Xpsm*409.1+36.7	**25.1**	90.4	-25.3	-20.3	OD
Jamnagar	2	*Xpsm*708.1+26.3	**12.7**	67.2	-27.5	-21.0	OD							
	4	*Xpsm*409.1+37.3	11.2	68.3	-29.2	-19.4	OD	2	*Xpsm*708.1+25.1			-27.6	-19.5	OD
	7	*Xpsmp*2203+29.9	7.7	66.8	-23.5	-25.6	OD	4	*Xpsm*409.1+40.1	**19.8**	88.8	-14.4	-7.4	OD

...contd. Table 49. Summary of significant QTLS for pearl millet downy mildew resistance against Indian and African Pathogen populations of *S. graminicola* from results of simple interval mapping as implemented in mapmaker/QTL

Pathogen populations	Best single QTL-models							Highest qualified multiple-QTL models						
	LG[a]	Position[b]	LOD[c]	Var[d]	Add[e]	Dom[f]	Inher[g]	LG[a]	Position[b]	LOD[c]	Var[d]	Add[e]	Dom[f]	Inher[g]
New Delhi	2	*Xpsm*708.1+29.9	5.6	56.2	-16.2	-14.4	OD							
	4	*Xpsm*409.139.0	**10.5**	63.6	-20.1	-12.7	OD	2	*Xpsm*708.1+26.2			-9.4	-8.8	OD
	7	*Xpsmp* 2203+29.9	3.5	54.6	-16.1	-17.2	OD	4	*Xpsm*409.1 +39.5	**17.0**	82.1	-19.5	-13.2	OD
African														
Maiduguri	2	*Xpsm*708.1+29.2	**16.2**	69.5	-24.6	-21.1	OD							
Bamako	1	*Xpsm*492+12.3	5.9	31.7	-20.3	-4.5	Dom							
	2	*Xpsmp*2201+14.2	3.2	15.8	-10.0	13.0	Rec	4	*Xpsm*409.1+19.8			-27.3	-5.2	PD
	4	*Xpsm*409.1+20.8	**15.5**	73.9	-28.9	-4.4	PD	7	*Xpsm*160+35.7			-5.6	-3.6	OD
	7	*Xpsm*160+42.1	5.0	15.0	-13.3	-0.6	Add	2	*Xpsmp*2201+20.5	**19.6**	80.3	-5.96	6.5	Rec

[a] Linkage groups; [b] Position of the QTL with the marker loci; [c] Log Likelihood score; [d] Percentage of variation explained for downy mildew resistance; [e] Additive effect; [f] Dominance effect; [g] estimated mode of inheritance for resistance; OD = Over-dominance, Rec = Receesive; Add = Additive

pathogen following their introduction from Africa into India around 3000 years ago (Purseglove, 1976).

The highest qualified multiple-QTL model achieved for the screen against the Durgapura pathogen population was a three-QTL model with a LOD score of 15.0. This QTL model detected two QTLs on LG2 and one QTL on LG4, which altogether explained 82.3% of observed phenotypic variance. But for the pathogen population from Patancheru, a four-QTL model was accepted as the highest qualified multiple-QTL model with a LOD score of 25.1 (>12.9+2+2+2=18.9), which explained the highest amount of observed phenotypic variation (90.4%) across screens of any of the eight pathogen populations from India and Africa included in the present study. LG2 (2 QTLs), LG3 and LG4 accommodated the QTLs detected by four-QTL model near the marker loci *Xpsm*708.1+26.2 cM, *Xpsmp*225+39.2 cM, *Xpsm*37+5.4 cM and *Xpsm*409.1+36.7 cM respectively. Possible explanations for the location of detected QTLs at larger map distances from the flanking marker loci is lack of availability of polymorphic markers to the mapping population parental lines in these genomic regions, occurrence of putative double crossovers and sparse genome coverage of markers in those regions of the pearl millet genetic map. The best single-QTL model explained 50% observed phenotypic variance for the screen against the pathogen population from Patancheru, as well as for the other pathogen populations used in this study. Over-dominant effects were observed for most of the identified QTLs and the resistance was inherited from male parent ICMB 90111-P6. Azhaguvel (2001) have also mentioned similar results from his study of QTL mapping for resistance to downy mildew in pearl millet cross IP 18293 × Tift 238D1.

The total downy mildew disease resistance variation among 172 $F_{2:}F_4$ self-bulk progenies, contributed by best two-QTL models for screen against pathogen populations from Jamnagar and New Delhi were 88.8% and 82.1%, respectively, with acceptable and qualifying LOD scores of 19.8 and 17.0. The positions of detected QTL on LG2 (*Xpsm*708.1) and LG4 (*Xpsm*409.1) controlling resistance against these two pathogen populations from India were similar to those detected for resistance to pathogen populations from Durgapura, Jamnagar, Patancheru and New Delhi. Two QTLs were detected for DM resistance effective against the pathogen population from Jalna, on LG2 (*Xpsmp*2225+30.8 cM) with LOD 4.5 and LG4 (*Xpsm*409.1+40.9 cM) with LOD 10.2. A total of 62.9% of observed phenotypic variation among progenies of 172 $F_{2:4}$ mapping

population for downy mildew reaction in screen against the Jalna pathogen population was explained by these two QTLs. However, the distribution of diseased progenies of mapping population was skewed toward one side and surprisingly, the resistant parent (ICMB 90111-P6) showed more DM infestation in screening against this pathogen population than the susceptible parent (ICMB 89111-P6) of the mapping population under study (Figure 10).

In the present study, for the pathogen population from Maiduguri (Nigeria, West Africa), only a single major QTL on LG2 (*Xpsm*708.1+29.2 cM) was detected. The distributions and segregation of individuals of mapping population progenies were skewed towards resistant parent for this pathogen population. The level of observed phenotypic variance explained by the QTL was 69.5% with a LOD value of 16.2, which qualifies it as a major QTL resistance QTL. None of multiple-QTL models could meet the criteria to be accepted for the screen against this pathogen population. The reasons for only a single QTL could be because of the effect of other QTLs of small effects were masked by the epistatic interaction of the genes, the number of marker loci may not be sufficient to locate the resistance QTLs, or the power and precision of the analysis may be insufficient to detect QTLs of more larger effect because of the relatively small mapping population size and short map length. Moreover, in this study, only those detected QTLs are included which recorded a minimum LOD score of 3.0 and above and QTLs below this LOD score are not reported.

In contrast to the above African pathogen population, four QTLs were identified from phenotypic data obtained from the screen against another pathogen population from Bamako (Mali, West Africa). These were located on LG1 (*Xpsm*492+12.3 cM), LG2 (*Xpsmp*2201+14.2 cM), LG4 (*Xpsm*409.1+20.8 cM) and LG7 (*Xpsm*160+42.1 cM). The highest LOD score for the best single-QTL identified on LG4 was 15.5 and for other single-QTLs detected varied from 3.2 to 5.9. The largest amount of observed phenotypic variation (73.9%) was contributed by this QTL detected on LG4. A three-QTL model with an acceptable LOD score of 19.6, detected QTLs on LG2, LG4 and LG7, and explained 80.3% of phenotypic variation of among progenies of the mapping population under study for DM reaction against this particular pathogen population.

It was very much clear from this study that a quite good number of different resistance QTLs effective against the Indian and African pathogen populations of *S.*

graminicola were detected. But interestingly, one DM resistance QTL common across all screen against the six pathogen populations from India and one from Africa (Bamako, Mali) was identified on LG4. Azhaguvel (2001) also mentioned a common DMR QTL on LG4 for both African pathogen populations in his study. In addition to the above results, there were other major DMR QTLs detected against these pathogen populations, which are either common to a particular position on a given linkage group or on different positions on different linkage groups.

Although the resistance and virulence factors studied here are not likely to be representative of the pearl millet downy mildew host-plant pathogen system as a whole, these results suggest that there are distinct geographical differences in the virulence and pathogenicity of *S. graminicola* populations. Ball and Pike (1983) showed that pearl millet host cultivars responded differently to different sources of downy mildew inoculum. Ball and Pike (1984) reported intercontinental variation of *S. graminicola.* The factors like level of resistance and virulence and trends of host pathogen population relations obtained in this study may differ across mapping populations. Jones *et al. (*1995) reported several downy mildew resistance QTL segregating in the (LGD 1-B-10 × ICMP 84510)-based mapping population that were effective against individual pathogen populations, but found only one possible block of resistance QTLs effective against more than one African downy mildew pathogen population. Azhaguvel (2001) and Kolesnikova-Allen (2001) also have reported similar common blocks of resistance QTLs effective against more than one pathogen population of *S. graminicola* in pearl millet.

For the pearl millet pathogen populations from India and Africa used in this study, a minimum of nine major host plant resistance QTL have been identified on linkage groups LG1, LG2, LG3, LG4, LG7 in the mapping population based on parental lines ICMB 89111-P6 and ICMB 90111-P6. The summary of these QTLs is presented in Table 49. Although, the previous studies have reported independent inheritance of DM resistance to different populations of *S. graminicola* across India and Africa, a common DMR QTL was obtained in this study against all six Indian and one of the two African pathogen populations in the present study. This common QTL was located on LG4 in the vicinity of marker locus *Xpsm* 409.1 with its position varying across pathogen populations with wide range 20 cM to 40 cM. The map positions of this QTL could not be more precisely located because of a shortage of polymorphic marker loci between two

markers flanking it on linkage group 4 in the consensus pearl millet map. For this common QTL as well as for many other QTLs, the mode of resistance was recorded to be over-dominance (except partially dominance in case of the pathogen population from Bamako). Failure to detect this QTL in the screen against the moderately virulent pathogen population from Maiduguri, Nigeria, may have been due to the lack of an appropriate virulence gene to overcoming the allele of susceptible parent ICMB 89111-P6 at this locus or due to presence in the pathogen population of virulence gene capable of overcoming the resistant allele from resistant parent ICMB 90111-P6. Further study will be required to determine which of these two alternatives best accounts for the phenotypic data set in hand. Several other studies (Singh *et al.*, 1978; Dass *et al.*, 1984; Jones *et al.*, 1995; Azhaguvel, 2001) have also revealed over-dominance as a component of downy mildew resistance inheritance in pearl millet. Recessive inheritance of resistance was also witnessed for QTLs effective against pathogen populations from Bamako (LG2, *Xpsmp*2201 + 14.2 cM) and Jodhpur (LG1, *Xpsm*280 + 36.2 cM and LG7, *Xpsm*160 + 40.2 cM), with additive and dominance modes of inheritance for two DMR QTLs effective against the pathogen population from Bamako (LG1 and LG7). Singh *et al.* (1978) studied downy mildew inheritance in pearl millet and reported a recessive resistance gene. This type of resistance has also been observed in other plant-pathogen systems (Day, 1974; de Wit 1992) and in pearl millet downy mildew QTL studies (Azhaguvel, 2001). Most previous reports on downy mildew resistance in pearl millet have suggested dominance as an important component of resistance (Appadurai *et al.* 1975; Gill *et al.*, 1975; Pethani *et al.*, 1980; Basavaraju *et al.*, 1981b; Shinde, 1984; Mehta and Dang, 1987). Interval mapping procedures implemented in Mapmaker/QTL along with composite interval mapping implemented in PlabQTL were used for QTL analysis in the present study. There were no major differences recorded in QTL maps obtained by these two procedures except minor differences in position of QTLs and changes of inheritance pattern in case of a few pathogen populations. There were few QTLs detected by MapMaker/QTL but not detected by PlabQTL and vice-versa because of the difference in algorithms of the two software packages. Jones (1994) observed significant differences between QTL maps produced by MapMaker/QTL and linear regression analysis. But the earlier studies (Paterson *et al.*, 1988; Stuber *et al.*, 1992; Bubeck *et al.*, 1993; Pè *et al.*, 1993; van Ooijen, 1994) compared different methods of identification of QTLs and observed no major differences in the QTL maps produced.

Dudley (1993) suggested that this was due to the normal distribution of data so that maximum likelihood estimates were reduced to least squares estimates. In present study, non-normal distribution of DMI value for several of the phenotypic data sets may therefore have contributed to the differences observed between results from simple interval mapping and composite interval mapping.

In the present study, it has been noticed that for some QTL loci, the mode of inheritance varied across different pathogen populations. The resistance QTL on LG4 (*Xpsm*409.1) was over-dominant for all pathogen populations under study, except against Bamako, where it was partially dominant. Other example is a QTL on LG7 (*Xpsm*160), for which resistance was recessively governed against the pathogen population from Jodhpur. This QTL showed additive inheritance of resistance against the Bamako pathogen population and over-dominant inheritance against that from Durgapura. In general, resistance against the pathogen population from Bamako showed different modes of inheritance for the DMR QTL detected at similar map positions in screens against other pathogen populations. This was repeated in multiple-QTL models for resistance against the pathogen populations from Bamako, Durgapura and Patancheru. The logical and possible explanations to such effects was the observed differences between putative and significant QTLs, the estimation of mode of inheritance theoretically explains this due to error in assessing QTLs of small effect, or due to effects of closely linked genes, different alleles at the same locus or inter-allelic dominance interactions affecting the apparent inheritance of resistance (Jones, 1994), and differences in the virulence of pathogen populations used to detect these QTLs.

5.5. MULTILOCATION TRIALS OF DIFFERENT VERSIONS OF HHB 94-LIKE HYBRIDS

5.5.1. Genotype × environment interaction studies

Genotypic-by-environment interactions (GEI) are almost unanimously considered to be among the major factors limiting response to selection and, in general, the efficiency of the plant and animal breeding programs. That is why the study of genotype-by-environment interactions has become imperative in crop cultivars/hybrids evaluation. GEI is composed of G × Location, G × Year and G × L × Y constituents but in the present study, sums of squares for genotypes, environments, and genotype × environment

interactions have been calculated, with year being confounded within the locations components. Significant GEI tends to hinder genetic progress in breeding programs, in particular, the crossover type of GEI makes it difficult to unambiguously select promising materials that perform consistantly better across a wide range of environmental conditions. The first step in dealing with the consequences of GEI is to assess its relative importance through pooled analysis of data across the testing sites.

Trials conducted at different locations to identify or elucidate the effect of the environment and assess the relative importance of genotype × environment interaction effects and genotypic effects, because differential expression of a phenotypic trait by genotypes across environments, or genotype × environment interaction, is an old problem of primary importance for quantitative genetics and plant breeding trials (Eberhart and Russell, 1966; Falconer, 1981; Via and Lande, 1987; Tiret *et al.,* 1993).

The estimates of mean sums of squares from eleven HHB 94-like hybrids and controls for yield and yield components were found statistically to be highly significant for genotypes, environments and G × E interactions (except for effective plant stand per square meter) across all the ten test environments (Table 21) suggesting the presence of genetic variability and environmental differences. Based upon analysis of the pooled data in this study, the environment represented the major source of variation, followed by genotypes and G × E interactions.

Results (Table 21-24) of cluster-wise pooled analyses carried out to assess the extent of G × E interactions in three different clusters of test environments (made depending upon the environmental similarity and physical closeness of locations as described in earlier studies by Rachaputi (2003) and Nigam *et al.* (2003) in groundnut). The multiple-test environment cluster for Andhra Pradesh revealed significant variation among genotypes (all observed traits except for effective plant stand), environments (all observed traits except for panicle diameter) and G × E interactions (for time to 50% flowering, panicle length and 1000-grain mass) (Table 22). The results of this study are slightly different from the usual pattern followed, where a major portion of variation is contributed by environment followed by G × E interaction and genotypes, as is usually been observed in multi-environmental trials in most crops. Similar results were obtained in the study earlier reported by Ali *et al.* (2001) in pearl millet. In contrast, the multiple-test environment clusters for Haryana and Rajasthan exhibited slightly different patterns

of sources of variation. For both the clusters, the environmental variance was greater than both those attributable to G × E and genotypes (as usual) but G × E variance was higher than the genotypic variance for some of the traits in this study. Consequently, the operational heritability (on both entry-mean basis and plot basis) values were observed to be surprisingly and strikingly variable ranging from some negative values (all of which were considered to be poor estimates of zero for the purposes of this study) to very high (>0.95) for many traits. The variation among G × E interactions across the multiple-test environment clusters was non-significant for most traits except grain yield, panicle length and harvest index in Rajasthan. Haryana test environments exhibited non-significant G × E interactions only for effective plant stand, panicle grain number and harvest index (Tables 23-24).

In previous studies G × E interactions were found to be significant for pearl millet grain yield and its component traits except for time to 50% flowering, plant height, and total and effective numbers of tillers per plant (Chikurte *et al.*, 2003). Similar results were also reported by Tyagi *et al.* (1979), Dass *et al.* (1985), Dahiya *et al.* (1987). Bhaviskar (1990), Suryavanshi *et al.* (1991) and Anarase *et al.* (2000) in pearl millet. Gupta *et al.* (1975) have observed occurrence of environmental (seasonal) and G × E interactions in pearl millet for fodder yield and its component traits. The presence of significant G × E interactions has been further reported by Gupta (1979), Mangat (1992), and Wilson *et al.* (1993). However, such studies simply give preliminary information about the phenotypic stability of genotypes, as is the objective of the present study.

In addition to estimating G × E interactions and agronomic mean performance of several HHB 94-like hybrids, the present multilocation trials study also aims at assessing cytoplasmic effects contributing to grain yield between hybrids of different sub-selections of ICMA 89111, ICMB 89111 and ICMB 90111 across multilocation trials.

Coefficient of variation (CV) were <20% for all multiple-test environment clusters for all traits except grain yield in Haryana and Rajasthan, fresh straw yield in Rajasthan, dry straw yield in the pooled analysis across all ten test environments in India and across the Haryana and Rajasthan clusters, panicle mass in Rajasthan, effective tiller number across all ten test environments and Haryana, and fresh straw yield, dry straw yield and total above-ground biomass yield in the Rajasthan test environment cluster. CV values were higher in Rajasthan than Haryana for most of traits, perhaps because of differences

in agroclimatic conditions in these test environment clusters for example, heavy rain on 10-15 days old crop, preventing maintenance of plant population and terminal drought stress cause heavy losses in yield and its component traits. Similar patterns were observed when close attention was given to test entry differences individual test environments. In contrast to these results, Ali *et al.* (2001) have reported CV values less than 18% for all grain and stover yield component traits from multilocation trials of pearl millet conducted in India.

Such multilocation/phenotypic studies help plant breeders in two ways: (1) to identify genotypes that perform better over a range of environments, and those that perform best under specific environmental conditions, and (2) to minimize the bias caused by genotype × environmental interactions in the estimates of different components of genetic variation (Khairwal and Singh, 1999). Regression procedures have been used to study multilocational performance of pearl millet populations, which allow partitioning of G × E interactions into linear (predictable) and non-linear (unpredictable) components.

5.5.2. Mean performance of HHB 94-like hybrids

The efficiency of any breeding program mainly depends upon the choice of parents. While choosing the parents of hybrids, high mean value is considered as the main selection criterion. In this study, the mean performance of HHB 94-like hybrids revealed no significant differences among genotypes across test environments, or across clusters of test environments among traits except for time to 50% flowering. The significance of any pair of two genotypes depends on difference of two genotypic means exceeding a critical value LSD (p=0.05) (Baker, 1990) irrespective of sign. But in this study, differences were compared with HSD (honestly significant differences, Tukey's test) for a more conservative testing of the significance of performance differences among genotypes across test environments.

G × E interactions become important when ranks of the genotypes changes in different environments. These changes in rank are defined as cross over G × E (Baker, 1988). Changes in ranking make it difficult for plant breeders to decide which genotypes to be selected (Nguyen *et al.*, 1980). The various versions of HHB 94-like hybrids in present study changed the ranks of mean performances for most traits in the different individual test environments as depicted in Figures 38-52 for grain and stover yield and

their component traits. Better rank orders (first, second and third etc.) were observed for hybrid versions based on ICMB 90111 for traits like grain yield, effective plant stand (ICMH 02005), panicle length, straw moisture content, effective tiller number, panicle yield, 1000-grain mass (ICMH 02005), and harvest index in most of the individual test environments, while hybrids based on sub-selections of ICMA 89111 scored better rank orders for fresh straw yield, dry straw yield and 1000-grain mass in some of these test environments. ICMB 89111-based hybrids could performed better for time to 50% flowering (HHB 94-P6B), plant height, panicle yield, panicle diameter, fresh straw yield, dry straw yield (HHB 94-P6B), panicle grain number and total above-ground biomass yield. In an earlier study (Rai *et al.*, 2000), combined analysis of variance for grain yield revealed highly significant differences among genotypes as well as G × E interactions ($p<0.01$) where F_1 hybrids had ICMB 89111 in their parentage. Closer inspection (from results of individual test environments) revealed the causes of G × E interactions were primarily related to changes in the magnitude but also included genotype rank changes.

In comparison to these nine hybrid versions of HHB 94-like hybrids, both hybrid control entries (HHB 94 original and HHB 181), involving ICMA 89111 in their parentage for F_1 hybrids, performed either better than/or at par with all/some of the new versions of HHB 94-like new hybrid versions for grain yield, time to 50% flowering, effective plant stand (HHB 94-original), plant height, straw moisture content, effective tiller number, panicle yield, 1000-grain mass, panicle grain number (Rajasthan and Haryana test environment clusters), total above-ground biomass yield (HHB 94-original), and harvest index in most of test environments. The mean performance of the HHB 94-like hybrids in these multi-environment trials conducted in ten different test environments as well as their multiple-test environment clusters varied for different traits.

Mean grain yield was higher at Patancheru followed by Haryana, and lowest in Rajasthan, without revealing any significant differences among HHB 94-like hybrids, but showing large differences because of the better performance of one location over the other. HHB 94-like hybrids based on sub-selections of ICMA 89111 (HHB 94-P5A) performed better at Patancheru and in Haryana than in Rajasthan (Table 25). In a previous study in sorghum, the comparison of F_1 hybrids and A-lines has shown that high yielding A-lines do not necessarily produce high yielding F_1 hybrids (Hookstra and Ross, 1982; Gorz *et al.*, 1984). In one of such study Rai *et al* (2000) have reported that the least

responsive F_1 hybrids involved at least one least responsive inbred line in their parentage. This suggests the possibility of producing more responsive F_1 hybrids can be considerably increased by including in their parentage at least one highly responsive inbred line or by excluding lines having poor responsiveness. Hybrid control entry HHB 181 was superior in grain yield to all of the newly developed versions of HHB 94 across all test environments included in this study. The observed differences in trial mean performance in the different test environments and test environment clusters were mainly because of environmental factors viz. sowing time, amount and time of rains, type of soil, weather conditions at different stages of the crop, agronomic practices followed at different test environments, and, of course, poor adaptation of hybrids to the harder production environments in Rajasthan.

As the trials were over sown and thinned to a uniform stand in each of the test environments, no differences between entries were expected for effective plant stand and the observed lack of significant pair-wise differences between entries in the pooled analysis across each of the state-wise clusters of these (Tables 25-28) can be taken as an indication that the crop establishment was well managed in each of the trial test environment s, despite ANOVAs suggesting significant genotypic variation for this trait in Haryana test environment cluster (Table 23) and across all ten test environment (Table 21).

Within a test environment cluster, differences were noticed in mean grain yield between different hybrid versions and control entries in spite of physical closeness and similarities of the locations and seasonal effects. Nagaur (Rajasthan) had the lowest grain yield because of lack of uniform and adequate plant population and numbers of effective tillers due to heavy rains when the crop was 10-15 days old followed by a pro-longed dry spell (terminal drought) in later stages of crop growth. HHB 94-like hybrids took comparatively less mean time to complete 50% flowering at Patancheru than in Haryana and in Rajasthan, mainly because of environmental and location effects which include photoperiod, temperature, wind velocity etc. Bawal and Hisar in *kharif* 2003 required 55 and 54 days, respectively, to complete 50% flowering followed by Nagaur (53 days) and RCE 24C (51 days) at Patancheru, also in *kharif* 2003.

The mean performance for effective plant stand was almost uniform with out any significant differences among HHB 94-like hybrids within each of the three clusters and

across India as a whole (Table 25). But across individual test environments, effective plant stand had a wide range from 8.0 plants m^{-2} (RP 9A K02) to 15.0 plants m^{-2} (Hisar K03). Hybrids based on ICMB 89111 produced slightly higher effective plant stands at Patancheru and in Haryana, but in Rajasthan ICMA 89111-based hybrids had higher plant stands. Mean performance for effective tiller number differed significantly across test environment clusters and individual test environments, but not among the HHB 94-like hybrids themselves. This trend indicated mainly environmental differences for this trait because of location effect and poor adaptability of the genotypes to a specific environment with no genotypic differences. The number of effective tillers ranged from 13.7 tillers m^{-2} (Nagaur) to 43.2 tillers m^{-2} (RP 6B K03) across individual locations and a grand mean of 16 tillers m^{-2} (Rajasthan cluster) to 37 tillers m^{-2} (Patancheru cluster) across clusters due to variation in plant stand, adaptability of genotypes and location effects. Effective tiller number of any cereal crop is of great importance for grain yield. In a previous study of yield and its components in pearl millet in Botswana by Karikari and Mosekiemang (2002) it has been stated that as the population increased, the development of tillers terminated earlier in growth of the plant resulting in reduced tiller survival rates therefore reduced number of productive tillers per plant. Consequently, grain yield per plant declined owing to reduced panicle numbers and also to lower grain numbers per panicle. The observed increase in grain yield as tiller numbers increased is in agreement with Kassam (1976) who reported that tillers contributed about 25% of the total grain yield. Carberry *et al.* (1985) and Crawfurd and Bidinger (1989) reported that primary pearl millet grain yield component affected by population was tiller numbers per plant. Karikari and Ngwako (1999) have advocated maintaining three tillers to have grain yield advantages.

After effective plant stand and effective tiller number, it is the plant height, which influences plant fresh and dry weight yields. In the present study, firstly HHB 94-like hybrids based on ICMB 89111 had slightly shorter plant heights compared to other versions across test environment clusters. Secondly, trials conducted at Patancheru recorded shorter plant height (perhaps due to earlier flowering induced by the shorter photoperiods in this lower latitude location) followed by the Rajasthan and Haryana test environment clusters. Fresh straw yield, dry straw yield and straw moisture content exhibited significant variation across test environment clusters and within clusters across the ten test environments. But the differences for fresh straw yield among genotypes were

not significant. RCE 24 K02 (Patancheru) and Nagaur (Rajasthan) were ranked as first and last, respectively, for fresh straw yields. The main reasons for the differences in trial mean performances for these three straw related traits included location effects, adaptation of the genotypes to different target locations, differences in effective plant stands and tiller numbers, and lastly, the agronomic practices followed along with different procedures used in taking dry straw weights. In test environments at ICRISAT-Patancheru, the dry straw weight was taken after over-drying the samples for 60 hours. But in Haryana and Rajasthan, the harvested material was left as such in the field and sun-dried for 2-3 weeks, which is a very crude method of estimating dry weight and calculating straw moisture content.

Traits like panicle length and diameter are important to achieve good mean performance of panicle yield. ICMB 89111 based hybrids demonstrated better mean performance for panicle diameter while hybrids based on sub-selections of ICMA 89111 had better performance for panicle length across all test environment clusters and pooled across all ten test environments in the present study. Higher panicle yields were observed from HHB 94-like hybrids based on ICMA 89111 across the Patancheru and Haryana test environment clusters but ICMB 89111-based hybrids gave higher values across the Rajasthan cluster. Reasons of the large variation in panicle yields were the variation in effective tiller numbers, plant populations, panicle diameter and length, and location effects. Rajasthan registered the lowest trial mean 1000-grain mass values, with terminal drought during grain-filling being the reason particularly at Nagaur K03 (Tables 25-28). Among the three different region-wise test environment clusters, Patancheru gave highest 1000-grain mass. HHB 94-like hybrids based on sub-selections ICMA 89111 recorded higher 1000-grain mass values than hybrids based on sub-selections of ICMB 89111 or ICMB 90111.

The calculation of average panicle grain number, total above-ground biomass yield, and harvest index values depend upon other component traits like plant stand, effective tiller numbers, grain yield, 1000-grain mass, and fresh and dry straw yields. In this study, Bawal K02 recorded the largest panicle grain number while Hisar K03 had the minimum. Among all test environment clusters, Rajasthan had the largest panicle grain number (Table 28). Total above-ground biomass yield was higher at Hisar K02 because of more vegetative growth due to early sowing and favorable growing conditions. Harvest

index values ranged from 19.7% (Hisar K03) to 41.5% (RP 6B K03, Patancheru). The Patancheru (AP) test environment cluster recorded a higher harvest index mean than did Haryana. In comparison to the experimental HHB 94-like hybrids, both control hybrids (which involve ICMA 89111 in their parentage) performed better for time to 50% flowering, effective plant stand, plant height, panicle diameter, fresh straw yield, dry straw weight (HHB 94-original), straw moisture content, and total above-ground biomass yield (HHB 94-original) in many of the test environments.

5.5.3. Character associations

Correlation studies among quantitative traits provides knowledge of intensity of linkage and pleiotropic effects occurring among traits, and thus facilitates assessment of the feasibility of joint selection for two or more traits and opportunities for selection for more highly heritable secondary trait(s) to achieve genetic gain for primary traits. The trial means from ten different test environments and across three clusters of these (ICRISAT-Patancheru, Haryana and Rajasthan) were computed and Spearman rank correlations were calculated using Genstat version 6.0.

Grain yield was taken as the dependent variable and all other traits were correlated with it. Fresh straw yield, straw moisture content, effective tiller number, panicle yield (Rao and Damodaran, 1964), 1000-grain mass, panicle grain number, total above-ground biomass yield, and harvest index had significant positive Spearman rank correlation with grain yield. Among these traits, panicle yield (0.885) and effective tiller number (0.778) recorded very strong and highly significant associations with grain yield. The positive correlations between effective tiller number and grain yield are in complete agreement with earlier studies [Ayyangar *et al.* (1936), Burton (1951), Ahluwalia and Patnaik (1963), Mahadevappa and Ponnaiya (1967), Gupta (1968), Gupta and Nanda (1971), Phul *et al.* (1974), Rao (1981), and Bhamre and Harinarayana (1992b)]. Shankar *et al.* (1963), Pokhriyal *et al.* (1967), Rao (1981), Burton (1983), Jindla and Gill (1984), Bidinger *et al.* (1993), and Bhamre and Harinarayana (1992b) have reported positive correlations between panicle size and grain yield which is in agreement with observations in the present study.

At the same time traits like time to 50% flowering, plant height, panicle length and diameter were negatively correlated with grain yield. Among these traits time to 50%

flowering (-0.486) registered the highest significant negative correlation followed by panicle length (-0.306) and panicle diameter (-0.221) (Table 44). These results conform to the findings of earlier studies by Gupta (1968), Gupta and Dhillon (1974) and Rao (1981) for negative correlations between days to heading and grain yield. But significantly positive correlations have been reported by Mahadevappa and Ponnaiya (1967) and Patil *et al.* (1989) in pearl millet. These differences are likely due to differences in the genotypes, photo period-temperature regimes, and period for which moisture availability was adequate in these various studies. Negative correlations between plant height and grain yield have also been reported by Mahadevappa and Ponnaiya (1967). But between these traits earlier workers have indicated a positive association between these two traits [Ayyanger *et al.* (1936), Burton (1951), Shankar *et al.* (1963), Rao and Damodraran (1964), Pokhriyal *et al.* (1967), Gupta and Sidhu (1972), Gupta and Dhillon (1974) and Rao (1981)]. Positive correlation between panicle number per plant and grain yield per plant have been reported by Pokhriyal *et al.* (1967), Burton (1983) and Jindla and Gill (1984). Virk (1988), Balakrishna and Vijendra Das (1995) and Khairwal and Singh (1999) have summarized the correlations between various quantitative yield-related traits in pearl millet.

Time to 50% flowering, plant height, panicle length, fresh straw yield, effective tiller number, panicle yield, 1000-grain mass and total above-ground biomass yield exhibited a positive correlation with dry straw yield. Manga and Saxena (1988a, b) also have reported positive correlation between dry shoot yield and total dry matter. In the present study, effective plant stand and dry straw yield were found to have positive but non-significant relationships with grain yield. Amongst all positively correlated traits, total above-ground biomass yield (0.826), fresh straw yield (0.758), and plant height (0.715) exhibited highly significant associations with dry straw yield. On the other hand, only harvest index showed a significant negative correlation (<0.05 probability level) with dry straw yield and other traits like grain yield, effective plant stand, panicle diameter, straw moisture content and panicle grain number displayed positive but non-significant associations with dry straw yield.

Grain yield (0.651), straw moisture content (0.751), effective tiller number (0.472), panicle yield (0.352), and 1000-grain mass (0.680) not only had positive correlations with harvest index but some of them also had highly significant associations.

In contrast, time to 50% flowering (-0.546), plant height (-0.524), panicle diameter (-0.182) and panicle length (-0.490), dry straw yield (-0.678) and total above-ground biomass yield (–0.446) were found to have significant negative relationships with harvest index (Table 43).

Significantly positive yet non-significant correlations were observed for time to 50% flowering with plant height, panicle diameter, and fresh and dry straw yields. Although effective plant stands, straw moisture content and total above-ground biomass yield. Very highly to moderately significant negative associations were observed for time to 50% flowering with harvest index (-0.546), grain yield (-0.486), panicle yield (-0.421), 1000-grain mass (-0.224), panicle grain number (-0.360), and effective tiller number (-0.273). Among earlier workers Phul (1963) reported a positive correlation between days to heading and plant height, while Gupta (1968), Gupta and Dhillon (1974), and Rao (1981) have reported the negative correlations between days to heading and grain yield, which are in agreement with the results of the present study.

5.6. YIELD QTL POTENTIAL FROM MULTILOCATION TRIALS

The multilocation trials of HHB 94-like hybrids conducted across ten different test environments (including both individual test environments and multiple-test environment clusters) exhibited no significant differences in entry mean grain yield performance except in a few test environments. The differences in mean grain yield between the hybrids based on different types of female parents suggest significant contribution of cytoplasm to grain yield within the HHB 94 background. This information in turn, could finally help in estimating the potential of yield QTLs from ICMB 90111 to improve the elite hybrid parental A-/B- pair ICMBA/B 89111 and thus finally improve HHB 94.

The ANOVA to test the significance of a single degree of freedom contrast for cytoplasmic effects on grain yield in HHB 94 background demonstrated little significance of differences in grain yield due to use of the A_1 male-sterility inducing cytoplasm of the A-lines compared with the normal male-fertile cytoplasm of the B-lines except significant differences between average performance of three hybrids based on sub-selections of ICMA 89111 (HHB 94-P2A, -P5A and -P6A) and average performance of three hybrids based on sub-selections of ICMB 89111 (HHB 94-P2B, -P5B and –P6B) from trials conducted at Bawal K02, Durgapura K03 and Patancheru, RCE 24 K03 (Table 47). The

comparison made between mean grain yield performance of HHB 94-P6A and HHB 94-P6B exhibited significant differences in grain yield at Bawal during *kharif* 2002 while between HHB 94-P2A and -P5A, and HHB 94-P2B and -P5B at Durgapura during *kharif* 2003 suggested that the A_1 male-sterile cytoplasm had a positive effect on grain yield in both of these test environments (Tables 45-47).

Similarly, no significant variation in grain yield was observed when comparison was made between each hybrid version based on ICMA 89111 and HHB 94-original, and between the average of HHB 94-P2, -P5A, and -P6A and the original HHB 94 in a majority of the test environments except at Durgapura during *kharif* 2003 for testing of the effect of selection for downy mildew resistance within ICMA background on grain yield performance. These results were confirmed by ANOVA for the single degree of freedom contrast between the grain yield of hybrid versions based on each of three sub-selections of ICMA 89111 and the original HHB 94. These results suggested that the contribution of cytoplasm to grain yield not large enough to cause significant differences between the two cytoplasm versions of hybrids of the three sub-selections of ICMA/ICMB 89111 within a location, but that this small effect of cytoplasm to grain yield was significant in the pooled analysis across locations. This indicates a major role of environment and G × E interactions rather than genotypes for the observed fluctuations in grain yield performance of the closely related hybrids in this trial across test environments. The single degree of freedom comparison made between the two hybrid controls (HHB 94-original and HHB 181) produced on a common CMS line (ICMA 89111) using different pollinators (G73/107 and H 77/833-2, respectively), showed significant differences in grain yield in trials conducted at Hisar (K02 and K03), and at Patancheru (RP 9A K02 and RP 6B K03) (Tables 45-47) with superior performance of HHB 181. The probable explanation for the observed variation in differences between these two control hybrids could be to due pollinator's effect and its interaction with environmental forces working differently in the different test environments.

Another comparison was carried out testing for yield potential QTL in the normal male-fertile cytoplasmic-genetic background of ICMB 90111 to contribute grain yield QTLs to ICMB 89111 that will in turn enhance the performance of HHB 94 (in the normal male-fertile cytoplasm). The ANOVA for this single degree of freedom contrast assessing the potential of ICMB 90111 demonstrated significant difference in grain yield

in three individual test environments (Bawal K03, Hisar K02, RCE 24 K02) and across the Patancheru multiple-test environment cluster. Significant differences were also recorded for mean performance across individual test environments at Bawal K03, Durgapura and RP 9B K03 and across multiple-test environments clusters for Rajasthan and Patancheru as well as for the pooled analysis across all ten test environments. The marked differences obtained were due to nuclear factors contributing to grain yield. These results indicated that ICMB 90111 had high yield potential QTLs that could be used to improve HHB 94 and use this as a parent in hybrids breeding program and as a parent in developing mapping populations based on its cross with ICMB 89111 (as in other parts of this study) could enhance the possibility of improving HHB 94 by marker-assisted selection to introgress QTLs from ICMB 90111 into the background of ICMB 89111 and its male-sterile counterpart ICMA 89111, which is the seed parent of the original HHB 94.

5.7. GENERAL DISCUSSION AND FUTURE PROSPECTS

5.7.1. QTL Mapping

QTL mapping in the present study has lead to the detection of about nine major QTLs for resistance to pearl millet downy mildew resistance that are individually effective against one or more of six Indian and two African pathogen populations of *S. graminicola*. A number of resistance QTLs effective against these pathogen populations were suggested to be common across them and some of these QTLs were found to confer resistance that is potentially non-specific and effective across pearl millet downy mildew pathogen populations. Such QTLs have been placed on LG2, LG4 and LG7 in present study. This kind of disease resistance has, in some other host-pathogen systems, proven to be durable (i.e. to maintain its effectiveness in time and space despite with speedy deployment). That is why these resistance QTLs with potentially greater durability are of considerable interest to plant breeders and plant pathologists. Durability is often conferred by polygenic or non-pathotype-specific resistance (Johnson, 1984).

At the same time, MapMaker/QTL and PlabQTL have also detected some DM resistance QTLs from the present mapping population based on cross ICMB 89111-P6 × ICMB 90111-P6 that appeared to be effective only against specific to pathogen populations. These host plant pathogen-specific DM resistance QTLs have been detected

on LG1, LG2, LG3 and LG7. This kind of disease resistance QTL is generally less likely to confer durable resistance because of host plant-pathogen specificity. Therefore, stability of such disease resistance QTL alleles, when transferred to different genetic backgrounds of our interest and evaluated across environments, is a matter of concern because of the limited knowledge about distribution patterns of virulence genes within and between *S. graminicola* populations. Tanksley and Hewitt (1988) and Witcombe and Hash (2000) have suggested breeding programs based on marker-assisted selection (MAS) and the implications of this kind of QTL into desired agronomically superior genetic backgrounds.

QTLs are considered to be a chromosomal segment containing individual genes of large effect or group of genes that together influence complex traits (Stuber *et al.*, 1999) like grain yield, downy mildew disease reaction and many others. Though a QTL is implicitly understood to represent a single genetic determinant (or a factor), but there are examples of individual QTLs that have been resolved into multiple-genetic factors by recombination (Graham *et al.*, 1997; Yamamoto *et al.*, 1998). Therefore, it may not be important to decide whether the QTL stands for a single genetic factor or cluster of tightly linked genes for manipulation of identified QTLs in applied plant breeding programs. However, if cloning of specific QTLs is vital to their utilization, then chromosomal locations must be limited to a convenient piece of DNA (Paterson *et al.*, 1988). QTL mapping has proven to be an extremely powerful technique for localizing QTLs contributing towards host plant disease resistance to particular regions of the chromosomes of crop species. This has enabled the identification of independent segregation of resistance to different pathogen populations of pearl millet downy mildew, as demonstrated in previous studies (Jones, 1994; Jones *et al.*, 1995, 2002; Kolesnikova-Allen, 2001 and Azhaguvel, 2001).

Simple interval mapping as implemented in Mapmaker/QTL detected a total of 24 single-QTLs for downy mildew resistance using phenotypic data generated by greenhouse seedling screens of 172 F_2 mapping population progenies against eight pathogen populations of *S. graminicola* of Indian (six) and African (two) origin. The detected DMR QTLs exhibited LOD score values of at least 3, included QTLs of both major and minor effect, and were placed across all pearl millet linkage groups except LG5 and LG6. Composite interval mapping as implemented in PlabQTL, a software package using a

different algorithm than Mapmaker/QTL, has also detected almost an equal number of DMR QTLs and confirmed those QTL identified by MapMaker/QTL with slightly changed positions between the same pair of flanking markers. A few putative QTLs, which were not identified by PlabQTL, have been detected in the present study by interval mapping with MapMaker/QTL, using single-QTL models as well as multiple-QTL models. The total estimates of observed phenotypic variation explained for downy mildew disease reaction among 172 $F_{2:4}$ mapping population progenies were higher by those single-major QTL detect by MapMaker/QTL than for QTL detected by PlabQTL. This is probably due to systematic over-estimation of the additive effects of individual QTLs (and multiple-QTL models) by the interval mapping procedure. MapMaker/QTL have also detected QTLs explaining as much as 90% of total observed phenotypic variation in particular screens even in case of qualified multiple-QTL models involving QTLs on LG2, LG3, and LG4 against the pathogen population from ICRISAT-Patancheru, India (*Sg*153).

The accepted multiple-QTLs models have passed a qualifying test indicating they were at least 100 times as probable as the best available model having one/or fewer QTLs, and recorded very high LOD values (as high as 25.1 against the ICRISAT-Patancheru pathogen population). If these multiple-QTL peaks were not false positives then concentration of QTLs along the linkage groups may be a result of tandem duplication. It has been suggested that tandem duplication, translocation and intra-allelic recombination are major components of evolution of multiple-resistance genes in plant (Crute, 1992). Tanksley and Rick (1980) found evidence for tandem duplication in tomato and suggested that diploid higher plants use tandem duplication in absence of ploidy to increase genome size. Linkage and allelism observed for pearl millet downy mildew resistance are commonly observed in other host-pathogen systems. For example, many resistance genes map to LG1 in lettuce against downy mildew (Crute, 1992), to the short arm of LG10 in maize against rust (Hooker, 1985), and to chromosome 5 in barley against powdery mildew (Jørgensen, 1992).

5.7.2. Marker-Assisted Selection

After detection and location of putative QTLs by QTL mapping procedures, the presence and potential of resistance genes that they represent needs to be confirmed by backcrossing single putative QTLs into a susceptible genetic background and assessing

the disease reaction of the introgression line(s) under controlled conditions. The mode of inheritance of resistance genes, their contributions toward resistance across environments, and their pathogen population-specificity all need to be investigated. The creation of near-isogenic lines (NILs) proves useful as resistance genes can be characterized easily in terms of corresponding avirulence genes in the pathogen and selected NILs then used as differential lines to monitor virulence in pathogen populations.

QTL mapping is usually considered to be a preliminary stage in detection of QTLs after successful construction of a genetic linkage map with a fairly good coverage of marker loci across the linkage groups. Marker-based methods applied to segregating populations have provided us with a means to locate QTLs to chromosomal regions and to estimate effects of QTL allele substitution (Lander and Botstein, 1989). The ability to estimate the effects for a qualitative trait can be very useful for designing and application of more efficient breeding strategies. Marker-assisted selection (MAS) for quantitative traits has been proposed by many authors (Tanksley, 1993; Lee, 1995; Kearsy and Pooni, 1996). In backcrossing breeding programs, MAS can efficiently reduce linkage drag and optimize population size by permitting effective selection against the donor genome except for allele(s) in the genomic region to be introduced from the donor.

Downy mildew resistance QTLs that individually explain a larger amount of observed phenotypic variation (>50%) in disease reaction in screens against one or more pathogen populations are of considerable interest to plant breeders and plant pathologists. Subsequent marker-assisted introgression of several such QTLs into genetic backgrounds of agronomically elite pearl millet hybrid parental lines is likely to be required for durable resistance to pearl millet downy mildew disease.

The greenhouse seedling downy mildew screens against most pathogen populations of *S. graminicola* in the present study have recorded high operational heritabilities. DMR QTLs identified on LG2, LG4 and LG7 explained high portions of the observed phenotypic variance for disease reaction among the 172 $F_{2:4}$ mapping population progenies across the eight pathogen populations used in the present study. High operational heritabilities are of paramount importance for effective QTL detection, yet MAS is considered to be most efficient for the selection of poorly heritable traits (Paterson, 1991a). An added advantage of dealing with a highly heritable trait is that it is less likely to have variable expression in different genetic backgrounds and across

different environments (Gale and Witcombe, 1992). Soller and Beckmann (1990) stated that MAS for quantitative traits with high heritability would not necessarily be as efficient as conventional breeding. Hash *et al.* (2000) discussed alternate methods of marker-assisted backcross transfer of QTLs detected for downy mildew resistance as well as drought tolerance in pearl millet. Use of MAS has more advantages for the construction of NILs, compared to conventional breeding methods. As well as allowing transfer of resistance donor chromosomal segments of interest, markers covering the remaining genome ensure minimal introgression of other genome segments from the resistance donor. Segments other than those containing the target resistance gene could considerably confuse further studies (Zeven *et al.*, 1983).

Published reports of successful application of MAS to improve hybrid yield and disease resistance are just beginning to appear. The first successful application and achievement of public-sector MAS after rice, is being accomplished in pearl millet for improvement of downy mildew resistance of inbred pollinator line H 77/833-2, elite parental line of popular pearl millet hybrid HHB 67 (Hash *et* al., 2003) from CCS Haryana Agricultural University Hisar, India. Sharma (2001) has developed several improved versions of this pearl millet pollinator by marker-assisted backcrossing, which has to be time and cost-effective for downy mildew resistance in pearl millet.

Mainly two cultivar types, open pollinated varieties bred by random-mating sets of selected inbred lines or progenies from recurrent selection schemes, and single-cross hybrids based on the A_1 cytoplasmic-genetic male-sterility system provide the major stock of commercial seed production in pearl millet in India. In this regard, MAS will enable us to achieve great progress to be made through incorporation of additional disease resistance into the inbred parents of agronomically superior high yielding hybrids of similar maturity, but lacking resistance durability. Firstly, gene pyramiding where a breeding strategy to increase resistance durability is expected to require more than one resistance gene to be incorporated from various sources (Jones *et al.*, 1995) into a single male-sterile line and its hybrids. Secondly, an alternative to gene pyramiding is the sequential deployment of resistance genes over a course of time developing hybrids/cultivars that are genetically heterogeneous for resistance genes (Witcombe and Hash, 2000). MAS can be utilized to establish the most appropriate resistant gene combination/deployment strategy that will allow resistance durability across

environments. Similar studies have been proposed by Pederson and Leath (1988) to evaluate the effectiveness of pyramided resistance to control *Puccinia graminis* in wheat. The parent ICMA 89111 serves as a female parent for producing commercial hybrids (HHB 94, HHB 181 *etc.*) that have released from CCS Haryana Agricultural University, Hisar (India). Improving the combining ability for downy mildew resistance and yield potential of ICMB 89111 (seed parent maintainer of recently release hybrid HHB 94) may usher in new ways of improving hybrids released for Haryana and Rajasthan. Therefore, the DMR QTLs identified from this study can be used in a marker-assisted backcrossing program for hybrid parental line improvement. Yadav *et al.* (2002) suggested a similar strategy in pearl millet to transfer a major drought tolerance QTL into elite pollinator inbred H 77/833-2 (male parent of early-flowering released hybrid, HHB 67) using marker-assisted backcrossing to introgress genomic regions from donor PRLT 2/89-33.

5.7.3. QTL-map-based cloning

Genetic markers are invariably linked to the resistance gene rather being homologous to it. The prior knowledge of segregation pattern of DNA markers (e.g. RFLP and SSRs) linked to the resistance gene will enhance selection efficiency a great deal. However, the progress made in biotechnology for resistance gene cloning has the potential to provide a solution to this problem. A number of genes have been cloned over the last years (Martin *et al.*, 1993; Jones *et al.*, 1994; Lawrence *et al.*, 1994). The possibility of sequence homology between some of the resistance genes combined with QTL mapping studies will enable genes encoding resistance to pearl millet downy mildew to be cloned. Once clones have been sequenced, PCR-based markers could be constructed so that similar resistance genes can be quickly and efficiently detected and selected by the plant breeder using limited technology.

The detection of precise and accurate location of QTLs by genetic linkage mapping provides a starting point for map-based cloning (Tanksley *et al.*, 1995). In many plant species, several agronomically important genes have been isolated using map-based cloning techniques, for example the gene controlling photoperiod-sensitive flowering response in *Arabidopsis* (Putternil *et al.*, 1995). From high-density maps of molecular marker in crops species, one could explore new gene-cloning approaches and opportunities, such as map-based cloning or positional cloning, which make it possible to

really identify those particular gene(s) responsible for the QTL. For such successful accomplishments, we need to have comprehensive genomic libraries of relatively large DNA fragments, especially BAC (Bacterial Artificial Chromosome) vectors, with closely linked DNA markers, ideally less than four hundred-kilo bases apart (Xu, 1997). Martin *et al.* (1993) isolated the tomato gene *Pto* conferring resistance to the bacterial pathogen *Psendomonas syringe*, by this approach.

The most recent advances in molecular biology will allow accelerating plant breeding programs but it will never completely substitute the techniques and methodologies of conventional plant breeding (Paterson *et al.*, 1991b; Lande and Thompson, 1990). At the same time, DNA markers can be expensive to use, relatively time consuming to assay, and require a substantial and relatively sophisticated laboratory system (Gale and Witcombe, 1992). While development of new technologies like automated DNA extraction for larger number of samples at a stretch and different kind of PCR (polymerase chain reaction)-based molecular markers will largely alleviate these difficulties, the problem associated with stability of gene expression under variable genetic backgrounds and environments still needs to be addressed. Tanksley and Hewitt (1988) have described the several phenotypic expressions of a chromosomal segment of *Lycopericon chmielewskii* in different genetic backgrounds. Environmental variation has also been observed to have significant effects on QTLs for yield and its components (Stuber *et al.*, 1987; Paterson *et al.*, 1991a; Beavis *et al.*, 1991; Bubeck *et al.*, 1993; Yadav *et al.*, 2003).

After considering the facts mentioned above, the need to look into the magnitude of these complications along with a multitude of other related characteristics that needs immediate solution, is obvious. The discerning eyes of plant breeders will retain their essential role in assessing phenotypic expression of improved varieties/hybrids from the laboratory to field trials. In future with integration of the artistic, scientific and technological skills an efficient plant breeder will continue to play a vital role in bringing new green revolution products in a ever-increasing population of resource-limited SAT regions of the world of the twenty-first century.

The first green revolution was targeted at few major crops and the major beneficiaries were the larger producers and consumers in western world and some parts of developing countries (e.g. the northern part of India), who possessed high operational and

capital funds and ready-to-use natural resources, and could invest the required-inputs in cultivating the improved crop cultivars. But this second green revolution needs to be targeted at subsistence farmers and crops like pearl millet, which provides the livelihood of millions of marginal and resource-limited poor farmers in relatively less fertile SAT regions of the world.

CHAPTER 6

SUMMARY

6. SUMMARY

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] ranks 6th among cereals and is the staple food grain crop for about 90 million people living in the semi-arid tropical regions of Africa and the India sub-continent. It is better adapted than other cereals to marginal lands of low fertility that receive minimal application of inputs. It is a multi-purpose cereal grown for grain, stover and green fodder on about 27 million hectares, primarily in Asia and Africa (FAO and ICRISAT, 1996). Pearl millet is also considered a good experimental plant for genetical studies because of its low-diploid chromosome number (2n = 14) with moderate high DNA content, short duration, large seed number, and protogynous flowering.

Among diseases affecting pearl millet, downy mildew, also known as green ear disease, is most devastating. It is caused by systemic infection by the obligate biotrophic pseudo-fungus *Sclerospora graminicola* (Sacc.) J. Schroet. The most efficient, environmental friendly and economical means to control pearl millet downy mildew is the use of resistant cultivars. Therefore, breeding for improvement of yield and resistance to downy mildew has been a prime concern of pearl millet breeders but the allogamous and highly variable nature of both the host and the pathogen have been considerable hindrance to breeding for host plant resistance to this disease. Earlier studies on this host-pathogen interaction have shown that resistance is polygenically controlled and often dominant with pathogen populations exhibiting host-specific virulence difference. Another major factor limiting response to selection and hence breeding efforts is Genotype-by-Environment interactions of which host specific virulence differences are an example.

The present study was based on a cross of parental lines ICMB 89111-P6 (d_2 dwarf, high tillering, maintainer with site-specific resistance to downy mildew) and ICMB 90111-P6 (tall, and high tillering, with highly stable resistance to downy mildew). This study was designed to construct a skeleton linkage map, based on ICMB 89111-P6 × ICMB 90111-P6, to identify and map QTLs controlling downy mildew resistance (DMR), to study inheritance of DMR and finally, to assess agronomic performance of versions of HHB 94-like hybrids having their seed parents sub-selections of ICMA/B 89111 and

ICMB 90111 in multilocation trials. To fulfill these objectives, the two pearl millet inbred parental lines were crossed to F_1, and the F_1 progenies were selfed to produced 172 segregating F_2 mapping population progenies for generating marker data using 46 SSR and RFLP markers exhibiting clear polymorphism between parental lines. The F_2 mapping population progenies were selfed and bulked for two generations to produce $F_{2:4}$ self-bulks for DM screening against eight pathogen populations from India and Africa. The downy mildew screening of segregating $F_{2:4}$ population progenies based on ICMB 89111-P6 × ICMB 90111-P6 against six downy mildew pathogen populations from India (Patancheru, Jodhpur, New Delhi, Jamnagar, Jalna and Durgapura) was done at ICRISAT-Patancheru and against two African pathogen populations (Maiduguri and Bamako) at Bangor, UK. The segregating $F_{2:4}$ mapping population progenies showed a continuous variation for DMI and inheritance patterns varied across the pathogen populations. Mendelian segregation patterns among the 172 $F_{2:4}$ self-bulks screened against pathogen populations from India and Africa showed that at least one to four (monogenic to tetragenic) genes were controlling resistance to this range of pathogen populations.

Spearman rank correlations revealed a general trend of stronger similarities among pathogen populations from India and between Indian and African populations than between the two African pathogen populations themselves. Pathogen populations from northern India were more highly correlated among themselves than those from southern India. The cluster analysis demonstrated that the pathogen population from Jalna was relatively dissimilar from all other pathogen populations included in this study. In contrast, the dendrogram based on entry mean DMI values revealed that the pathogen population from Jamnagar was closely related with that from Durgapura and that from Patancheru with that from New Delhi.

The construction of genetic linkage maps and QTL mapping for economical traits in fields crops are very important tools for studying genome structure, identifying introgression between genomes and localizing genes of interest in genomic regions. Co-dominant markers such as RFLPs and SSRs have simple genetic segregation patterns and are potentially abundant in number. The first RFLP-based genetic linkage map of pearl millet was constructed by Liu *et al.* (1994). The parental lines were screened against a set of 80 SSR and 35 RFLP markers following standardized protocols and 40% of these

exhibiting clear and scorable polymorphism was used to generate the mapping population marker data.

Chi square estimates revealed the majority of selected polymorphic marker loci used for genotyping the mapping population segregated as per expected Mendelian segregation ratios of 1:2:1. However, about 60% of marker loci exhibited distorted segregation, with the abundance of heterozygotes and homozygotes for alleles of the resistant male parent (ICMB 90111-P6). The marker data from 46 marker loci was used to construct a skeleton linkage map of pearl millet cross ICMB 89111-P6 × ICMB 90111-P6. The number of linkage groups and marker orders in each of the seven newly constructed linkage group were the same as that of base map (Liu *et al.*, 1994), integrated consensus map (Qi *et al.*, 2004), and other previously constructed pearl millet marker-based genetic linkage maps.

A genetic linkage map of 747.9 cM (Haldane) was constructed for these 46 marker loci using MapMaker/Exp version 3.0. Among seven linkage groups LG3 was the shortest and LG7 was the largest one. For QTL mapping, interval mapping implemented in MapMaker/QTL and composite interval mapping method as implemented in PlabQTL were used. A total of nine different major disease resistance QTLs were identified from eight screens of the $F_{2:4}$ mapping population against pathogen populations from India and Africa. Among these, a common resistance QTL was identified on LG4, which was effective against all six Indian and one (Bamako) of the African pathogen populations. Another common QTL was identified on LG2, which was effective against four Indian (Durgapura, Patancheru, Jamnagar and New Delhi) and one African (Maiduguri) pathogen populations. Such disease resistance, appropriately backstopped by pyramiding with additional resistances, expected to be durable for longer periods. Several pathogen population-specific DM resistance QTLs were also identified on LG1, LG2, LG4 and LG7, but such disease resistances are not considered likely to be durable ones.

Accurate knowledge of inheritance patterns of disease resistance is of paramount importance in breeding disease resistant cultivars. DNA markers (especially co-dominant markers) and QTL mapping provide insights into facets of quantitative inheritance patterns. In present study, a majority of downy mildew resistance QTLs detected exhibited over-dominant modes of inheritance, and most of DMR resistant QTLs come from resistant male parent ICMB 90111-P6. These identified DMR QTLs from ICMB

90111-P6 can now be transferred to genetic backgrounds of elite pearl millet hybrid parental lines (*e.g.* ICMB 89111) through marker-assisted backcrossing programs. Flanking markers of the identified QTLs can facilitate selection of resistant progenies during the backcrossing process, where as other marker loci can be used in reducing the length of the donor segments carried along with the introgressed DM resistance genes and/or selecting for recovery of recurrent parent alleles on non-carrier chromosomes. Marker-assisted selection can also be used to pyramid several disease resistance genes into a single male-sterile line (and its maintainer) or pollinator line.

The pathogen populations elicited significantly different downy mildew reactions among the 172 $F_{2:4}$ self-bulks across all screens against eight pathogen populations. The pathogen population from Jodhpur (India) and Bamako (Africa) were observed as the most highly virulent among all Indian and African pathogen populations included in this study. All screens against individual pathogen populations, as well as across all eight pathogen populations exhibited high heritabilities which were sufficient to permit the use of these in QTL mapping. The pooled ANOVA from 172 $F_{2:4}$ self-bulks screened against six Indian (Asian) and two African pathogen populations revealed significant intracontinental pathogenic variability while variance component analysis revealed significant intercontinent variability in DMI.

The agronomic performance of HHB 94-like hybrids in multilocation field trials in Haryana, Rajasthan and Andhra Pradesh showed highly significant differences among genotypes (HHB 94-like hybrids), genotype × environment interactions, and environments for grain, and stover yield and their component traits across all ten field environments and across four multiple-field environment clusters. Environments represented the major source of variation in this study, followed by genotypes and genotypes × environment interactions. The average performance of HHB 94-like hybrids was observed to be better in the Andhra Pradesh multiple field-environment cluster followed by those for Haryana and Rajasthan. Hybrid versions based on sub-selections of ICMA 89111 produced higher grain yield while performance of other yield components trait was found to be variable across sub-selections of ICMA/B 89111 and ICMB 90111. Heritability estimates for a majority of traits ranged from high to very high across individual as well as across groups of field trial environments.

Correlation studies provide estimates of the intensity of linkage and pleiotropic effects occurring among quantitative traits. In the present study, grain yield showed strong positive correlation with effective tiller numbers, panicle yield, 1000-grain mass, panicle grain number and harvest index. Breeding efforts to improve these key components of grain yield can be expected to increase crop productivity and production. Negative correlations were recorded between grain yield and both time to 50% flowering and plant height.

Test for yield potential QTL results indicated that ICMB 90111 had high yield potential QTLs that could be used to improve HHB 94 and use this as a parent in hybrids breeding program and as a parent in developing mapping populations based on its cross with ICMB 89111 (as in other parts of this study) could enhance the possibility of improving HHB 94 by marker-assisted selection to introgress QTLs from ICMB 90111 into the background of ICMB 89111 and its male-sterile counterpart ICMA 89111, which is the seed parent of the original HHB 94.

The use of markers in breeding programs can range from facilitating appropriate choice of parents for crosses, to mapping/tagging of gene blocks associated with economically important traits. Molecular markers tightly linked to different disease resistance genes having potential importance in facilitating selection procedures particularly for pyramiding two or more resistance genes with the intension of producing a more durable and broad-spectrum resistance. In spite of these many uses, DNA markers, of course, have demerits, preventing their general use in breeding programs. They are more expensive, require a sophisticated laboratory set up, and consume a lot of time to setup in their initial stages. New technologies like automated DNA extraction, high through-put genotyping systems, and PCR based non-radioactive visualization techniques can overcome many of these difficulties. Undoubtly, the most recent advances in molecular breeding, along with new innovations in bioinformatics, will complement conventional plant breeding methods and permit their acceleration to realize crop improvement and enhance crop productivity in a more directed manner and in less time to fulfill the need of ever increasing human and livestock populations in a resource limited world, especially for SAT regions in the twenty-first century.

CHAPTER 7

CHAPTER 7

BIBLIOGRAPHY

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Agrama, H.A., Wilde, G.E., Reeses, J.C., Campbell, L.R. and Tuinstra, M.R., 2002. Genetic mapping of QTLs associated with green bug resistance and tolerancein *Sorghum bicolor. Theor. Appll. Genet.*, ***104***: 1373-1378.

Ahluwalia, M. and Patnaik, M.C., 1963. A study of heterosis in pearl millet. *Indian J. Genet. Plant Breed.*, ***23***: 34-38.

Ahmad, R., Shetty, H.S. and Safeeulla, K.M., 1978. Existence of pathogenic races in [*Sclerospora graminicola* (Sacc.) Schroet.] attacking pearl millet [*Pennisetum typhiodes* (Burm.) Stapf & Hubb.] 3rd Intl. Congress of Plant Path., Munich. Pp. 123.

Akkaya, M.S., Bhagwat, A.A. and Cregan, P.B., 1992. Length polymorphisms of simple sequence repeat DNA in soybean. *Genetics*, ***132***: 1131-1139.

Akkaya, M.S., Shoemaker, R.C., Specht, J.E., Bhagwat, A.A. and Cregan, P.B., 1995. Integration of simple sequence repeat DNA markers into a soybean linkage map. *Crop Sci.*, ***35***: 1439-1445.

Aldrich, P.R., Hamrick, J.L., Chavarriaga, P. and Kochert, G., 1998. Microsatellite analysis of demographic genetic structure in fragmented populations of the tropical tree *Symphonia globulifera. Mol. Ecol.*, *7*: 933-944.

Ali, A.M., Hash, C.T., Ibrahim, A.E.S. and Bhasker Raj, A.G., 2001. Population diallel of elite medium- and late-duration pearl millet composites: I. Populations and their F_1 crosses. *Crop Sci.*, ***41***: 705-711.

Allouis, S., Qi, X., Lindup, S., Gale, M.D. and Devos, K.M., 2001. Constructions of BAC library of pearl millet, *Pennisetum glaucum. Theor. Appl. Genet.*, ***102***: 1200-1205.

Anand Kumar, K., 1989. Pearl millet: Current status and future potential. *Outlook on Agriculture*, ***18***: 46-53.

Anand Kumar, K., Andrews, D.J., Jain, R.P. and Singh, S.D., 1984. ICMA-1 and ICMB-2 pearl millet parental lines with A_1 cytoplasmic-genic male sterile system. *Crop Sci.*, ***24***: 832.

Anarase, S.A., Ugale, S.D. and Moholkar, N.D., 2000. Phenotypic stability of yield and yield components in pearl millet. *J. Maharashtra Agric. Univ.*, ***25(3)***: 258-261.

Andrews, D.J., King, S.B., Witcombe, J.R., Singh, S.D., Rai, K.N., Thakur, R.P., Talukdar, B.S., Chavan, S.B. and Singh, P., 1985. Breeding for disease resistance and yield in pearl millet. *Field Crops Res.*, ***11***: 241-258.

Appadurai, R., Parambaramani, C. and Natarajan, V.S., 1975. Note on the inheritance of susceptibility to pearl millet to downy mildew. *Indian J. Agric. Sci.*, ***45***: 179-180.

Appadurai, R., Raveendran, T.S. and Nagarajan, C., 1982. A new male-sterility system in pearl millet. *Indian J. Agric. Sci.*, ***52***: 832-834.

Arunachalam, V. and Chandrashekaran, S., 1993. RFLP approach to breeding for quantitative traits in plants: A critique. *J. Genet.*, ***72***: 73-83.

Ayyangar, G.N.R., Hariharan, P.V. and Rajabhooshanam, D.S., 1936. The relation of some plant characters to yield in *cumbu* [*Pennisetum typhoids* (Burm.) Stapf & Hubbard]. *Madras Agric. Sci.*, ***40***: 895-900.

Azhaguvel, P., 2001. Linkage map construction and identification of QTLs for downy mildew (*Sclerospora graminicola*) resistance in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Ph.D. Thesis, TNAU, Coimbatore.

Azhaguvel, P., Hash, C.T., Rangaswamy, P. and Sharma, A., 2003. Mapping the d_1 and d_2 dwarfing genes and the purple foliage color locus *P* in pearl millet. *J. Heredity*, ***94(2)***: 155-159.

Baker, R.J., 1988. Test crossover for genotype-by-environmental interactions. *Can. J. Plant Sci.*, ***68***: 405-410.

Baker, R.J., 1990. Crossover genotype-environmental interactions in spring wheat. *In*: Genotype-by-environment interactions and plant breeding (Kang, M.S., Ed.). Louisiana State Univ., Banton Rouge. Pp. 42-51.

Balakrishna, A. and Vijendra Das, L.D., 1995. Character association in pearl millet. *Madras Agric. Journal*, ***82***: 59-60.

Ball, S.L. and Pike, D.J., 1983. Pathogenic variability of downy mildew (*Sclerospora graminicola*) on pearl millet. II. Statistical techniques for analysis of data. *Ann. Appl. Biol.*, ***102***: 265-273.

Ball, S.L. and Pike, D.J., 1984. Intercontinental variation of *Sclerospora graminicola*. *Ann. Appl. Biol.*, ***104***: 41-51.

Ball, S.L., 1983. Pathogenic variability of downy mildew (*Sclerospora graminicola*) on pearl millet. I. Host cultivar reactions to infection by different pathogen isolates. *Ann. Appl. Biol.*, ***102***: 257-264.

Ball, S.L., Pike, D.J. and Burridge, C.Y., 1986. Characterization of populations of *Sclerospora graminicola*. *Ann. Appl. Biol.*, ***108***: 519-526.

Basavaraju, R., 1978. The genetics of resistance to downy mildew in pearl millet. Unpub. Ph.D. Thesis, University of Mysore, Mysore. Pp. 215.

Basavaraju, R., Safeeulla, K.M. and Murthy, B.R., 1980. The role of gene effects and heterosis for resistance to downy mildew in pearl millet. *Indian J. Genet.*, ***40(3)***: 537-548.

Basavaraju, R., Safeeulla, K.M. and Murty, B.R., 1981a. Inheritance of resistance to downy mildew in pearl millet. *Indian J. Genet.*, ***41(1)***: 144-149.

Basavaraju, R., Safeeulla, K.M. and Murty, B.R., 1981b. Genetic variance and heritability for resistance to downy mildew in pearl millet. *Indian J. Genet.*, ***41(1)***: 137-143.

Basten, C.J., Zeng, S.B. and Weir, B.S., 1994. ZMAP-A QTL cartographer. *In*: Proceedings of the 5th World Congress on Genetics Applied to Livestock Production: Computing strategies and software. (Smith, C., Gavora, J.S., Benket, B., Chesnais, J., Fairfull, W., Gibson, J.P., Kennedy, B.W. and Burnside, E.B., Eds.). Guelph, Ontario, Canada. ***22***: 65-66.

Basten, C.J., Zeng, S.B. and Weir, B.S., 1997. QTL Cartographer: A reference manual and tutorial for QTL mapping. Department of Statistics, North Carolina State University, Raleigh, North Carolina, USA.

Beavis, W.D., 1998. QTL analysis: Power, precision and accuracy. *In*: Molecular dissection of complex traits (Paterson, A.H., Ed.). CRC Press, Boca Raton, FL. USA. Pp. 145-162.

Beavis, W.D., Grant, D., Albertson, M. and Fincher, R., 1991. Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci. *Theor. Appl. Genet.*, ***83***: 141-145.

Beckmann, J. and Soller, M., 1983. Restriction fragment length polymorphisms in genetic improvement: methodologies, mapping and costs. *Theor. Appl. Genet.*, ***67***: 35-43.

Beckmann, J. and Soller, M., 1986. Restriction fragment length polymorphisms and genetic improvement of agricultural species. *Euphytica*, ***35***: 111-124.

Bell, C.J. and Ecker, J.R., 1994. Assignment of 30 microsatellite loci to linkage map of *Arabidopsis*. *Genomics*, ***19***: 137-144.

Bennett, M.D., 1976. DNA amount, latitude and crop plant distribution. *Environ. Exp. Bot.*, ***16***: 93-108.

Bentolila, S., Hardy, T., Guitton, C. and Freyssinet, G., 1992. Comparative genetic analyses of F_2 plants and anther culture derived plants of maize. *Genome*, ***30***: 575-582.

Bernatzky, R. and Tanksley, S.D., 1986. Towards a saturated linkage map in tomato based on isozymes and random cDNA sequences. *Genetics*, ***112***: 887-898.

Bhamre, D.N. and Harinarayana, G., 1992b. Changes in correlations and partial regression of pearl millet populations under different mating. *J. Maharashtra Agric. Univ.*, ***17***: 192-194.

Bhat, S.S., 1973. Investigations on the biology and control of *Sclerospora graminicola* on bajra. Ph.D. Thesis, University of Mysore, Karnataka, India.

Bhatnagar, S.K., 2003. Coordinator's review of All India Coordinated Pearl Millet Improvement Project 2002-2003. *In:* Indian Council of Agricultural Research, Mandor, Jodhpur, India. Pp. 7.

Bhattacharjee, R., Bramel, P.J., Hash, C.T., Kolesnikova-Allen, M. and Khairwal, I.S., 2002. Assessment of genetic diversity within and between pearl millet landraces. *Theor. Appl. Genet.*, ***105***: 666-673.

Bhaviskar, A.P., 1990. Genetic studies on grain yield and its component trait in pearl millet [*Pennisetum americanum* (L.) Keeke]. Ph.D. Thesis, MPKV, Rahuri, India.

Bidinger, F.R., Alagarswamy, G. and Rai, K.N., 1993. Use of grain number components as selection criteria in pearl millet. *Crop Improv.*, ***20***: 21-26.

Bonamigo, L.A., 1999. Pearl millet crop in Brazil: Implementation and development in the Cerrado savanahas. *In*: Proceedings of the International Pearl Millet Workshop (Neto, A.L. de F., Amabile, R.F., Netto, D.A.M., Yamashita, T. and Gocho, H., Eds). Empbrapa Cerrados: Planaltina, D.F., Brazil. Pp. 31-66.

Bonierbale, M.W., Plaisted, R.L. and Tankley, S.D., 1988. RFLP maps based on common sets of clones reveal modes of chromosome evolution in potato and tomato. *Genetics*, ***120***: 1095-1103.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W., 1980. Construction of a genetic map in man using restriction fragment length polymorphisms. *American J. Hum. Genet.*, ***32***: 314-331.

Bowcock, A.M., Ruiz-Linares, A., Tomfohrde, J., Minch, E., Kidd, J.R. and Cavalli-Sforza, L.L., 1994. High resolution human evolutionary trees with polymorphic microsatellites. *Nature*, ***368***: 455-457.

Bramel-Cox, P.J., 1996. Breeding for reliability of performance across unpredictable environments. *In*: Genotype-by-environment interaction. (Kang, M.S. and Gauch Jr., H.G., Eds.) CRC Press, Inc., Boca Raton, FL. Pp. 309-339.

Breese, W.A., Hash, C.T., Devos, K.M. and Howarth, C.J., 2002. Pearl millet genomics: an overview with respect to breeding for resistance to downy mildew. *In*: Sorghum and Millets Pathology. (Leslie, J.F., Ed.). Ames, Iowa, USA: Iowa State Press. Pp. 243-246.

Brink-Mann, B., Klintschsar, M., Neuhuber, F., Huhne, J. and Rolf, B., 1998. Mutation rate in human microsatellites: Influence of the structure and length of the tandem repeat. *American J. Hum. Genet.*, ***62***: 1408-1415.

Bruford, M.W. and Wayne, R.K., 1993. Microsatellites and their application to population genetic studies. *Current Opinion in Genetics and Development*, ***3***: 939-943.

Brunken, J.N., 1977. A systematic study of *Pennisetum* sect. *Pennisetum* (Gramineae). *American J. Bot.*, ***64***: 161-176.

Bryan, G.J., Collins, A.J., Stephenson, P., Orry, A., Smith, J.B. and Gale, M.D., 1997. Isolation and characterisation of microsatellites from hexaploid bread wheat. *Theor. Appl. Genet.*, ***94***: 557-563.

Brzustowicz, L.M., Merette, C., Xie, X., Townsend, L., Gilliam, T.C. and Ott, J., 1993. Molecular and statistical approaches to the detection and correction of errors in genotypes databases. *American. J. Human Genet.*, ***53***: 1137-1145.

Bubeck, D.M., Goodman, M.M., Beavis, W.D. and Grant, D., 1993. Quantitative trait loci controlling resistance to gray leaf spot in maize. *Crop Sci.*, ***33***: 838-847.

Budak, H.F., Pedraza, P.B., Cregan, P.S., Baenziger and Dweikat, I., 2003. Development and utilization of SSRs to estimate the degree of genetic relationships in a collection of pearl millet germplasm. *Crop Sci.*, ***43***: 2284-2290

Burr, B. and Burr, F.A., 1991. Recombinant inbreds for molecular mapping in maize. *Trends in Genet.*, ***7***: 55-60.

Burr, B., 2001. Some concepts and new methods for molecular mapping in plants. *In*: DNA based markers in plants (Phillips, R.L. and Vasil, I.K., Eds.). Kluwer Academic Publishers, The Netherlands. Pp. 1-8.

Burr, B., Burr, F.A., Thompson, K.H., Albertson, M.C. and Stuber, C.W., 1988. Gene mapping with recombinant inbreds in maize. *Genetics.*, ***118***: 519-526.

Burton, G., 1995. History of hybrid development in pearl millet. *In*: Proceedings of First Grain Pearl Millet Symp. Tifton, GA. 17-18 Jan, 1995. Univ. of Georgia, Tifton. Pp. 5-8.

Burton, G.W. and Wells, H.D., 1981. Use of near-isogonic host populations to estimate the effect of three foliage diseases on pearl millet forage yield. *Phytopathol.*, ***71***: 311-333.

Burton, G.W., 1951. Quantitative inheritance in pearl millet (*Pennisetum glaucum*). *Agronomy Journal.*, ***43***: 409-417.

Burton, G.W., 1952. Quantitative inheritance in grasses. *In*: Proceedings of 6th International Grassland Congress, ***1***: 277-283.

Burton, G.W., 1969. Registration of pearl millet inbreds Tift $23B_1$, Tift $23A_1$, Tift $23DB_1$ and Tift $23BA_1$ (Reg. Nos. PL 1, PL 2, PL 3 and PL 4). *Crop Sci.*, ***9***: 397.

Burton, G.W., 1983. Breeding pearl millet. *Plant Breed. Rev.*, ***1***: 162-182.

Buscot, F., Wipf, D., Battista, C., Munch, J.D., Botton, B. and Martin, F., 1996. DNA spacers and microsatellite-primed PCR. *Mycological Res.*, ***100***: 63-71.

Busso, C.S., Liu, C.J., Hash, C.T., Witcombe, J.R., Devos, K.M., de Wet, J.M.I. and Gale, M.D., 1995. Analysis of recombination rate in female and male gametogenesis in pearl millet (*Pennisetum glaucum*) using RFLP markers. *Theor. Appl. Genet.*, ***90***: 242-246.

Butcher, P.A., Bell, C.J. and Moran, G.F., 1992. Patterns of genetic diversity and nature of breeding system in *Melaleuca alternifolia* (Myrtaceae). *Aust. J. Bot.*, ***40***: 365-375.

Butler, E.J., 1907. Some diseases of cereals caused by *Sclerospora graminicola.* Memories of Department of Agriculture in India. *Botanical Series*, ***2***: 1-24.

Caetano-Anolles, G., 1997. Nucleic scanning by amplification with mini-hairpin and microsatellite oligonucleotide primers. *In:* DNA markers: protocols, applications and overviews (Caetano-Anolles, G. and Gresshoff, P.M., Eds.). Wiley-Liss, Inc., New York. Pp. 91-114

Cameron, D.R. and Moav, R., 1957. Inheritance in *Nicotiana tobacum* XXVII. Pollen killer, an alien genetic locus inducing abortion of microspores not carrying it. *Genetics*, ***42***: 326-335.

Carberry, P.S., Campbell, L.C. and Bidinger, F.R., 1985. The growth and development of pearl millet as affected by plant population. *Field Crops Res.*, ***11***: 193-205.

Causse, M.A., Fulton, T.M., Cho, Y.G., Ahn, S.N., Chunwongse, J., Wu, K., Xiao, J., Yu, Z., Ronald, P.C., Harrington, S.E., Second, G., McCouch, S.R. and Tanksley, S.D., 1994. Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics*, ***138***: 1251-1274.

Cavalli, L.L., 1952. An analysis of linkage in quantitative inheritance. *In*: Quantitative inheritance. (Reeve, E.C.R. and Waddington, C.H., Eds.). His Majesty's Stationary Office, London. Pp. 135-144.

Chao, S., Sharp, P.J., Worland, A.J., Warham, E.J, Koebner, R.M.D and Gale, M.D., 1989. RFLP based genetic maps of wheat homeologus group 7 chromosomes. *Theor. Appl. Genet.*, ***78***: 495-504.

Chase, M.R., Moller, C., Kesseli, R. and Bawa, K.S., 1996. Distant gene flow in tropical trees. *Nature*, ***383***: 398-399.

Chikurte, K.N., Desale, J.S. and Anarase, S., 2003. Genotype × Environment interaction for yield and yield components in pearl millet. *J. Maharashtra Agric. Univ.*, ***28(1)***: 30-33.

Chin, E.C.L., Senior, M.L., Shu, H. and Smith, J.H.C., 1996. Maize simple repetitive DNA sequence: abundance and allele variation. *Genome*, ***39***: 866-873.

Choumane, W., Winter, P., Weigand, F. and Kahl, G., 2000. Conservation and variability of sequence-tagged microsatellite sites (STMSs) from chickpea [*Cicer aerietinum (sic)* L.] within the genus *Cicer*. *Theor. Appl. Genet.*, ***101***: 269-278.

Churchill, G.A. and Doerge, R.W., 1994. Empirical threshold values for quantitative trait mapping. *Genetics*, ***138***: 963-971.

Cloutier, S. and Landry, B.S., 1994. Molecular markers applied to plant tissue culture, *In vitro. Cell Dev Biol.*, ***30***: 32-39.

Cloutier, S., Landry, B.S. and Cappadocia, M., 1991. RFLP analyses of a doubled haploid population of *Brassica napus*. *In*: Proceedings 6th Can. Soc. Plant. Mol. Biol. Ann. Meet University, Laval, Quebec City, Pp. 17.

Collins, V.P., Cantor, A.H., Pescatore, A.J., Straw, M.L. and Ford, M.J., 1997. Pearl millet in layer diets enhances egg yolk n-32 fatty acids. *Poult. Sci.*, ***76(3)***: 326-330.

Conneally, P.M., Edwards, J.H., Kidd, K.K., Lalouel, J.M. and Morton, N.E., 1985. Reports of the committee on methods of linkage analysis and reporting. *Cytogenet. Cell Genet.*, ***40***: 356-359.

Costa, N.L., 1992. Estabelecimento, formacao e manejo de pastagens de milheto. *Lav Arrozeira*, ***45***: 7-12.

Craufurd, P.Q. and Bidinger, F.R., 1989. Potential and realized yield in pearl millet (*Pennisetum americanum)* as influenced by plant population density and life-cycle duration. *Field Crops Res.*, ***22***: 211-225.

Cregan, P. and Quigley, C.V., 1998. Simple sequence repeats DNA marker analysis. *In*: DNA markers: protolcs, applications and overviews. (Caetano-Anolles, G. and Gresshoff, P.M. Eds.). Wiley-Liss, Inc., New York. Pp. 173-185.

Crute, I.R., 1992. From breeding to cloning (and back again?): A case of study with lettuce downy mildew. *Annual Rev. Phytopathol.*, ***30***: 485-506.

Dahiya, B.N., Deswal, O.P., Yadav, H.P. and Kharab, R.P.S., 1987. Stability analysis for grain yield in some advance cross of pearl millet. *Indian J. Hered.*, ***19(3-4)***: 24-29.

Dallas, J.F., 1988. Detection of DNA 'fingerprints' of cultivated rice by hybridization with a human minisatellite DNA probe. *Proc. Natl. Acad. Sci., USA,* ***85***: 6831-6835.

Danesh, D., Aarons, S., McGill, G.E. and Young, N.D., 1994. Genetics dissection of oligogenic resistance to bacterial wilt in tomato. *Mol. Plant-Microbe Interact.*, *7*: 464-471.

Darvasi, A. and Soller, M., 1992. Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus. *Theor. Appl. Genet.*, ***85***: 353-359.

Darvasi, A., Weinreb, A., Minke, V., Weller, J.I. and Soller, M., 1993. Detecting marker-QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. *Genetics*, ***134***: 943-951.

Dass, S., Kapoor, R.L., Jatasra, D.S. and Kumar, P., 1985. Regression analysis of general adaptation of grain yield in pearl millet. *Indian J. Agric. Sci.*, ***55(4)***: 223:227.

Dass, S., Kapoor, R.L., Paroda, R.S. and Jatasra, D.S., 1984. Gene effects for downy mildew (*Sclerospora graminicola*) resistance in pearl millet. *Indian J. Genet.*, ***44***: 280-285.

Dave, H.R., 1987. Pearl millet hybrids. *In*: Proceedings of International Pearl Millet Workshop. (Witcombe, J.R. and Beckerman, S.R., Eds.). ICRISAT, Patancheru, India. Pp. 121-126.

Day, P.R., 1974. Genetics of host parasite interactions. W.H. Freeman and Company, San Francisco, USA.

Dayanandan, S., Rajora, O.P. and Bawa, K.S., 1998. Isolation and characterisation of microsatellites in trembling aspen (*Populus tremuloides*). *Theor. Appl. Genet.*, ***96***: 950-956.

de Bary, A., 1881. Zur Kenntniss der Peronosporeen. (Schluss). *Botanische Zeitung*, **39:** 517-625.

de Wit, P.J.G.M., 1992. Molecular characterization of gene-for-gene system in plant-fungus interactions and the application of avirulence genes in control of plant pathogens. *Ann. Rev. Phytopathol.*, ***30***: 391-418.

Dellaporta, S.L., Wood, J. and Hicks, J.B., 1983. A plant DNA minipreparation: version II. *Plant Mol. Biol. Rept.*, ***1***: 19-21.

Deswal, D.P. and Govila, O.P., 1994. Genetics of resistance to downy mildew *(Sclerospora graminicola)* in pearl millet *(Pennisetum glaucum)*. *Indian J. Agric. Sci.*, ***64***: 661-663.

Devos, K.M., Atkinson, M.D., Chinoy, C.N., Liu, C.J. and Gale, M.D., 1992. RFLP-based genetic map of the homeologus group-3 chromosomes of wheat and rice. *Theor. Appl. Genet.*, ***83***: 931-939.

Devos, K.M., Pittaway, T.S., Busso, C.S. and Gale, M.D., 1995. Molecular tools for the pearl millet nuclear genome. *International Sorghum and Millets Newsletter*, ***36***: 64-66.

Devos, K.M., Pittaway, T.S., Reynolds, A. and Gale, M.D., 2000. Comparative mapping reveals a complex relationship between the pearl millet genome and those of foxtail millet and rice. *Theor. Appl. Genet.*, ***100***: 190-198.

Diers, B.W., Keim, P., Fehr, W.R. and Shoemaker, R.C., 1992. RFLP analysis of soybean seed protein and oil content. *Theor. Appl. Genet.*, ***83***: 608-612.

Dirlewanger, E., Isacc, P.G., Ranade, S., Belajouza, M., Cousin, R. and de Vienne, D., 1994. Restriction fragment length polymorphism analysis of loci associated with disease resistance genes and development developmental traits in *Pisum sativum* (L). *Theor. Appl. Genet.*, ***88***: 17-27.

Don, R.H., Cox, P.T., Wainwright, B.J., Baker, K. and Mattick, J.S., 1991. Touchdown PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Res.*, ***19***: 4008.

Dudley, J.W., 1993. Molecular markers in plant improvement: Manipulation of genes affecting quantitative traits. *Crop Sci.*, ***33***: 660-668.

Dweikat, I., Ficus, E. and Cregan, P., 2004. Development of CT-based microsatellites in pearl millet. *International Sorghum and Millets Newsletter* (*In press*).

Eberhart, S.A. and Russell, W.A., 1966. Stability parameters for comparing varieties. *Crop Sci.*, ***6***: 36-40.

Edwards, M.D., Helentjaris, T., Wright, S. and Stuber, C.W., 1992. Molecular marker facilitated investigations of quantitative trait loci in maize. *Theor. Appl. Genet.*, ***83***: 765-774.

Edwards, M.D., Stuber, C.W. and Wendel, J.F., 1987. Molecular marker facilitated investigations of quantitative trait loci in maize. I. Numbers, genomic distribution and types of gene action. *Genetics*, ***115***: 113-125.

Ejeta, G., Hansen, M.M. and Mertz, E.T., 1987. *In vitro* digestibility and amino acid composition of pearl millet (*Pennisetum typhoides*) and other cereals. *Proc. Nat. Acad. Sci. USA*, ***84***: 6016-6019.

Ellis, T.H.N., 1986. Restriction fragment length polymorphism markers in relation to quantitative characters. *Theor. Appl. Genet.*, ***72***: 1-2.

Elston, R.D. and Stewart, J., 1971. A general method for the genetic analysis of pedigree data. *Hum. Hered.*, ***21***: 523-542.

Endo, T.R., 1982. Gametocidal chromosomes of three *Aegilops* species in common wheat. *Can. J. Genet. Cytol.*, ***24***: 201-206.

Evan, L.T., 1993. Crop evolution, adaptation and yield. Cambridge University Press, Cambridge, Great Britain.

Everson, E.H. and Schaller, C.W., 1995. The genetics of yield difference associated with awn barbing in barley hybrid (Lion × Altas^{10}) × Altas. *Agronomy Journal*, ***47***: 276-280.

Faires, E.W., Dawson, J.R., LaMaster, J.P. and Wise, G.H., 1941. Experiments with annual crops and permanent pastures to provide grazing for dairy cows in the sandhill regions of the southeast. *In*: Technical Bulletin No. 805, USDA: Washington, D.C.

Falconer, D.S., 1981. Introduction to Quantitative Genetics. Second Edition. Longman Group Limited, London, the UK.

Fancher, B.I., Jensen, L.S., Smith, R.L. and Hanna, W.W., 1987. Motabolizable energy content of pearl millet [*Pennisetum americanum* (L.) Leeke]. *Poult. Sci.*, ***66***: 1693-1696.

FAO and ICRISAT, 1996. The world sorghum and millet economics: Facts, trends and outlook. FAO, Rome, Italy and ICRISAT, Patancheru-502324, Andhra Pradesh, India.

FAO, 2000. Bulletin of Statistics. Vol. 1, FAO, Rome, Pp. 16-36.

Fatokun, C.A., Menancio-Hautea, D.I., Danesh, D. and Young, N.D., 1992. Evidence for orthologous seed weight genes in cowpea and mung bean based on RFLP mapping. *Genetics*, ***132***: 841-846.

Feinberg, A.P. and Vogelstein, S., 1983a. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.*, ***132***: 6-13.

Feinberg, A.P. and Vogelstein, S., 1983b. Addendum: A technique for radiolabelling DNA restriction fragments to a high specific activity. *Anal. Biochem.*, ***137***: 266-267.

Fisher, P.J., Richardson, T.E. and Gardner, R.C., 1998. Characteristics of single- and multi-copy microsatellites from *Pinus radiate*. *Theor. Appl. Genet.*, ***96***: 1069-1076.

French, M.H., 1948. Local millets as substitutes for maize in feeding of domestic animals. *East Afr. Agric. Journal*, ***13***: 213-220.

Freymark, P.J., Lee, M., Woodman, W.L. and Martinson, C.A., 1993. Quantitative and qualitative trait loci affecting host-response to *Exserohilum turcicum* in maize (*Zea mays* L.). *Theor. Appl. Genet.*, ***87***: 537-544.

Gale, M.D. and Witcombe, J.R., 1992. DNA markers and marker-mediated applications in pearl millet breeding. *In*: Biotechnology and crop improvement in Asia. (Moss, J.P., Ed.). ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), Patancheru-502 324, Andhra Pradesh, India. Pp. 323-332.

Gates, R.N., Hanna, W.W. and Hill, G.M., 1999. Pearl millet as a forage. *In*: Proceedings of the International Pearl Millet Workshop. (Neto, A.L. de F., Amabile, R.F., Netto, D.A.M., Yamashita, T. and Gocho, H., Eds.). Embrapa Cerrados, Planaltina, D.F., Brazil. Pp. 85-92.

Gebhardt, C., Ritter, E., Barone, T., Debener, T., Walkemeier, B., Schachtschabel, U., Kaufman, H., Thompson, R.D., Bonierbale, M.W., Ganal, M.W., Tanskley, S.D. and Salamini, F., 1991. RFLP maps of potato and their alignment with the homeologous tomato genome. *Theor. Appl. Genet.*, ***83***: 49-57.

Gelaye, S., Terril, T.H., Amoah, E.A., Miller, S., Gates, R.N. and Hanna, W.W., 1997. Nutritional value of pearl millet grain for lactating and growing goats. *J. Ani. Sci.*, ***75(10)***: 1409-1414.

Gelderman, H., 1975. Investigation on inheritance of quantitative characters in animals by gene markers. I. Methods. *Theor. Appl. Genet.*, ***46***: 300-319.

GENESTAT 6th Edition, 2002. The guide to Genstat ® release 6.1. Payne, R.W. 2002. Part 2: VSN Iinternational Limited, Oxford, UK.

Gibson, T.B. and Thoday, J.M., 1962. Effect of disruptive selection VI. A second chromosome polymorphism. *Heredity*, ***17***: 1-26.

Gill, B.S., Phul, P.S., Chahal, S.S. and Singh, N.B., 1975. Inheritance of resistance to downy mildew in pearl millet: A preliminary report. *Crop Improv.*, ***2***: 128-129.

Gill, B.S., Phul, P.S., Chahal, S.S. and Singh, N.B., 1978. Inheritance of resistance to downy mildew in pearl millet. *Cereals Res. Comm.*, ***6***: 71-74.

Goldman, D. and Merril, C.R., 1982. Silver staining of DNA in polyacrylamide gels: linearity and effect of fragment size. *Electrophoresis*, ***3***: 24-26.

Goral, H. and Spiss, L. 1997. Utilization cytoplasmic male-sterility in obtaining triticale hybrids. *Zoszyty Naukowo Academii Rolniezej w Szezecinic, Rolnictwo*, ***65(1)***: 109-114.

Gorz, H.J., Toy, J.J., Haskins, F.A. and Ross, W.M., 1984. Comparison of F_1s and inbreds as female parents for sorghum-sundangrass seed production. *Crop Sci.*, ***24***: 1134-1137.

Graham, G.I., Wolff, D.W. and Stuber, C.W., 1997. Characterization of a yield quantitative trait locus on chromosome five of maize by fine mapping. *Crop Sci.*, ***37***: 1601-1610.

Graner, A., Jahoor, A., Schondelmaier, J., Siedler, H., Pillen, K., Fischbeck, G., Wenzel, G. and Herrmann, R.G., 1991. Construction of an RFLP map of barley. *Theor. Appl. Genet.*, ***83***: 250-256.

Gupta, M., Chyi, Y.S., Romero-Severson, J. and Owen, J.L., 1994. Amplification of DNA markers from evolutionary diverse genomes using single primers of simple-sequence repeats. *Theor. Appl. Genet.*, ***89***: 998-1006.

Gupta, P.K. and Varshney, R.K. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*, ***113***: 163-185.

Gupta, S.K., 1979. Genetic analysis of continuous variation from generation means in pearl millet [*Pennisetum typhoides (Burm)* Stapf and C.E. Hubb.]. Ph.D. Thesis. Punjab Agricultural University, Ludhiana, India.

Gupta, V.P. and Dhillon, B.S., 1974. Variation and covariation of some plant and grain traits in pearl millet. *Indian J. Agric. Sci.*, ***44***: 213-216.

Gupta, V.P. and Nanda, G.S., 1971. Role of grain, plant and head characters in improving grain yield of pearl millet. *Indian J. Genet. Plant Breed.*, ***31***: 128-131.

Gupta, V.P. and Sidhu, P.S., 1972. Component analysis for grain yield in bajra. *Plant Sci.*, ***4***: 12-14.

Gupta, V.P., 1968. Basic concepts in the improvement of pearl millet. *Indian J. Genet. Plant Breed.*, ***28(A)***: 287-294.

Gupta, V.P., Sidhu, P.S. and Nanda, G.S., 1975. Genotype-environmental interactions for green fodder yield and its components in pearl millet. *Crop Improv.*, ***2***: 52-54.

Hackett, C.A., 1997. Model diagnostics for fitting QTL models to trait and marker data by interval mapping. *Heredity*, ***79***: 319-328.

Haley, C.S. and Knott, V., 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*, ***69***: 315-324.

Hanna, W.W., 1996. Improvement of millets-Emerging trends. *In*: Proceedings. 2nd Intl. Crop Sci. Congress, Delhi, India. Pp. 139-146.

Hanna, W.W., Dove, R., Hill, G.M. and Smith, R., 1991. Pearl millet as an animal feed in the US. *In*: Abstracts of technical papers, 1991, Annu. Meet. S. Branch, ASA, 18th, Fort Worth, TX. 2-6 Feb. 1991. ASA: Madison, WI. Pp. 7.

Hanson, W.D., 1956. The theoretical distribution of lengths of parental gene blocks in the gametes of an F_1 individual. *Genetics, **44***: 197-209.

Harrison, B.J. and Mather, K., 1950. Polygenic variability in chromosomes of *Drosophilla melanogaster* obtained from the wild. *Heredity, **4***: 295-312.

Hash, C.T. and Blake, T.K., 1981. Half-seed determination of hordeins associated with known Ml-a alleles conferring race-specific resistance to barley powdery mildew. *Barley Genetics Newsletter, **11***: 74-76.

Hash, C.T. and Bramel-Cox, P.J., 2000. Marker applications in pearl millet. *In*: Application of molecular markers in plant breeding: Training manual for a seminar held at IITA, Ibadan, Nigeria, 16-17 Aug. 1999. (Haussmann, B.I.G., Geiger, H.H., Hess, D.E., Hash, C.T. and Bramel-Cox. P., Eds.). International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. Pp. 112-127.

Hash, C.T. and Witcombe, J.R., 1994. Pearl millet mapping populations at ICRISAT. *In*: Use of Molecular Markers in Sorghum and Pearl Millet Breeding for Developing Countries. Proceedings of an ODA. Plant Science Research Programme Conference, (Witcombe, J.R. and Duncan, R.R., Eds.). 29th March- 1st April 1993, Norwich, UK. ODA: London, UK. Pp. 69-75.

Hash, C.T. and Witcombe, J.R., 2002. Gene management and breeding for downy mildew resistance. *In*: Sorghum and Millets Pathology, 2000. (Leslie, J.F., Ed.). Ames, Iowa, USA: Iowa State Press. Pp. 27-36.

Hash, C.T., 1991. ICRISAT pearl millet breeding and the potential use of RFLPs. *In*: Rockefeller Foundation Conference: The establishment of a sorghum and millet RFLP network to support breeding in developing countries, 6-10 May 1991. Report of meeting. Pp. 28-29.

Hash, C.T., 1997. Research on downy mildew of pearl millet. *In*: Integrating research evaluation efforts: Proceedings of an International Workshop, 14-16 Dec 1994, (Bantilan, M.C.S. and Joshi, P.K., Eds.). ICRISAT, Patancheru, India. Pp. 121-128.

Hash, C.T., 2000. Concepts for application of marker techniques in Africa. *In*: Application of molecular markers in plant breeding: Training manual for a seminar held at IITA, Ibadan, Nigeria, 16-17 Aug. 1999. (Haussmann, B.I.G., Geiger, H.H., Hess, D.E., Hash, C.T. and Bramel-Cox, P. Eds.). International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. Pp. 155-169.

Hash, C.T., Cavan, C.P., Bidinger, F.R., Howarth, C.J. and Singh, S.D., 1995. Downy mildew resistance: QTLs from a seedling heat tolerance mapping population. *International Sorghum and Millet Newsletter*, **36**: 66-67.

Hash, C.T., Sharma, A., Kolesnikova-Allen, M.A., Serraj, R., Thakur, R.P., Bidinger, F.R., Bhasker Raj, A.G., Rizwi, S.M.H., Beniwal, C.R., Yadav, H.P., Yadav, Y.P., Srikant, Bhatnagar, S.K., Yadav, R.S., Howarth, C.J., Breese, W.A. and Witcombe, J.R., 2003. Marker-assisted breeding to improve pearl millet hybrid HHB 67: Lab to field. *In:* Abstracts and Final Program, In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution. Bologna, Itlay, May 27-31, 2003. Pp. 82.

Hash, C.T., Singh, S.D., Thakur, R.P. and Talukdar, B.S., 1999. Breeding for disease resistance. *In*: Pearl Millet Breeding (Khairwal, I.S., Rai, K.N., Andrews, D.J. and Harinarayana, G., Eds.). Oxford. & IBH: New Delhi, India. Pp. 337-379.

Hash, C.T., Witcombe, J.R., Thakur, R.P., Bhatnagar, S.K., Singh, S.D. and Wilson, J.P., 1997. Breeding for pearl millet disease resistance. *In*: Proceedings of the International Conference on Genetic Improvement of Sorghum and Pearl Millet, September 22-27, 1996. INTSORMIL Publication, No. 95-5 INTSORMIL and ICRISAT. Pp. 337-372.

Hash, C.T., Yadav, R.S., Cavan, G.P., Howarth, C.J., Liu, H., Qi, X., Sharma, A., Kolesnikova-Allen, M.A., Bidinger, F.R. and Witcombe, J.R., 2000. Marker-assisted backcrossing to improve terminal drought tolerance in pearl millet. *In*: Proceedings of a Strategic Planning Workshop on "Molecular Approaches for the Genetic Improvement of Cereals for stable Production in Water-limited Environment", June 21-25, 1999, CIMMYT, El Batan, Maxico (Ribaut, J.M. and Poland, D., Eds.). CIMMYT, Mexico, D.F., Mexico. Pp. 114-119.

Hearne, C.M., Ghosh, S. and Todd, J.A., 1992. Microsatellite for linkage analysis of genetic traits. *Trends Genet.*, ***8***: 288-294.

Helentjaris, T., 1987. A genetic linkage map for maize based on RFLPs. *Trends Genet.*, ***3***: 217-221.

Helentjaris, T., Slocum, M., Wright, S., Schaefer, V. and Nienhuis, J., 1986a. Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. *Theor. Appl. Genet.*, ***72***: 761-769.

Helentjaris, T., Weber, D.F. and Wright, S., 1986b. Use of monosomics to map cloned DNA fragments in maize. *Proc. Natl. Acad. Sci. USA*, ***83***: 6035-6039.

Hernandez, P., Laurie, D.A., Martin, A. and Snape, J.W., 2002. Utility of wheat simple sequence repeats (SSR) markers for genetic analysis of *Hordeaum chilense* and *Tritordeum. Theor. Appl. Genet.*, ***104***: 735-739.

Heun, M., Kennedy, A.E., Anderson, J.A., Laptiton, N.L.V., Soller, M.E. and Tanksley, S.D., 1991. Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare). Genome, **34***: 437-447.

Hill, A.P., 1975. Quantitative linkage: A statistical procedure for its detection and estimation. *Ann. Hum. Genet.*, ***38***: 439-449.

Hill, G.M. and Hanna, W.W., 1990. Nutritive characteristics of pearl millet grain in beef cattle diets. *J. Anim. Sci.*, ***68***: 2061-2066.

Holloway, J.L. and Knapp, S.J., 1994. Gmendel 3.0 User Guide. Department of Crop and Soil Science, Oregon State University: Corvallis, OR 97331, USA.

Hooker, A.L., 1985. Corn and sorghum rusts. *In*: The cereal rusts. Vol. 2. Diseases, Distribution, Epidemiology and Control. (Roelfs, A.P. and Bushnell, W.R., Eds.) Academic Press, Orlando. Pp. 207-233.

Hookstra, G.H. and Ross, W.M., 1982. Comparison of F_1's and inbreds as female parents for hybrid sorghum seed production. *Crop Sci.*, ***22***: 147-150.

Hoseney, R.C., Andrews, D.J. and Clark, H., 1987. Sorghum and pearl millet. *In*: Nutritional quality of Cereal Grains: Genetic and Agronomic Improvement. ASA, Monograph. ***28***: 397-456.

Hospital, F., Moreau, L., Lacoudre, A., Charcosset, A. and Gallais, A., 1997. More on the efficiency of marker-assisted selection. *Theor. Appl. Genet.*, ***95***: 1181-1189.

Howarth, C.J., Cavan, G.P., Scot, K.P., Layton, R.W.H., Hash, C.T. and Witcombe, J.R., 1994. Mapping QTLs for heat tolerance in pearl millet. *In*: Use of molecular markers in sorghum and pearl millet breeding for developing countries: Proceedings of an ODA Plant Sciences Research Programme Conference, 29 Mar.-1 Apr. 1993, Norwich, UK (Witcombe, J.R. and Duncan, R.R., Eds.). London, UK: Overseas Development Administration. Pp. 80-85.

Hüttel, B., Winter, P., Weising, K., Choumane, W., Weigand, F. and Kahl, G., 1999. Sequence-tagged microsatellite site markers for chickpea (*Cicer arietinum* L.). *Genome,* ***42***: 210-217.

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), 1989. Annual Report 1988. ICRISAT, Patancheru-502 324, AP, INDIA. Pp. 30.

Jansen, R.C. and Stam, P., 1994. High resolution of quantitative traits into multiple loci via interval mapping. *Genetics*, ***136***: 1437-1445.

Jansen, R.C., 1993. Interval mapping of multiple quantitative trait loci. *Genetics*, ***135***: 205-211.

Jarne, P. and Lagoda, P.J.L., 1996. Microsatellites from molecules to populations and back. *Trends in Ecology and Evolution*, ***11***: 424-429.

Jauhar, P.P. and Hanna, W.W., 1998. Cytogenetics and genetics of pearl millet. *Adv. Agron.*, ***64***: 1-26.

Jauhar, P.P., 1981. Cytogenetics and breeding of pearl millet and related species. Progress and topics in cytogenetics. Vol.1. Alan R Liss: New York. Pp. 1-289.

Jayakar, S.D., 1970. On the detection and estimation of linkage between a locus influencing a quantitative character and a marker locus. *Biometrics*, ***26***: 441-464.

Jensen, J., 1989. Estimation of recombination parameters between a quantitative trait locus (QTL) and two markers gene loci. *Theor. Appl. Genet.*, ***78***: 613-618.

Jindla, L.N. and Gill, K.S., 1984. Inter-relationship of yield and its component characters in pearl millet. *Crop Improv.*, ***11***: 43-46.

Johnson, H.W., Robinson, H.F. and Comstock, R.E., 1955. Estimates of genetic and environmental variability in soybean. *Agronomy Journal*, ***47***: 314-318.

Johnson, R., 1984. A critical analysis of durable resistance. *Annual Rev. Phytopathol.*, ***22***: 309-330.

Jones, C.J., Edwards, K.J., Castaglione, S., Winfield, M.O., Sala, F., Van de Wiel, C., Bredemeijer, G., Vosman, B., Matthes, M., Dalx, A., Brettschneider, R., Bettini, P., Buiatti, M., Maestri, E., Malcevschi, A., Marmiroli, N., Aert, R., Volckaert, G., Rueda, J., Linacero, R., Vazquez, A. and Karp, A., 1997. Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Mol. Breed.*, ***3***: 381-390.

Jones, D.A., Balint-Kurti, P.J., Hammond-Kosach, K.A., Norcott, C.M., Thomas, C.M. and Jones, J.D.G., 1994. Transposon tagging and characterization of the tomato *Cf*-9 gene for resistance to *Cladosporium fulvum.* Abstracts of the Seventh International Symposium on Molecular Plant-Microbe Interaction. University of Edinburgh, Scotland, June 26th-July 1st 1994. Pp. 85.

Jones, E.S., 1994. Mapping quantitative trait loci for resistance to downy mildew in pearl millet. Ph.D. Thesis, University of Wales, Bangor, UK.

Jones, E.S., Breese, W.A. and Shaw, D.S., 2001. Infection of pearl millet by the downy mildew fungus *Sclerospora graminicola*: chilling inoculum to prevent zoospore release and subsequent spray damage to zoospores. *Plant Pathol.*, ***50***: 1-8.

Jones, E.S., Breese, W.A., Liu, C.J., Singh, S.D., Shaw, D.S. and Witcombe, J.R., 2002. Mapping quantitative trait loci for resistance to downy mildew in pearl millet: Filed and greenhouse screens detect the same QTL. *Crop Sci.*, ***42***: 1316-1323.

Jones, E.S., Liu, C.J., Gale, M.D., Hash, C.T. and Witcombe, J.R., 1995. Mapping quantitative trait loci for downy mildew resistance in pearl millet. *Theor. Appl. Genet.*, ***91***: 448-456.

Jørgensen, J.H., 1992. Multigene families of powdery mildew resistance genes in locu *Mla* on barley chromosome 5. *Plant Breed.*, ***108***: 55-59.

Joshi, V.J. and Ugale, S.D., 2002. Involvement of higher order interactions addressing complex polygenetically controlled inheritance of downy mildew [*Sclerospora graminicola* (Sacc.) Schrot] resistance in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Euphytica, **127***: 149-161.

Karikari, S.K. and Mosekiemang, T., 2002. Yield and yield component compensation in pearl millet (*Pennisetum glaucum* L.) in Botswana. *Crop Res.*, ***23(1)***: 27-33.

Karikari, S.K. and Ngwako, S., 1999. Effect of plant population and tiller number on the growth and yield of pearl millet (*Pennisetum typhoides). UNISWA Res. J. Agric., Sci. and Technol.*, ***3***: 62-68.

Kassam, A.H., 1976. Sorghum and millet project report. *In*: ACHROACYTE In-House Review. Pp. 121-126.

Kataria, R., Yadav, H.P. and Dass, S., 1993. Inheritance of downy mildew resistance in pearl millet. *Agric. Sci. Dig.*, ***13(3/4)***: 128-130.

Kataria, R.P., Yadav, H.P., Beniwal, C.R. and Narwal, M.S., 1994. Genetics of incidence of downy mildew (*Sclerospoora graminicola*) in pearl millet (*Pennisetum glaucum). Indian J. Agric. Sci.*, ***64***: 664-666.

Kearsey, M.J. and Farquhar, A.G.L., 1998. QTL analysis in plants; where are we now? *Heredity*, ***80***: 137-142.

Kearsey, M.J. and Hyne, V., 1994. QTL analysis: A simple 'marker-regression' approach. *Theor. Appl. Genet.*, ***89***: 698-702.

Kearsey, M.J. and Pooni, H.S., 1996. The Genetical Analysis of Quantitative traits. Chapman & Hall, London.

Kearsey, M.J., 1998. The principals of QTL analysis (a minimal mathematical approach). *J. Exp. Bot.*, ***49(327)***: 1619-1623.

Keim, P., Diers, B.W., Olson, T.C. and Shoemaker, R.C., 1990. RFLP mapping in soybean: association between marker loci and variation in quantitative traits. *Genetics*, ***126***: 735-742.

Kenneth, R.G., 1981. Downy mildew of graminaceous crops. *In*: Downy mildew. (Spencer, D.M., Ed.). Academic press, London, New York, San Fransisco. Pp. 903-912.

Kerns, M.R., Dudley, J.W. and Rufener II, G.K., 1999. Tester and type of progeny affect QTL detection in maize. *Maydica*, ***44***: 69-83.

Khairwal, I.S. and Singh, S., 1999. Quantitative and qualitative traits. *In* Pearl millet breeding (Khairwal, Rai, Andrews and Harinarayana, Eds.). Oxford and IBH Publishing Co. Pvt. Ltd. Pp. 119-155.

Kicherer, S., Backes, G., Walther, U. and Jahoor, A., 2000. Localising QTLs for leaf rust resistance and agronomics traits in barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.*, ***100***: 881-888.

Klopfenstein, C.F., Hoseney, R.C. and Leipold, H.W., 1983a. Goitrogenic effects of pearl millet diets. *Nutr. Rep. Int.*, ***27(5)***: 1039-1047.

Klopfenstein, C.F., Hoseney, R.C. and Leipold, H.W., 1983b. Further studies on the goitrogenic effects of pearl millet diets. *Nutr. Rep. Int.*, ***28(5)***: 1137-1144.

Klopfenstein, C.F., Hoseney, R.C. and Leipold, H.W., 1985. Nutritional quality of pearl millet and sorghum grain diets and pearl millet weanling food. *Nutr. Rep. Int.*, ***31(2)***: 287-297.

Knapp, S.J., Bridges Jr., W.C. and Birkes, D., 1990. Mapping quantitative trait loci using molecular marker linkage maps. *Theor. Appl. Genet.*, ***79***: 583-592.

Knapp, S.J., Bridges Jr., W.C. and Liu, B.H., 1992. Mapping quantitative trait loci using non-simultaneous and simultaneous estimators and hypothesis tests. *In*: Plant Genomes: Methods for Genetic and Physical Mapping. (Beckmann, J.S. and

Osborn, T.S., Eds.). Kluwer Academic Publishers, Dordrecht, The Netherlands. Pp. 209-237.

Koduru, P.R.K. and Krishna Rao, M., 1983. Genetics of qualitative traits and linkage studies in pearl millet. *Z. Pflanzenzücht*, ***90***: 1-22.

Kolesnikova Maria-Allen, 2001. Mapping new quantitative trait loci (QTL) for downy mildew resistance in pearl millet. Ph.D. Thesis, Russian Academy of Sciences, Moscow.

Kreike, C.M., de Koning, J.R.A., Vinke, J.H, Van Ooijen, J.W., Gebhardt, C. and Stiekema, W.J., 1993. Mapping of loci involved in quantitatively inherited resistance to the potato cyst-nematode *Globodera rostochiensis* pathotype Rol. *Theor. Appl. Genet.*, ***87***: 464-470.

Kresovich, S., Lamboy, W.F., Li, R., Ren, J., Szewc-McFadden, A.K. and Bliek, S.M., 1994. Application of molecular methods and statistical analyses for discrimination of accessions and clones of vetiver grass. *Crop Sci.*, ***34***: 805-809

Lande, R. and Thompson, R., 1990. Efficiency of marker-assisted selection in improvement of quantitative traits. *Genetics*, ***124***: 743-756.

Lander, E.S. and Botstein, D., 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, ***121***: 185-199.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J, Lincoln, S.E. and Newburg, L., 1987. MAPMAKER: An interactive computer program for constructing genetic linkage maps of experimental and natural populations. *Genetics*, ***1***: 174-181.

Landry, B.S. and Michelmore, R.W., 1987. Methods and applications of restriction fragment length polymorphism analysis to plants. *In*: Tailoring genes for crop Improvement: An agricultural perspective. (Bruening, G., Harada, J. and Hollaender, A., Eds.). Plenum Press, New York.

Landry, B.S., Kesseli, R.V., Barry Farrara and Michelmore, R.W., 1987. A genetic map of lettuce (*Lactuca sativa* L.) with restriction fragment length polymorphism, isozyme, disease resistance and morphological markers. *Genetics*, ***116***: 331-337.

Law, C.N., 1967. The location of factors controlling a number of quantitative characters in wheat. *Genetics*, ***56***: 445-461.

Lawrence, C.G., Ellis, J.G. and Finnegan, E.S., 1994. Cloning a rust resistance gene in flax. Abstracts of the Seventh International Symposium on Moleculat Plant-Microbe Interactions. University of Edinburgh, Scotland, June 26th-July 1st 1994. Pp-86.

Lebowitz, R.J., Soller, M. and Beckmann, J.S., 1987. Trait-based analyses for the detection of linkage between marker loci and quantitative trait loci in crosses between inbred lines. *Theor. Appl. Genet.*, ***73***: 556-562.

Lee, M., 1995. DNA markers in plant breeding programs. *Adv. Agron.*, ***55***: 265-344.

Lenz, M.C. and Atkins, R.E., 1981. Comparisons of agronomic and morphologic characters in sorghum having different cytoplasm. *Crop Sci.*, ***21***: 946-950

Leonards-Schippers, C., Gieffers, W., Schaufer-Pregl, R., Ritter, E., Knapp, S.J., Salamini, F. and Gebhardt, C., 1994. Quantitative resistance to *Phytophthora infestans* in potato: A case study of QTL mapping in an allogamous plant species. *Genetics*, ***137***: 67-77.

Lewers, K.S., Crane, E.H., Bronson, C.R., Schupp, J.M., Keim, P. and Shoemaker, R.C., 1999. Detection of linked QTL for soybean brown stem rot resistance in 'BSR 101' as expressed in a growth chamber environment. *Mol. Breed.*, ***5***: 33-42.

Lin, Y.R., Schertz, K.F. and Paterson, A.H., 1995. Comparative analysis of QTLs affecting plant height and maturity across the *Poaceae*, in reference to an intraspecific sorghum population. *Genetics*, ***140***: 391-411.

Lincoln, S., Daly, M. and Lander, E., 1992a. Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1. Whitehead Institute Technical Report. 2nd edition.

Lincoln, S., Daly, M. and Lander, E., 1992b. Constructing genetic maps with MAPMAKER/EXP 3.0. Whitehead Institute Technical Report. 3rd edition.

Liu C.J., Devos, K.M., Witcombe, J.R., Pittaway, T.S. and Gale, M.D., 1996. The effect of genome and sex on recombination rates in *Pennisetum* species. *Theor. Appl. Genet.*, ***93***: 902-908.

Liu, B.H. and Knapp, S.J., 1992. QTLSTAT.1.0. A software for mapping complex trait using nonlinear models, Oregon State University.

Liu, C.J., Witcombe, J.R., Pittaway, T.S., Nash, M., Hash, C.T., Busso, C.S. and Gale, M.D., 1994. An RFLP-based genetic linkage map of pearl millet (*Pennisetum glaucum). Theor. Appl. Genet.*, ***89***: 481-487.

Lloyd, G.L., 1964. Use of munga to replace maize in a broiler ration. *Rhodesia Agric. Journal*, ***61***: 50-51.

Louvel, D., 1982. Breeding millet (*Pennisetum*) for disease resistance. Centre National de Recherches Agronomiques: Bambey, Senegal.

Lu, Y.Y. and Liu, B.H., 1995. PGRI, a software for plant genome research. Plant Genome III conference, San Diego, CA. Pp. 105.

Lynch, M. and Walsh, B., 1998. Genetics and Analysis of Quantitative Traits. Sinauer Associates, Sunderland, MA, USA.

Lyttle, T.W., 1991. Segregation distorters. *Annu. Rev. Genet.*, ***25***: 511-557.

Mace, E.S., Buhariwalla, H.K. and Crouch, J.H., 2003. A high-throughput DNA extraction protocol for tropical molecular breeding programs. *Plant. Mol. Biol. Rep.*, ***21***: 459-460.

Maciel, G.A. and Tabosa, J.N., 1982. Tecnologia de producao para milheto. *In*: Cultura de milheto apre extensionista agricola. Pernambuco, Brazil: Empresa Pernambucana de Pesquisa Agropecuaria/Universidada Federal de Pernambuco/Banco do Nordeste. Pp. 23-25.

Mackill, D.J. and Junjian, Ni, 2001. Molecular mapping and marker assisted selection for major gene traits in rice. *In*: Proceedings of the 4th International Rice Genetics Symposium (Khush, G.S., Brar, D.S. and Hardy, B., Eds.). International Rice Research Institute. Los Banos, Philippines.

Mahadevappa, M. and Ponnaiya, B.W., 1967. Discriminant functions in the selection of pearl millet (*Pennisetum typhoides* Stapf and Hubb.) population for grain yield. *Madras Agric. Journal*, ***54***: 211-222.

Maiti, R.K. and Bidinger, F.R., 1981. Growth and development of pearl millet. Research Bulletin, No. 6. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India.

Maiti, R.K. and Singh, V.P., 2004. Biotic factors affecting pearl millet [*Pennisetum glacum* (L.) R. Br.] growth and productivity-A review. *Crop Res.*, ***27(1)***: 30-39.

Maliepaard, C. and Van Ooijen, J.W., 1994. QTL mapping in a full-sib family of an out crossing species. *In*: Biometrics in Plant Breeding: Applications of molecular markers. (Van Ooijen, J.W. and Jansen, J., Eds.). Proceedings of the ninth meeting of the EUCARPIA, Section Biometrics in Plant Breeding, 6-8 July, 1994. Wageningen: The Netherlands. Pp. 140-146.

Manga, V.K. and Saxena, M.B.L., 1988a. Association of root and related traits at different growth stages in pearl millet. *Crop Improv.*, ***15***: 105-106.

Manga, V.K. and Saxena, M.B.L., 1988b. Variability for seedling vigour and its association with yield in pearl millet. *Crop Improv.*, ***17***: 83-84.

Mangat, V.K., 1992. Stability analysis of grain yield in pearl millet using standard variety mean as environmental index. *Indian J. Genet. Plant Breed.*, ***52***: 111-113.

Mangin, B., Goffinet, B. and Rebai, A., 1994. Constructing confidence intervals for QTL location. *Genetics*, ***138***: 1301-1308.

Maniatis, T., Fritsch, E.R. and Sambrook, J., 1982. Quantification of DNA and RNA. *In*: Molecular cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press: NY. Pp. 448-469.

Manly, K.F. and Olsen, J.M., 1999. Overview of QTL mapping software and introduction to Mapmanager QT. *Mamm. Genome*, ***10***: 327-334.

Manly, K.F., 1993. A Macintosh program for storage and analysis of experimental genetic mapping data. *Mamm. Genome*, ***1***: 123-126.

Manzanares-Dauleux, M.J., Delourme, R. and Baron, F., 2000. Mapping of one major gene and QTLS involved in resistance to clubroot in *Brassica napus*. *Theor. Appl. Genet.*, ***101***: 885-889.

Marshall, S.P., Sanchez, A.B., Somers, H.L. and Arnold, P.T.D., 1953. Value of pearl millet pasture for dairy cattle. University of Florida Agricultural Experiment Stations Bulletin No. 527.

Martel, E., De Nay, D., Silijak-Yakovlev, S., Brown, S. and Sarr, A., 1997. Genome size variation and basic chromosome number in pearl millet and fourteen related *Pennisetum* species. *J. Hered.*, ***88***: 139-143.

Martin, B., Nienhuis, J., King, G. and Schaefer, A., 1989. Restriction fragment length polymorphisms associated with water use efficiency in tomato. *Science*, ***243***: 1725-1728.

Martin, G.B., Brommonschenkel, S.H., Chunwongse, J., Fray, A., Ganal, M.W., Spivey, R., Wu, T., Earle, E.D. and Tanksley, S.D., 1993. Map-based cloning of a protein kinase gene conferring disease resistance in tomato. *Science*, ***262***: 1432-1436.

Martinez, O. and Curnow, R.N., 1992. Estimation the locations and sizes of the effects of quantitative trait loci using flanking markers. *Theor. Appl. Genet.*, ***85***: 480-488.

Masi, P., Zeuli, P.L.S. and Donini, P., 2003. Development and analysis of multiplex microsatellite markers sets in common bean (*Phaseolus vulgaris* L.). *Mol. Breed.*, ***11***: 303-313.

Masjoe, Myskow, B. and Milczarski, P., 2001. Expending an RFLP-based map of rye using random polymorphic DNA (RAPD) and isozyme markers. *Theor. Appl. Genet.*, ***102***: 1273-1279.

Mather, K. and Harrison, R.J., 1949. The manifold effect of selection. *Heredity*, ***3***: 1-52.

Mather, K., 1949. Biometrical genetics, 1st edn. Methuen: London.

McCouch, S.R., Kochert, G., Yu, Z.H., Wang, Z.Y., Khush, G.S., Coffman, W.R. and Tanksley, S.D., 1988. Molecular mapping of rice chromosomes. *Theor. Appl. Genet.*, ***76***: 815-824.

McDonough, C.M., 1986. Structural characteristics of the mature pearl millet (*Pennisetum americanum)* caryopsis. M.Sc. Thesis, Texas A&M University, College Station, USA.

Mcintosh, M.S., 1983. Analysis of combined experiments. *Agronomy Journal, **75***: 153-155.

Mehta, N. and Dang, J.K., 1987. Studies on the inheritance of downy mildew, ergot and smut of pearl millet. *Indian J. Mycol. Pl. Pathol., **17***: 200-203.

Melchinger, A.E., 1990. Use of molecular markers in breeding for oligogenic disease resistance. *Pl. Breed., **104***: 1-19.

Melchinger, A.E., Utz, H.F. and Schon, C.C., 1998. Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. *Genetics, **149***: 383-403.

Michelmore, R.W., Paran, I. and Kesseli, R.V., 1991. Identification of markers linked to disease resistance genes by bulked-sergeants analysis; a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA, **88***: 9828-9832.

Miles, J.T., Cowsert, W.C., Lusk, J.W. and Owen, J.R., 1956. Most milk from Sudan in state college dairy tests. *Mississippi Farm Res., **19***: 6-7.

Miller, J.C. and Tanksley, S.D., 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon. Theor. Appl. Genet., **80***: 437-448.

Mohan, M., Mukerji, K.G. and Rani, K., 1978. Disease of bajra. *Seeds and Farms, **4***: 35-38.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T.G., Yano, M., Bhatia, C.R. and Sasaki, T., 1997. Genome mapping, molecular markers and marker-assisted selection in the improvement of quantitative traits. *Mol. Breed., **3***: 87-103.

Moreau L., Charcosset, A., Hospital, F. and Gallais, A., 1998. Marker-assisted selection efficiency in populations of finite size. *Genetics, **148***: 1353-1365.

Morgan, R.N., Wilson, J.P., Hanna, W.W. and Ozias-Akin, P., 1998. Molecular markers for rust and Pyricularia leaf spot disease resistance in pearl millet. *Theor. Appl. Genet., **96***: 413-420.

Morgante, M. and Olivieri, A.M., 1993. PCR-amplified microsatellites as markers in plant genetics. *Plant Journal, **3**:* 175-182.

Moxon, E.R., Wills, C., 1999. DNA microsatellites: agents of evolution. *Scientific American, January*: 72-77.

Muranty, H. and Goffinet, B., 1997. Multitrait and multi-population QTL search using selective genotyping. *Genet. Res., **70***: 259-265.

Murray, M. and Thompson, W.F., 1984. Rapid isolation of high molecular weight plant DNA. *Nucl. Acids Res., **8***: 4321-4325.

Nene, Y.L. and Singh, S.D., 1976. Downy mildew and ergot of pearl millet. *PANS, **22***: 366-385.

Nepolean, T., 2002. Identification of QTLs for yield and its component traits and downy mildew [*Sclerospora graminicola* (Sacc.) J. Schrot.] resistance in pearl millet [*Pennistum glaucum* (L.) R. Br.] Ph.D. Thesis, TNAU, Coimbtore, India.

Nguyen, H.T., Sleper, D.A. and Hunt, K., 1980. Genotype × Environment interactions and stability analysis for herbage yield of tall fescue synthetics. *Crop Sci., **20***: 221-224.

Niemann-Sorenson, A. and Robertson, A., 1961. The association between blood groups and several production characters in three Danish cattle breeds. *Acta agric. Scand., **11***: 163-196.

Nienhuis, J., Helentjaris, T., Slocum, M., Ruggero, B. and Schaefer, A., 1987. Restriction fragment length polymorphism analysis of loci associated with insect resistance in tomato. *Crop Sci., **27***: 797-803.

Nigam, S.N., Chandra, Rupa Sridevi, K. and Manohar Bhukta, 2003. Multi-environment analysis for Indian sites. *In*: Breeding of drought-resistant peanuts. Proceedings of collaborative review meeting, ACIAR (Cruickshank, A.W., Rachaputi, N.C., Wright, G.C. and Nigam, S.N., Eds). Pp. 67-71.

Nilsson, N.O., Hansen, M., Panagopoulous, A. H., Turesson, S., Ehlde, M., Christiansson, M., Rading, T.M., Rissler, M. and Kraft, T., 1999. QTL analysis of *Cercospora* leaf spot resistance in sugar beet. *Plant Breed., **118***: 327-334.

Nilsson, N.O., Sall, J. and Bengtsson, B.O., 1993. Chiasma data and recombination in plants-are they compatible? *Trends Genet.*, ***9***: 344-348.

O' Donoughue, L.S., Wang, Z., Rõder, M., Kneen, B., Leggett, M., Sorrells, M.E. and Tanksley, S.D., 1992. An RFLP-based linkage map of oats based on a cross between two diploid taxa (*Avena atlantica* × *A. hirtula*). *Genome*, ***35***: 765-771.

Ouedraogo, J.T., Gowda, B.S., Jean, M., Close, T.J., Ehlers, J.D., Hall, A.E., Gillaspie, A.G., Roberts, P.A., Ismail, A.M., Bruning, G., Gepts, P., Timko, M.P. and Belzile, F.J., 2002. An improved genetic map for cowpea (*Vigna unguiculata* L.) combining AFLP, RFLP, RAPD and Biochemical Marker and Biological Resistance Traits. *Genetics*, ***45***: 178-188.

Panse, V.G. and Sukhatme, P.V., 1967. Statistical methods of agricultural research workers. ICAR Publication, 152-155.

Paran, I., Goldman, I., Tanksley, S.D. and Zamir, D., 1995. Recombinant inbred lines for genetic mapping in tomato. *Theor. Appl. Genet.*, ***90***: 542-548.

Paterson, A.H., Damon, S., Hewitt, J.D., Zamir, D., Rabinowitch, H.D., Lincoln, S.E., Lander, E.S. and Tanksley, S.D., 1991a. Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments. *Genetics*, ***127***: 181-197.

Paterson, A.H., Lander, E., Hewitt, J.D., Peterson, S., Lincoln, S.E. and Tanksley, S.D., 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature*, ***335***: 721-726.

Paterson, A.H., Tanksley, S.D. and Sorrels, M.E., 1991b. DNA markers in plant improvement. *Adv. Agron.*, ***46***: 39-90.

Patil, B.D., Gupta, S.K., Premachandran, M.N. and Coubey, R.N., 1989. Genetics and breeding of forage *Pennisetum* (L.). Leeke: A review. *Agric. Rev.*, ***10***: 12-32.

Pê, M.E., Gianfranceschi, L., Taramino, G., Tarchini, R., Angelini, P., Dani, M. and Binelli, G., 1993. Mapping quantitative trait loci (QTLs) for resistance to *Giberella zeae* infection in maize. *Mol. Gen. Genet.*, ***241***: 11-16.

Pederson, W.L. and Leath, S., 1988. Pyramiding major genes for resistance to maintain residual effects. *Ann. Rev. Phytopathol.*, ***26***: 369-378.

Pereira, M.G., Lee, M., Bramel-Cox, P., Woodman, W., Doebley, J. and Whitkus, R., 1994. Construction of an RFLP map in sorghum and comparative mapping in maize. *Genome*, ***37***: 236-243.

Pethani, K.V., Kapoor, R.L. and Chandra, S., 1980. Gene action and phenotypic stability for incidence of downy mildew disease in pearl millet. *Indian J. Agrl. Res.*, ***14***: 217-223.

Phillips, R.L., 1999. Unconventional sources of genetic diversity: de novo variation and elevated epitasis. *In:* Plant Breeding in the Turn of the Millennium. (Borem, A., Giudice, M.P. and Sakiyama, N.S., Eds.). University of Vicosa, Brazil. Pp. 103-131.

Phul, P.S., 1963. A study of quantitative heredity in pearl millet. M.Sc. Thesis, Punjab Agricultural University, Ludhiana, India.

Phul, P.S., Gupta, S.K. and Gill, K.S., 1974. Association analysis of some morphological and physiological traits in pearl millet. *Indian J. Genet. Plant Breed.*, ***34***: 346-353.

Pokhriyal, S.C., Mangat, K.S. and Gangal, L.K., 1967. Genetic variability and correlation studies in pearl millet [*Pennisetum typhiodes* (Burm.) Stapf and C.E. Hubb.]. *Indian J. Agric. Sci.*, ***37***: 77-82.

Poncet, V., Lamy, F., Devos, K.M., Gale, M.D., Sarr, A. and Robert, T., 2000. Genetic control of domestication traits in pearl millet (*Pennisetum glaucum* L. Poaceae) *Theor. Appl. Genet.*, ***100***: 147-159.

Poncet, V., Lamy, F., Enjalbert, J., Joly, H., Sarr, A. and Robert, T., 1998. Genetic analysis of the domestication syndrome in pearl millet (*Pennisetum glaucum* L. Poaceae): inheritance of major characters. *Heredity*, ***81***: 648-658.

Poncet, V., Martel, E., Allious, S., Devos, K.M., Lamy, F., Sarr, A. and Robert, T., 2002. Comparative analysis of QTLs affecting domestication traits between two domesticated × wild pearl millet (*Pennisetum glaucum* L., *Poaceae*) crosses. *Theor. Appl. Genet.*, ***104***: 965-975.

Price, A.H., Steele, K.A., Moore, B.J., Barraclough, P.B. and Clark, L.J., 2000. A combined RFLP and AFLP linkage map of upland rice (*Oryza sativa* L.) used to identify QTLs for root-penetration ability. *Theor. Appl. Genet.*, ***100***: 49-56.

Prioul, J.L., Quarrie, S., Causse, M. and de Vienne, D., 1997. Dissecting complex physiological functions through the use of molecular quantitative genetics. *J. Exp. Bot.*, ***48(311)***: 1151-1163.

Provan, J., Russell, J.R., Boothm, A. and Powell, W., 1999. Polymorphic chloroplast simple sequence repeat primers for systematic and population studies in the genus Hordeum. *Mol. Ecol.*, ***8***: 505-511.

Purseglove, J.W., 1976. Millets. *In*: Evolution of Crop Plants (Simmonds, N.W., Ed.). Longman, London, New York. Pp. 91-93.

Putternil, J., Robinson, K. L., Simon, R. and Coupland, G., 1995. The CONSTANS gene of *Arabidopsis* promotes flowering and encodes a protein showing similarity to zinc finger transcription factors. *Cell*, ***80***: 847-857.

Qi, X., Lindup, S., Pittaway, T.S., Allouis, S., Gale, M.D. and Devos, K.M., 2001. Development of simple sequence repeats markers from bacterial artificial chromosomes without subcloning. *Bio Techniques*, ***31***; 355-361.

Qi, X., Pittaway, T.S., Liu, H., Waterman, E., Padi, F.K., Hash, C.T., Zhu, J., Gale, M.D. and Devos, K.M., 2004. An integrated genetic map of pearl millet, *Pennisetum glaucum*. (*in press*).

Rachaputi, N.C., 2003. Environmental characterisation of experimental sites in India and Australia. *In*: Breeding of drought-resistant peanuts. Proc. of collaborative review meeting, ACIAR (Cruickshank, A.W., Rachaputi, N.C., Wright, G.C. and Nigam, S.N., Eds). Pp. 61-66.

Rachie, K.O. and Majumdar, J.V., 1980. Pearl Millet, Pennsylvania Univ. Press, University Park, PA, USA.

Rai, K.N. and Anand Kumar, K., 1994. Pearl Millet improvement at ICRISAT-an update. *International Sorghum and Millet Newsletter*, ***35***: 1-29.

Rai, K.N. and Hanna, W.W., 1990. Morphological characteristics of tall and dwarf pearl millet isolines. *Crop Sci.*, ***30***: 23-25.

Rai, K.N. and Singh, S.B., 1987. Breeding pearl millet male-sterile lines. *In*: Proceedings International pearl millet Workshops. (Witcombe, J.R. and Beckerman, S.R., Eds.). ICRISAT: Patancheru, India. Pp. 127-137.

Rai, K.N., Bidinger, F.R., Sahib, K.H. and Rao, A.S., 1998. Registration of ICMP 94001 pearl millet germplasm. *Crop Sci.*, ***38(5)***: 01411.

Rai, K.N., Chandra, S. and Rao, A.S., 2000. Potential advantages of male-sterile F_1 hybrids for use as seed parent of three-way hybrids in pearl millet. *Field Crop Res.*, ***68***: 173-181.

Rai, K.N., Talukdar, B.S., Singh, S.D., Rao, A.S., Rao, A.M. and Andrews, D.S., 1994. Registration of ICMP 423 parental line of pearl millet. *Crop Sci.*, ***34***: 1430.

Rao, D.V.N. and Damodaran, G., 1964. Studies on correlation of certain plant characters to yield in pearl millet (*Pennisetum typhiodeum*). *Andhra Agric. Journal*, ***11***: 22-25.

Rao, P.V., 1981. Genetic analysis of grain yield components in pearl millet [*Pennisetum typhoides* (Burm.) S. & H.]. M.Sc. Thesis, Punjab Agric. Univ., Ludhiana, India.

Rasmusson, J.M., 1935. Studies on the inheritance of quantitative characters in *Pisum*: I. Preliminary note on the genetics of flowering. *Hereditas*, ***20***: 161-180.

Reddy, M.P., Sarla, N. and Siddiq, E.A., 2002. Inter simple sequence repeats (ISSR) Polymorphism and its application in plant brteeding. *Euphytica*, ***128***: 9-17

Rick, C.M., 1966. Abortion of male and female gametes in the tomato determined by allelic interaction. *Genetics*, ***53***: 85-96.

Riedel, G.E., Swanberg, S.L., Kuranda, K.D., Marquette, K., La Pan, P., Bledoe, P., Kennedy, A. and Lin, B.Y., 1990. Denaturing gradient gel electrophoresis identities genomic DNA polymorphism with high frequency in maize. *Theor. Appl. Genet.*, ***80***: 1-10.

Rivard, S.Y., Cappadocia, M. and Landry, B.S., 1996. A comparison of RFLP maps based on anther culture derived, selfed, and hybrid progenies of *Solanum chacoense*. *Genome*, ***39***: 611-621.

Roark, D.B., Miles, J.T., Lusk, J.W. and Cowsert, W.C., 1952. Milk production from pearl millet, grain sorghum, and Tift Sudan. *Proc. Assos. South. Agric. Workers*, ***46***: 76-77.

Romagosa, I., Han, F., Ullrich, S.E., Hayes, P.M. and Wesenberg, D.M., 1999. Verification of yield QTL through realized molecular marker-assisted selection responses in a barley cross. *Mol. Breed.*, ***5***: 143-152.

Rongwen, J., Akkaya, M.S., Bhagwat, A.A., Lavi, U. and Cregan, P.B., 1995. The use of microsatellite DNA markers for soybean genotype identification. *Theor. Appl. Genet.*, ***90***: 43-48.

Rooney, L.W. and McDonough, C.M., 1987. Food quality and consumer acceptance in pearl millet. *In*: Proceedings of the International Pearl Millet Workshop (Witcombe, J.R. and Beckerman, S.R., Eds.). ICRISAT, Patancheru, India. Pp. 43-61.

Ross, W.M. and Kofoid, K.D., 1979. Effect on non-milo cytoplasm on the agronomic performance of sorghum. *Crop Sci.*, ***19***: 267-270.

Rossetto, M., Slade, R.W., Baverstock, P.R., Henry, R.J. and Lee, L.S., 1999. Microsatellite variation and assessment of genetic structure in teatree (Melaleuca alternifolia - Myrtaceae). *Mol. Ecol.*, ***8***: 633-643.

Saccardo, P.A., 1876. Fungi Veneti novi vel critici. Series V. *Nuovo Giornale Bot. Italiano*, ***8***: 162-211.

Safeeulla, K.M., 1976. Biology and control of the downy mildews of pearl millet, sorghum and finger millet. Downy Mildew Research Laboratory, University of Mysore, Mysore, India.

Sandler, L. and Golic, K., 1985. Segregation distortion in *Drosophila*. *Trends Genet.*, ***1***: 181-185.

Sandler, L. and Novitski, E., 1957. Meiotic derive as an evolutionary force. *American Nat.*, ***41***: 105-110.

Sanghai-Maroof, M.A., Biyashev, R.M., Yang, G.P., Zhang, Q. and Allard, R.W., 1994. Extraordinarily polymorphic microsatellite DNA in barley: Species diversity, chromosomal loacations and population dynamics. *Proc. Natl. Acad. Sci. USA,* ***91***: 5466-5470.

SAS version 8.0, 1999. SAS Institute Inc., Cary, NC, USA.

Sastry, J.G., Ramakrishna, W., Sivaramakrishnan, S., Thakur, R.P., Gupta, V.S. and Ranjekar, P.K., 1995. DNA fingerprinting detects genetic variability in the pearl millet downy mildew pathogen (*Sclerospora graminicola). Theor. Appl. Genet.*, ***91***: 856-861.

Sastry, J.G., Sivaramakrishnan, S., Rao, V.P., Thakur, R.P., Singru, R.S., Gupta, V.S. and Ranjekar, P.K., 2001. Genetic basis of host-specificity in *Sclerospora graminicola*, the pearl millet downy mildew pathogen. *Indian J. Phytopathol.*, ***54***: 323-328.

Saturnino, H.M. and Landers, J.N., 1997. Plantio direto a transferencia tcnologica nos tropicos e subtropics. *In*: O meio ambiente e o plantio direto. Brasila-Embrepa-APDC. Pp 89-112.

Sax, K., 1923. The association of size differences with seed coat pattern and pigmentation in *Phaseolus vulgaris. Genetics*, ***8***: 552-560

Scalea, M., 1999. Millet-growing and its use in no-till planting in Brazil's Cerrado Savannas. *In:* Proceedings of the International Pearl Millet Workshop. (Neto, A.L. de F., Amabile, R.F., Netto, D.A.M., Yamashita, T. and Gocho, H., Eds.). Embrapa Cerrados: Planaltina, D.F., Brazil. Pp. 75-82.

Schröter, J., 1879. Protomyces graminicola Sacc. *Hedwigia*, ***18***: 83-87.

Scoles, G.J. and Kibirge-Sebunya, I.N., 1983. Preferential abortion of gametes in wheat induced by an *Agropyron chromosome. Can. J. Genet. Cytol.*, ***25***: 1-6.

Senior, M.L. and Heun, M., 1993. Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. *Genome*, ***36***: 884-889.

Setiawan, A., Koch, G., Barens, S.R. and Jung, C., 2000. Mapping quantitative trait loci (QTLs) for resistance to *Cercospora* leaf spot disease (*Cercospora beticola Sacc.*) in sugar beet (*Beta vulgaris* L.). *Theor. Appl. Genet.*, ***100***: 1176-1182.

Shankar, K., Ahluwalia, M. and Jain, S.K., 1963. The use of selection indices in the improvement of pearl millet population. *Indian J. Genet. Plant Breed.*, ***23***: 30-33.

Sharma, A., 2001. Marker-assisted improvement of downy mildew resistance in elite pearl millet (*Pennisetum glaucum*) parental line H 77/883-2. Ph.D. Thesis, CCS HAU, Hisar, India.

Sharma, B.B., Sadagopan, V.R. and Reddy, V.R., 1979. Utilization of different cereals in broiler rations. *British Poult. Sci.*, ***20***: 371-388.

Sharp, P.J., Chao, S., Desai, S. and Gale, M.D., 1989. The isolation, characterization and application in the Triticeae of a set of wheat RFLP probes identifying each homoeologous chromosome arm. *Theor. Appl. Genet.*, ***78***: 342-348.

Sharp, P.J., Kries, M., Sherry, P.R. and Gale, M.D., 1988. Location of β-amylase sequences in wheat and its derivatives. *Theor. Appl. Genet.*, *75*: 286-290.

Shetty, H.S. and Ahmad, R., 1981. Physiologic specialization in *Sclerospora graminicola*. *Indian Phytopathol.*, ***34(3)***: 307-309.

Shetty, H.S., 1987. Biology and epidemiology of downy mildew in pearl millet. *In*: Proceedings of the International Pearl Millet Workshop. (Witcombe, J.R. and Beckerman, S.R., Eds.). ICRISAT, Patancheru, AP, India. Pp. 147-160

Shetty, H.S., Neergaard, P., Mathur, S.B. and Safeeulla, K.M., 1980. Variability in *Sclerospora graminicola* and seed borne nature in pearl millet. *Newsletter, Int. Working Group on Graminaceous Downy Mildew*, ***2***: 5-6.

Shinde, R.B., Patil, F.B. and Sangave, R.A., 1984. Resistance to downy mildew in pearl millet. *J. Maharashtra Agric. Univ.*, ***9***: 337-338.

Sidhu, G.S., 1986. Host-parasite genetics. *Plant Breed. Rev.*, **5**: 393-433.

Sillanpaa, M.J. and Arjas, E., 1998. Bayesian mapping of multiple quantitative trait loci form incomplete inbred line cross data. *Genetics*, **148**: 1373-1388.

Simwemba, C.G., Hoseney, R.C., Varriano-Marson, E. and Zeleznak, K., 1984. Certain B-viatmins and phytic acid contents of pearl millet (*Pennisetum americanum* L. Leeke). *J. Agric. Food Chem.*, **32**: 31-34.

Singh, F., Singh, R.K., Singh, R.M. and Singh, R.B., 1978. Genetic analysis of downy mildew (*Sclerospora graminicola*) resistance in pearl millet [*Pennisetum typhoides* (Burm.) S. & H.]. *Z. Pflanzenzücht*, **81**: 54-59.

Singh, F., Singh, R.M., Singh, R.B. and Singh, R.K., 1980. Genetics studies of downy mildew resistance in pearl millet. *In:* Trends in Genetical Research on *Pennisetums*. (Gupta, V.P. and Minocha, J.L., Eds.). Punjab Agricultural University, Ludhiana.

Singh, S.D. and Barsaul, C.S., 1976. Replacement of maize by coarse grains for growth and production in White Leghorn and Rhode Island Red birds. *Indian J. Anim. Sci.*, **46**: 96-99.

Singh, S.D. and Gopinath, R., 1985. A seedling inoculation procedure for detecting downy mildew resistance. *Plant Dis.*, **69**: 582-584.

Singh, S.D. and Govind Singh, 1987. Resistance to downy mildew in pearl millet hybrid NHB 3. *Indian Phytopathol.*, **40**: 178-180

Singh, S.D. and Talukdar, B.S., 1998. Inheritance of complete resistance to pearl millet downy mildew. *Plant Dis.*, **82**: 791-793.

Singh, S.D., 1974. Studies on downy mildew disease [*Sclerospora graminicola (Sacc.)* Scort.] of bajra [*Pennosetum typhoides* (Burm. F.) Stapf and C.F. Hubb]. Ph.D. Thesis, IARI, New Delhi, India, Pp. 126.

Singh, S.D., 1995. Downy mildew of pearl millet. *Plant Dis.*, **79**: 545-550.

Singh, S.D., Alagarswamy, G., Talukdar, B.S. and Hash, C.T., 1994. Registration of ICML 22 photoperiod insensitive, downy mildew resistant pearl millet germplasm. *Crop Sci.*, ***34(5)***: 1421.

Singh, S.D., Ball, S.L. and Thakur, R.P., 1987. Problems and strategies in the control of downy mildew. *In*: Proceedings International Pearl Millet Workshop. (Witcombe, J.R. and Beckerman, S.R., Eds.). ICRISAT, Patancheru, A.P., India. Pp. 161-172.

Singh, S.D., de Milliano, W.A.J., Mtisi, E. and Chingombe. P., 1990. Brown leaf spot of pearl millet caused by *Bipolaris urochloae* in Zimbabwe. *Plant Dis.*, ***74***: 931-932.

Singh, S.D., King, S.B. and Werder, J., 1993a. Downy mildew disease of pearl millet. Information Bulletin no. 37. International Crops Research Institute for the Semi-Arid Tropics: Patancheru-502324, A.P., India. Pp. 36.

Singh, S.D., Lal, S. and Pande, S., 1993b. The changing scenario of maize, sorghum and pearl millet disease. *In:* Pests and Pests Management in India-The changing Scenario. (Sharma, H.C. and Veerabhadra, M., Eds.). Plant Protection Association of India, NPRTI: Hyderabad, India. Pp. 130-139.

Singh, S.D., Wilson, J.P., Navi, S.S., Talukdar, B.S., Hess, D.E. and Reddy, K.N., 1997. Screening techniques and sources of resistance to downy mildew and rust in pearl millet. Information Bulletin no. 48. ICRISAT: Patancheru-502324, A.P., India.

Singru, R., Sivaramakrishnan, S., Thakur, R.P., Gupta, V.S. and Ranjekar, P.K., 2003. Detection of genetic variability in pearl millet downy mildew (*Sclerospora graminicola*) by AFLP. *Biochemical Genetics.*, ***41 (11-12)***: 361-374.

Sivaramakrishnan, S., Thakur, R.P., Kannan, S. and Rao, V.P., 2003. Pathogenic and genetic diversity among Indian isolates of *Sclerospora graminicola* from pearl millet. Inofrmation Bulletin no. 48. ICRISAT, Patancheru-502324, A.P., India.

Soller, M. and Beckmann, J.S., 1983. Genetics polymorphism in varietals identification and genetic improvement. *Theor Appl. Genet.*, ***67***: 25-33.

Soller, M. and Beckmann, J.S., 1990. Marker-based mapping of quantitative trait loci using replicated progenies. *Theor Appl. Genet.*, ***80***: 205-208.

Soller, M., Genizi, A. and Brody, T., 1976. On the power of experimental designs for the detection of linkage between marker loci and quantitative loci in crosses between inbred lines. *Theor Appl. Genet.*, ***47***: 35-39.

Sorrells, M.E., La Rota, M., Bermudez-Kandianis, C.E., Greene, R.A., Kantety, R., Munkvold, J.D., Miftahudin, M., Mahmoud, A., Ma, X. and Gustafson, P.J., 2003. Comparative DNA Sequence Analysis of Wheat and Rice Genomes. *Gen. Res.*, ***13***:1818-1827.

Southern, E.M., 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.*, ***98***: 503-517.

Spehar, C.R. and Landers, J.N., 1997. Characteristicas,limitacoes e futuro do plantio directo nos cerrados. *In*: Seminario Internacional Do Sistema Plantio Directo. Embrapa-Trigo: Anais, Passo Fundo, RS. Pp. 157-161.

Spehar, C.R., 1999. Pearl millet production systems in the Brazilian savannahs (Cerrados). *In*: Proceedings International Pearl Millet Workshop. (Neto, A.L. de F., Amabile, R.F., Netto, D.A.M., Yamashita, T. and Gocho, H., Eds.).Embrapa Cerrados: Planaltina, D.F., Brazil. Pp. 181-189.

Stam, P. and Van Ooijen, J.W., 1995. Joinmap™ version 2.0: Software for the calculation of genetic linkage maps CPRO-DLO: Wageningen, The Netherlands.

Stam, P., 1993. Construction of integrated genetic linkage maps by means of computer package-Joinmap. *The Plant Journal*, ***5***: 739-744.

Statistical Abstract of India. 2002. Agriculture. Pp. 17-33.

Steel, R.G.D. and Torrie, J.H., 1960. Principles and Procedures of Statistics with special reference to biological sciences. McGraw-Hill Book Company, Inc., NY. Pp. 109-110.

Stegmeier, W.D., Andrews, D.J., Rai, K.N. and Hash, C.T., 1998. Pearl millet parental lines 843A and 843B. *International Sorghum and Millet Newsletter*, ***39***: 129-130.

Strand, M., Prolla, T.A., Liskay, R.M. and Petes, T.D., 1993. Destabilisation of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature*, ***365***: 274-276.

Streiff, R., Ducousso, A., Lexer, C., Steinkellner. H., Glössl, J. and Kremer, A., 1999. Pollen dispersal inferred from paternity analysis in a mixed oak stand of Quercus robur L. and Quercus petraea (Matt.) Liebl. *Mol. Ecol.*, ***8***: 831-841.

Stringhini, J.H. and de Sousa Franca, A.F., 1999. Use of pearl millet grains in the formulation of animal rations. *In*: Proceedings International Pearl Millet Workshop. (Neto, A.L. de F., Amabile, R.F., Netto, D.A.M., Yamashita, T. and Gocho, H., Eds.). Embrapa Cerrados: Planaltina, D.F., Brazil. Pp. 127-139.

Stuber, C.W., Edwards, M. and Wendel, J., 1987. Molecular marker-facilitated investigations of quantitative trait loci in maize. II. Factors influencing yield and its component traits. *Crop Sci.*, ***27***: 639-648.

Stuber, C.W., Goodman, M. and Moll, R., 1982. Improvement of yield and ear number resulting from selection at allozyme loci in a maize population. *Crop Sci.*, ***22***: 737-740.

Stuber, C.W., Lincoln, S.E., Wolff, D.W., Helentjaris, T. and Lander, E.S., 1992. Identification of genetic factors contributing to heterosis in a hybrid from elite maize inbred lines using molecular marker. *Genetic*, ***132***: 823-839.

Stuber, C.W., Moll, R., Goodman, M., Schaffer, H. and Weir, B., 1980. Allozyme frequency changes associated with selection for increased grain yield in maize (*Zea mays* L.). *Genetics*, ***95***: 225-236.

Stuber, C.W., Polacco, M. and Lynn Senior, M., 1999. Synergy of empirical breeding, marker-assisted selection and genomics to increase crop yield potential. *Crop Sci.*, ***39***: 1571-1583.

Subudhi, P.K. and Nguyen, H.T., 2000. Linkage group alignment of sorghum RFLP maps using a RIL mapping population. *Genome*, ***43***: 240-249.

Suiter, K.A., Wendel, J.F. and Case, J.S., 1983. Linkage-l: A PASCAL computer program for the detection and analysis of genetic linkage. *J. Hered.*, ***74***: 203-204.

Sullivan, T.W., Douglas, J.H., Bond, P.L. and Andrews, D.J., 1990. Nutrition value of pearl millet in broiler diets. *Poultry Sci.*, ***62***: 132.

Sunil, K.L., 1999. DNA markers in plant improvement: an overview. *Biotechnol. Adv.*, ***17***: 143-182.

Suryavanshi, Y.B., Ugale, S.D. and Patil, R.B., 1991. Phenotypic stability of yield and yield components in pearl millet. *J. Maharashtra Agric. Univ.*, ***16(2)***: 218-221.

Sushil Kumar, Chahal, G.S., Virk, D.S. and Kumar, S., 1996. Combining ability of diverse male sterile sources in pearl millet. *Crop Improv.*, ***23***: 151-154.

Tabosa, J.N., Brito, M.B., de Lima, G.S., Neto, A.D. de A., Simplicio, J.B., Lira, M. de A., Maciel, G.A. and Galindo, F.A.T., 1999. Prospects for pearl millet in Brazil, Northeast region. *In*: Proceedings International Pearl Millet Workshop. (Neto, A.L. de F., Amabile, R.F., Netto, D.A.M., Yamashita, T. and Gocho, H., Eds.). Embrapa Cerrados: Planaltina, D.F., Brazil. Pp. 163-179.

Tai, G.C.C., Seabrook, J.E.A. and Aziz, A.N., 2000. Linkage analysis of anther-derived monoploids showing distorted segregation of molecular markers. *Theor. Appl. Genet.*, ***101***: 126-130.

Tai, T.H. and Tanksley, S.D., 1990. A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. *Plant Mol. Biol. Rep.*, ***8***: 297-303.

Tanksley, S.D. and Hewitt, J., 1988. Use of molecular markers in breeding for soluble solids content in tomato a re-examination. *Theor. Appl. Genet.*, ***75***: 811-823.

Tanksley, S.D. and Rick, C.M., 1980. Isozymes gene linkage map of the tomato: Applications in genetics and breeding. *Theor. Appl. Genet.*, ***57***: 161-170.

Tanksley, S.D., 1993. Mapping polygenes. *Ann. Rev. Genet.*, ***27***: 205-233.

Tanksley, S.D., Ganal, M.W. and Martin, G.B., 1995. Chromosomal landing : a paradigm for map-based gene cloning in plants with large genomes. *Trends. Genet.*, ***11***: 63-68.

Tanksley, S.D., Medina-Filho, H. and Rick, C.M., 1982. Use of naturally occurring enzymes variation to detect and map gene controlling quantitative traits in an interspecific backcross of tomato. *Heredity*, ***49***: 11-25.

Tanksley, S.D., Young, N.D., Paterson, A.H. and Bonierbale, M.W., 1989. RFLP mapping in plant breeding: New tools for an old science. *Bio Technology*, **7**: 257-264.

Tautz, D. and Ranz, M., 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acids Res.*, **12**: 4127-4138.

Thakur, R.P. and Hash, C.T., 2004. Biotechnology in the management of pearl millet downy mildew. *In*: Biotechnological Approaches for the Integrated Management of Crop Diseases. (Mayee, C.D., Manoharachary, C., Tilak, K.V.B.R., Mukadam, D.S. and Deshpande, J., Eds.), Daya Publishing House, Delhi. Pp. 247-260.

Thakur, R.P. and Rao, V.P., 1993. Intra-population genetic diversity for virulence and aggressiveness in *Sclerospora graminicola*. The Sixth Int. Congress of Plant Pathol. Montreal. Pp. 166.

Thakur, R.P. and Rao, V.P., 1997. Variation in virulence and aggressiveness among pathotypes of *Sclerospora graminicola* on pearl millet. *Indian Phytopathol.*, **50**: 41-47.

Thakur, R.P., Mangil, C.W., Sivaramakrishnan, S., Hash, C.T., Shetty, H.S. and Hess, D.E., 2002. Variability in *Sclerospora graminicola*, the pearl millet downy mildew pathogen. *In*: Sorghum and Millets Diseases, (Leslie, J.F., Ed.), Iowa State press, Ames, Iowa. Pp. 51-56.

Thakur, R.P., Rai, K.N., Rao, V.P. and Rao, A.S., 2001. Genetic resistance of pearl millet male-sterile lines of diverse Indian pathotypes of *Sclerospora graminicola*. *Plant Dis.*, **85(6)**: 621-626.

Thakur, R.P., Rao, V.P. and Hash, C.T., 1998. A highly virulent pathotype of *Sclerospora graminicola* from Jodhpur, Rajasthan, India. *International Sorghum and Millets Newsletter*, **39**: 140-142.

Thakur, R.P., Rao, V.P., Sastry, J.G., Sivaramakrishnan, S., Amruthesh, K.N. and Barbind, L.D., 1999. Evidence for a new virulent pathogtypes of *Sclerospora graminicola* on pearl millet. *J. Mycol. Plant Pathol.*, **29**: 61-69.

Thakur, R.P., Rao, V.P., Singh, S.D. and Navi, S.S., 1997. Characterization of downy mildew resistance in pearl millet. *Jour. Mycol. Pathol.*, ***27(1)***: 6-16.

Thakur, R.P., Shetty, K.G. and King, S.B., 1992. Selection for host-specific virulence in asexual population of *Sclerospora graminicola*. *Plant Pathol.*, ***41***: 626-632.

Thoday, J.M., 1961. Location of polygenes. *Nature*, ***191***: 368-370.

Tiret, L., Abel, L. and Rakotovao, R., 1993. Effect of ignoring genotype-environment interaction on segregation analysis of quantitative traits. *Genetic Epidemiology*, ***10***: 581-586.

Tsujimoto, H. and Tsunewaki, K., 1985. Gametocidal genes in wheat and its relatives. II. Suppressor of the chromosome 3C gametocidal gene of *Aegilops triuncialis*. *Can. J. Genet. Cytol.*, ***27***: 178-185.

Tukey, J.W., 1953. The problem of multiple comparisons, Ditto, Princeton University, Princton, N.J.

Tyagi, C.S. and Iqbal Singh, 1989. Genetics of downy mildew resistance in pearlmillet. *Bhartiya Krishi Anusandhan Patrika*, ***4***: 24-30.

Tyagi, C.S., Paroda, R.S. and Lal, S., 1979. Genotype × environment interactions for yiller and ear number and ear length in pearl millet [P. americanum (l.) Sachum]. *Crop Improv.*, ***6(2)***: 110-119.

Utz, H.F. and Melchinger, A.E., 1994. Comparison of different approaches to interval mapping of quantitative trait loci. *In*: Biometrics in Plant Breeding: Applications of Molecular Markers. Proc. of the 9th Meeting of the EUCARPIA Section Biometrics in Plant Breeding. (Van Ooijen, J.W. and Jansen, J., Eds.). Wageningen, The Netherlands. Pp. 195-204.

Utz, H.F. and Melchinger, A.E., 2000. PLABQTL: A computer program to map QTL, Version 1.1, University of Hohenheim, Germany.

Utz, H.F., Melchinger, A.E. and Schön, C.C., 2000. Bias and sampling error of the estimated proportion of genotypic variance explanted by QTL determined from experimental data in maize using cross validation and validation with independent samples. *Genetics*, ***154***: 839-849.

Val, A.J. do., 1994. Um italino no cerrado; novidade no Mato Grosso, o milheto, plantado no meio da soja, da bom ao gado. *Globe Rural*, ***9(99)***: 7-10.

van der Plank, J.E., 1963. Plant diseases: epidemics and control. New York Academic press. Pp. 349.

van der Plank, J.E., 1968. Disease resistance in plants. New York: Academic. 206 pp. (2nd Ed.), Orlando: Academic, 1984. Pp. 194.

van Ooijen, J.W. and Maliepaard, C., 1996. MapQTL™ verson 3.0: Software for the calculation of QTL positions in genetic maps. CPRP-DLO, Wageningen, The Netherlands.

van Ooijen, J.W., 1992. Accuracy of mapping quantitative trait loci in autogamous species. *Theor. Appl. Genet.*, ***84***: 803-811.

van Ooijen, J.W., 1994. Comparison of a single-QTL model with an approximate multiple-QTL model for QTL mapping. *In*: Biometrics in Plant Breeding: Applications of Molecular Markers, Proceedings of the Ninth Meeting of the EUCARPIA section biometrics in plant breedings, 6-8th July 1994, (Van Ooijen, J.W. and Jansen, J., Eds.). Wageningen, The Netherlands. Pp. 205-212.

van Ooijen, J.W., 1999. LOD significance thresholds for QTL analysis in experimental populations of diploid species. *Heredity*, ***83***: 613-624.

Via, S. and Lande, R., 1987. Evolution of genetic variability in a spatially heterogeneous environment: effects of genotype-environment interaction. *Genet. Res.*, ***49***: 147-156.

Virk, D.S., 1988. Biometrical analysis in pearl millet-a review. *Crop Imp.*, ***15***: 1-29.

Vision, T.J., Shmoys, D.G., Durret, R.T. and Tanksley, S.D., 2000. Selective mapping: A strategy for optimizing the construction of high-density linkage maps. *Genetics*, ***155***: 407-420.

Vogel, J.M. and Scolnik, P.A., 1997. Direct amplification from microsatellite: detection of simple sequence repeat-based polymorphisms without cloning. *In*: DNA markers: protocols, applications and overviews (Caetano-Anolles G. and Gresshoff, P.M., Eds.) Wiley-Liss, Inc., New York. Pp. 133-150.

Wang, G.L., Mackill, D.J., Bonman, J.M., McCouch, S.R., Champoux, M.C. and Nelson, R.J., 1994. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. *Genetics*, ***136***: 1421-1434.

Weber, D. and Helentjaris, T., 1989. Mapping RFLP Loci in maize using B-A translocations. *Genetics*, ***121***: 583-590.

Weber, J.L. and May, P.E., 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase reaction. *American Journal of Human Genetics*, ***44***: 388-396.

Weising, K., Atkinson, R.G. and Gardner, R.C., 1995. Genomic fingerprinting by microsatellite-primed PCR: a critical evaluation. *PCR Methods and Applications*, ***4***: 249-255.

Weising, K., Weigand. F., Driesel. A., Kahl, G., Zischler, H. and Epplen, J.T., 1989. Polymorphic GATA/GACA repeats in plant genomes. *Nucl. Acids Res.*, ***17***: 10-28.

Weller, J., Soller, M. and Brody, T., 1988. Linkage analysis of quantitative traits in an interspecific cross of tomato (*Lycopersicon esculentum* × *L. pimpinellifolium*) by means of genetic markers. *Genetics*, ***118***: 329-339.

Weller, J.I., 1987. Mapping and analysis of quantitative trait loci in *Lycopersicon* (tomato) with the aid of genetic markers using approximate maximum likelihood methods. *Heredity*, ***59***: 413-421.

Wells, H.D. and Hanna, W.W., 1988. Genetics of resistance to *Bipolaris setariae* in pearl millet. *Phytopathol.*, ***78***: 1179-1181.

Weltzien, R.E. and King, S.B., 1995. Recurrent selection for downy mildew resistance in pearl millet. *Plant Breed.*, ***114***: 308-312.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acid Res.*, ***18***: 6531-6535.

Williams, R.J. and Andrews, D.J., 1983. Breeding for disease and pest resistance in pearl millet. *FAO Pl. Protect. Bull.*, ***31***: 136-158.

Williams, R.J., 1983. Downy mildews of tropical cereals. *In*: Advances in Plant Pathology, (Ingram, D.S., Ed.). Academic Press, New York. Vol. ***2***: 1-103.

Williams, R.J., 1984. Downy mildews of tropical cereals. *Adv. Plant Pathol.*, ***2***: 1-103.

Williams, R.J., Singh, S.D. and Pawar, M.N., 1981. An improved field screening technique for downy mildew resistance in pearl millet. *Plant Dis.*, ***65***: 239-241.

Wilson, J.P., 1999. Pearl millet disease: A compilation of information on the known pathogens of pearl millet [*Pennisetum glaucum* (L) R. Br.] Agriculture Handbook No. 716. U.S. Department of Agriculture, Agriculture Research Service, National Technical Information Service: Springfield, VA, USA.

Wilson, J.P., Hanna, W.W. and Bondari, K., 1993. Directed use of germplasm resources for breeding rust resustant pearl millet. *Plant Pathol. Trends Agrl. Sci.*, ***1***: 67-74.

Winter, P. and Kahl, G., 1995. Molecular marker technologies for plant improvement. *World J. Microbiol. Biotech.*, ***11***: 438-448.

Winter, P., Pfaff, T., Udapa, S.M., Hüttel, B., Sharma, P.C., Sahi, S., Arreguin-Espinoza, R., Weigand, F., Muehlbauer, F.J. and Kahl, G., 1999. Characterization and mapping of sequence-tagged microsatellite sites in the chichpea (*Cicer areitinum* L.) genome. *Mol. Gen. Genet.*, ***262***: 90-101.

Witcombe, J.R. and Hash, C.T., 2000. Resistance gene deployment strategies in cereal hybrid using marker-assisted selection: Gene pyramiding, three-way hybrids and synthetic parent population. *Euphytica*, ***112***: 175-186.

Wu, K.S. and Tanksley, S.D., 1993. Abundance polymorphism and genetic mapping of microsatellites in rice. *Mol .Genet.*, ***241***: 225-235.

Wu, W.R. and Li, W.M., 1994. A new approach for mapping quantitative trait loci using complete genetic marker linkage maps. *Theor. Appl. Genet.*, ***89***: 535-539.

Xu, S. and Vogl, C., 2000. Maximum likelihood analysis of quantitative trait under selective genotyping. *Heredity*, ***84***: 525-537.

Xu, X., Peng, M., Fang, Z. and Xu, X., 2000. The direction of microsatellite mutations is dependent upon allele length. *Nat. Genet.*, ***24***: 396-399.

Xu, Y., 1997. Quantitative triat lici: Separating, pyramiding and cloning. *Plant Breed. Rev.*, ***15***: 85-139.

Yadav, R.S., Bidinger, F.R., Hash, C.T., Yadav, Y.P., Yadav, O.P., Bhatnagar, S.K., and Howarth, C.J., 2003. Mapping and characterization of QTL × E interactions for traits determining grain and stover yield in pearl millet. *Theor. Appl. Genet.*, ***106***: 512-520.

Yadav, R.S., Hash, C.T., Bidinger, F.R., Cavan, G.P. and Howarth, C.J., 2002. Quantitative trait loci associated with traits determining grain and stover yield in pearl millet under terminal drought stress conditions. *Theor. Appl. Genet.*, ***104***: 67-83.

Yadav, R.S., Hash, C.T., Bidinger, F.R., Devos, K.M. and Howarth, C.J., 2004. Genomic regions associated with grain yield and aspects of post-flowering drought tolerance in pearl millet across stress environments and tester background. *Euphytica, **136 (3)***: 265-277.

Yamagishi, M., Yano, M., Fukuta, Y., Fukui, Y., Otani, M. and Shimada, M., 1996. Distorted segregation of RFLP markers in regenerated plants derived from anther culture of an F_1 hybrid rice. *Gene Genet. Syst.*, ***71***: 37-41.

Yamamoto, T., Kuboki, Y., Lin, S.Y., Sasaki, T. and Yano, M., 1998. Fine mapping of quantitative trait loci *Hd1, Hd2* and *Hd3,* controlling heading date of rice, as single Mendelian factors. *Theor. Appl. Genet.*, ***83***: 813-820.

Young, N.D. and Tanksley, S.D., 1989a. Graphic-based whole genome selection using RFLPs. *In*: Current Communication in Molecular Biology: Development and Application of Molecular Markers to Problems in Plant Genetics, (Helentjaris, T. and Burr, B., Eds.). Cold Spring Harbor Press: Cold Spring Harbor, NY. Pp. 123-129.

Young, N.D. and Tanksley, S.D., 1989b. Restriction fragment length polymorphism maps and the concept of graphical genotypes. *Theor. Appl. Genet.*, *77*: 95-101.

Young, N.D., 1994. Constructing a plant genetic linkage map with DNA markers. *In*: DNA-Based Markers in Plants. (Phillips, R.I. and Vasil, I.K., Eds.). Pp. 39-57.

Young, N.D., 1996. QTL mapping and quantitative disease resistance in plants. *Annu. Rev. Phytopathol.*, ***34***: 479-501.

Young, N.D., 1999. A cautiously optimistic vision for marker-assisted breeding. *Mol. Breed.*, ***5***: 505-510.

Young, N.D., 2001. Constructing a plant genetics linkage map with DNA markers. *In*: DNA based markers in plants (Phillips, R.L. and Vasil, I.K., Eds.) Kluwer Academic Publisher. The Netherlands. Pp. 31-48.

Young, N.D., Miller, J. and Tanksley, S.D., 1987. Rapid chromosomal assignment of multiple genomic clones in tomato using primary trisomics. *Nucl. Acids Res.*, ***15***: 9339-9348.

Yu, Z.H., Mackill, D.J., Bonman, J.M. and Tanksley, S.D., 1991. Tagging genes for blast resistance in rice via linkage to RFLP markers. *Theor. Appl. Genet.*, ***81***: 471-476.

Zeng, Z.B., 1993. Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. *Proc. Natl. Acad. Sci. USA*, ***90***: 10972-10976.

Zeng, Z.B., 1994. Precision mapping of quantitative trait loci. *Genetics*, ***136***: 1457-1468.

Zeven, A.C., Knott, D.R. and Johnson, R., 1983. Investigation of linkage drag in near isogenic lines of wheat by testing for seedling reaction to races of stem rust, leaf rust and yellow rust. *Euphytica*, ***32***: 319-327.

Zhang, X.Q., Ross, K. and Gustafson, J.P., 2000. Physical location of homoeologous groups 5 and 6 molecular markers mapped in *Triticum aestivum* L. *Genetics*, ***91***: 441-445.

Zhonggi, L. and Youchun, L., 1994. Heterosis on grain weight in wheat hybrid with *Triticum timopheevii* cytoplasm. *Euphytica*, ***75***: 189-193.

APPENDICES

Appendix 1. Genotypic data points scored from 172 F_2 mapping population progenies using 46 marker loci (including SSRs and RFLPs) polymorphic betwwen pearl millet parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer/probe	[a] LG [b]	2 1	3 2	4 3	5 4	6 5	7 6	8 7	10 8	11 9	12 10	13 11	15 12	16 13	17 14	18 15	19 16	20 17	21 18	22 19	24 20	25 21	26 22	27 23	28 24	30 25
1	*Xpsm* 280	1	H	A	A	B	B	B	H	H	H	H	H	A	H	H	A	-	H	H	A	D	B	B	H	H	A
2	*Xpsm* 492	1	H	A	-	B	B	H	H	-	H	H	H	H	H	C	H	H	H	-	-	B	-	-	H	H	A
3	*Xpsmp* 2273	1	B	B	A	B	B	H	H	H	H	B	B	H	H	H	A	A	A	A	H	B	B	B	-	-	-
4	*Xpsm* 17	1	B	-	A	B	B	H	H	H	H	B	B	H	C	H	A	-	A	A	A	B	B	B	H	H	H
5	*Xpsmp* 2080	1	B	B	A	B	B	H	H	H	H	B	B	-	A	H	A	A	A	A	A	B	B	-	H	B	H
6	*Xpsmp* 2030	1	B	B	A	B	B	H	H	H	H	B	B	H	A	H	A	A	A	A	A	B	B	B	H	B	H
7	*Xpsm* 196.1	1	H	H	H	H	H	H	B	H	B	H	D	D	H	D	H	A	A	A	A	B	-	H	H	H	H
8	*Xpsm* 708.1	2	D	H	C	H	-	-	H	H	B	A	A	H	H	C	A	B	B	H	H	H	D	H	B	B	H
9	*Xpsmp* 2237	2	H	H	H	B	B	B	A	H	B	A	A	H	H	H	A	B	B	B	C	A	H	D	H	H	H
10	*Xpsmp* 2072	2	H	A	H	B	B	B	A	H	B	A	A	H	H	H	A	H	H	H	H	A	H	H	H	H	H
11	*Xpsmp* 2077	2	H	A	H	B	B	B	A	H	B	H	H	A	H	H	-	H	H	H	-	H	H	H	H	H	H
12	*Xpsmp* 2201	2	H	B	-	B	B	H	A	H	B	H	H	A	H	D	A	H	H	H	A	H	B	H	H	H	H
13	*Xpsmp* 2232.2	2	H	B	H	B	B	H	A	H	H	B	B	A	A	A	A	H	H	H	H	D	B	B	-	H	H
14	*Xpsmp* 2225	2	H	B	H	B	B	H	H	H	H	B	B	H	A	H	A	H	B	A	H	H	B	H	H	-	H
15	*Xpsmp* 2089	2	H	H	B	B	H	H	H	B	H	H	H	H	C	-	A	B	H	H	H	H	H	B	-	B	H
16	*Xpsm* 37	3	H	H	H	H	B	B	A	B	A	A	A	H	H	H	A	H	C	A	H	D	D	B	A	B	H
17	*Xpsm* 18	3	H	H	H	H	H	B	A	B	A	A	A	H	H	A	A	-	B	A	D	H	A	H	H	-	B
18	*Xpsmp* 2070	3	H	H	H	H	H	B	A	B	A	A	-	H	H	-	A	H	B	A	A	H	A	H	A	B	B
19	*Xpsmp* 2267	3	H	-	H	H	H	B	A	B	A	A	A	H	C	A	A	H	B	A	H	H	D	H	C	B	B
20	*Xpsm* 409.1	4	B	B	H	H	B	A	H	H	B	H	H	H	H	H	H	A	H	-	H	H	C	H	H	B	A
21	*Xpsm* 648	4	H	A	-	H	B	H	H	H	H	H	H	A	B	H	H	H	B	B	H	A	A	B	H	H	H
22	*Xpsm* 344	4	H	A	H	B	B	H	H	A	H	H	H	H	B	H	H	H	B	B	B	A	A	H	H	H	A
23	*Xpsm* 84	4	H	A	H	B	B	D	H	A	B	H	H	H	B	H	H	-	C	B	B	A	A	H	H	H	A
24	*Xpsmp* 2084	4	H	A	H	B	B	D	H	A	B	H	-	H	B	H	-	H	-	B	B	A	A	H	C	H	A
25	*Xpsm* 837.2	4	H	A	H	B	B	H	H	A	B	H	H	H	H	B	A	H	B	-	B	A	A	H	H	H	H
26	*Xpsm* 416.3	4	H	H	H	B	B	H	H	A	-	H	H	H	-	H	H	H	B	B	B	A	D	H	H	H	A

...contd. Appendix 1. Genotypic data points scored from 172 F_2 mapping population progenies using 46 marker loci (including SSRs and RFLPs) polymorphic betwwen pearl millet parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer/probe	LG	[a] 31	32	33	34	35	37	38	39	41	42	44	45	46	47	48	49	50	51	52	53	55	56	57	58	59
			[b] 26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
1	*Xpsm* 280	1	B	B	A	H	H	A	A	A	A	H	H	H	H	H	A	H	A	A	B	A	H	-	H	B	D
2	*Xpsm* 492	1	B	B	A	H	B	H	A	A	H	H	B	H	-	H	A	H	-	-	-	H	H	C	H	B	C
3	*Xpsmp* 2273	1	H	H	A	H	-	H	H	H	A	-	H	H	B	B	H	B	H	H	H	A	A	H	H	C	A
4	*Xpsm* 17	1	B	B	A	H	B	H	H	H	A	B	H	H	H	B	H	H	A	-	-	-	-	-	-	-	-
5	*Xpsmp* 2080	1	H	H	A	-	H	H	H	H	A	B	B	B	-	B	H	B	A	A	H	H	B	H	B	B	H
6	*Xpsmp* 2030	1	C	C	A	H	H	H	H	H	A	B	H	B	H	B	H	-	A	A	H	H	B	H	B	-	H
7	*Xpsm* 196.1	1	H	C	A	A	H	H	B	H	-	H	B	A	B	H	B	H	H	A	H	H	B	-	B	H	H
8	*Xpsm* 708.1	2	H	B	H	H	A	B	H	H	B	A	B	H	A	H	H	A	H	A	A	A	B	-	H	H	H
9	*Xpsmp* 2237	2	H	H	H	H	A	H	H	H	B	C	D	B	H	-	H	-	H	H	A	D	D	-	C	H	D
10	*Xpsmp* 2072	2	H	H	H	A	A	H	A	B	B	A	H	H	H	B	H	H	A	H	H	A	H	B	A	B	H
11	*Xpsmp* 2077	2	H	H	H	A	A	H	A	B	B	A	B	-	H	H	D	A	A	H	A	H	H	B	A	-	H
12	*Xpsmp* 2201	2	H	H	H	A	A	H	A	B	B	A	B	H	H	H	H	A	A	H	A	H	H	B	A	A	H
13	*Xpsmp* 2232.2	2	B	B	H	A	A	H	A	-	H	B	B	B	H	C	H	A	H	A	A	-	B	D	A	A	C
14	*Xpsmp* 2225	2	B	B	H	H	A	H	A	H	-	B	-	B	H	-	H	-	A	A	H	H	B	H	-	A	H
15	*Xpsmp* 2089	2	B	C	A	H	H	H	A	H	H	D	B	H	A	A	H	-	A	H	A	H	B	B	H	A	B
16	*Xpsm* 37	3	B	A	H	H	A	H	A	A	H	B	H	H	B	B	H	A	H	A	H	H	B	B	B	H	A
17	*Xpsm* 18	3	D	A	H	A	A	-	H	H	H	-	H	-	B	B	A	A	H	A	H	H	H	-	B	H	A
18	*Xpsmp* 2070	3	A	H	H	A	A	A	H	A	H	B	-	H	-	B	H	H	H	H	H	A	H	B	B	H	A
19	*Xpsmp* 2267	3	A	H	H	A	A	A	D	A	H	B	A	B	B	B	H	A	H	H	H	A	H	B	B	H	A
20	*Xpsm* 409.1	4	A	B	H	H	H	A	-	A	A	H	B	H	H	H	A	H	H	-	A	A	-	-	B	H	B
21	*Xpsm* 648	4	B	B	H	H	B	C	H	H	H	H	B	H	H	C	H	B	B	B	A	H	A	D	H	B	B
22	*Xpsm* 344	4	B	B	A	H	B	H	A	A	H	H	B	H	A	H	A	H	B	B	A	H	H	H	H	B	B
23	*Xpsm* 84	4	H	B	A	H	C	H	A	A	H	H	B	H	A	H	A	H	C	-	A	A	H	H	H	C	C
24	*Xpsmp* 2084	4	-	B	A	H	B	H	A	A	A	H	-	A	H	H	H	H	-	C	A	A	H	H	H	H	B
25	*Xpsm* 837.2	4	H	H	-	H	H	H	A	A	H	H	B	H	H	B	H	H	B	H	A	H	H	H	B	D	B
26	*Xpsm* 416.3	4	-	B	A	H	-	-	A	A	H	H	B	H	A	H	A	H	B	B	A	H	H	H	-	B	B

...contd. Appendix 1. Genotypic data points scored from 172 F_2 mapping population progenies using 46 marker loci (including SSRs and RFLPs) polymorphic betwwen pearl millet parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer/probe	LG	[a] 31 / [b] 26	32 / 27	33 / 28	34 / 29	35 / 30	37 / 31	38 / 32	39 / 33	41 / 34	42 / 35	44 / 36	45 / 37	46 / 38	47 / 39	48 / 40	49 / 41	50 / 42	51 / 43	52 / 44	53 / 45	55 / 46	56 / 47	57 / 48	58 / 49	59 / 50
27	*Xpsmp* 2202	5	B	H	A	A	H	A	H	-	H	H	H	A	H	H	B	H	-	H	B	H	B	H	-	A	A
28	*Xpsmp* 2274	5	B	H	A	A	H	H	H	H	H	H	H	A	H	H	B	H	A	H	B	C	B	H	A	H	A
29	*Xpsmp* 2220	5	B	H	A	-	H	-	H	H	H	A	-	H	H	A	B	H	-	H	B	H	B	-	A	H	A
30	*Xpsmp* 2078	5	B	C	A	A	H	H	H	H	H	A	H	H	H	A	B	D	A	H	B	H	B	H	A	H	A
31	*Xpsmp* 2208	5	B	H	A	-	H	H	H	H	H	A	A	H	H	A	B	H	A	H	B	-	B	H	A	H	A
32	*Xpsmp* 2276	5	B	H	A	A	H	H	H	H	-	A	A	H	H	A	B	H	A	H	B	H	B	H	A	H	A
33	*Xpsmp* 2277	5	B	B	A	A	H	H	H	H	H	A	A	H	H	A	B	H	A	H	B	H	B	H	A	H	A
34	*Xpsmp* 2261	5	B	B	A	A	A	H	A	H	H	A	A	H	H	A	B	H	H	H	B	H	B	H	A	H	A
35	*Xpsm* 588	6	H	H	H	H	B	B	H	A	B	H	B	B	C	H	H	H	H	B	H	H	B	H	H	H	H
36	*Xpsm* 696	6	B	H	H	H	B	B	H	A	B	H	B	B	B	H	B	H	H	H	H	H	C	H	H	A	B
37	*Xpsmp* 2018	6	H	H	B	H	B	B	H	A	H	H	H	B	B	H	B	H	H	B	B	H	H	B	H	A	B
38	*Xpsm* 575	6	H	H	B	A	B	B	H	A	H	H	H	B	B	H	B	H	H	-	B	H	H	B	H	A	B
39	*Xpsm* 718	7	C	H	A	A	B	B	B	H	B	B	A	H	H	H	H	H	H	-	A	B	A	A	H	B	B
40	*Xpsm* 269	7	H	H	H	H	H	B	B	H	B	B	A	H	A	-	A	H	H	A	H	H	-	H	H	-	B
41	*Xpsmp* 2224	7	H	H	H	H	H	B	B	H	H	B	-	B	A	H	H	H	A	H	H	H	H	H	H	H	B
42	*Xpsmp* 2074	7	H	D	H	H	H	B	-	H	D	B	C	B	A	A	-	H	-	D	-	H	H	H	H	-	B
43	*Xpsmp* 2263	7	H	H	H	H	D	H	-	B	H	-	H	C	H	-	H	H	A	H	H	A	-	H	A	B	H
44	*Xpsmp* 2203	7	H	A	H	A	H	H	B	C	H	H	H	B	A	A	A	H	-	A	-	H	A	A	A	B	B
45	*Xpsm* 160	7	A	A	H	A	H	H	H	H	H	H	H	A	H	H	H	H	B	A	C	H	-	-	H	-	H
46	*Xpsm* 190	7	A	H	H	H	H	H	H	A	H	H	H	A	H	B	H	H	B	C	A	H	H	H	H	B	B

[a]Original plant DNA sample number from mapping population; [b]Serial number given to them; LG-Linkage group

A = Homozygote for the allele a from parental strain P_1 (ICMB 89111-P6) at this locus

B = Homozygote for the allele b from parental strain P_2 (ICMB 90111-P6) at this locus

H = Heterozygote (F_1) carrying alleles from both P_1 and P_2 parental strains

C = Not a homozygote for allele A (i.e. either B or H)

D = Not a homozygote for allele B (i.e. either A or H)

- = Missing data for the individual at this locus

Xpsmp indicates SSR (microsatellite) primers and *Xpsm* indicates RFLP probes

...contd. Appendix 1. Genotypic data points scored from 172 F_2 mapping population progenies using 46 marker loci (including SSRs and RFLPs) polymorphic betwwen pearl millet parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer/probe	LG	[a] 60	61	62	63	64	65	66	67	69	70	72	73	74	75	76	78	79	80	82	83	85	86	87	88	89
			[b] 51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
1	*Xpsm* 280	1	H	A	A	H	H	A	H	H	H	H	H	H	B	H	B	H	B	H	H	A	H	C	H	A	H
2	*Xpsm* 492	1	B	A	H	H	H	H	H	H	B	H	B	-	B	H	H	H	H	H	H	A	H	A	-	-	H
3	*Xpsmp* 2273	1	H	A	A	-	A	H	A	H	H	H	H	H	H	H	H	B	B	H	-	-	H	H	H	A	H
4	*Xpsm* 17	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	*Xpsmp* 2080	1	H	H	H	-	-	A	H	A	H	B	-	B	H	B	B	-	B	H	H	H	H	H	H	A	H
6	*Xpsmp* 2030	1	-	H	A	A	A	A	H	A	H	B	B	B	B	B	B	B	B	H	H	H	H	H	H	A	H
7	*Xpsm* 196.1	1	H	B	H	H	-	A	A	A	H	H	H	-	B	B	-	B	H	A	B	-	-	H	B	H	A
8	*Xpsm* 708.1	2	H	B	H	D	H	H	A	H	H	H	H	B	H	-	B	A	B	B	-	B	B	H	H	H	A
9	*Xpsmp* 2237	2	H	H	A	B	A	H	A	H	D	D	H	H	H	H	H	H	D	H	H	H	H	D	H	D	H
10	*Xpsmp* 2072	2	B	A	A	D	A	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	B
11	*Xpsmp* 2077	2	B	A	A	B	A	H	A	H	H	A	H	H	A	H	A	A	H	H	H	H	H	A	A	H	B
12	*Xpsmp* 2201	2	B	A	A	B	A	H	A	H	H	A	H	H	A	H	A	A	H	H	A	H	B	A	A	A	B
13	*Xpsmp* 2232.2	2	H	H	H	B	A	A	H	A	H	H	H	H	H	B	H	C	D	H	H	H	-	A	H	-	H
14	*Xpsmp* 2225	2	H	H	H	H	-	A	H	A	H	B	A	B	H	B	C	C	B	H	B	H	H	H	H	H	H
15	*Xpsmp* 2089	2	H	B	B	H	H	C	H	C	H	B	H	B	-	B	B	D	-	B	H	B	B	B	H	A	B
16	*Xpsm* 37	3	H	B	B	H	A	H	B	H	H	H	H	A	B	H	D	A	B	B	B	A	H	A	H	H	B
17	*Xpsm* 18	3	H	B	B	C	A	H	B	H	H	H	B	D	-	H	D	H	B	H	B	H	-	A	H	H	B
18	*Xpsmp* 2070	3	H	B	H	C	A	H	B	H	H	H	B	H	B	H	A	H	B	B	-	H	H	A	H	H	B
19	*Xpsmp* 2267	3	H	B	B	H	A	H	B	C	H	H	B	H	B	H	A	-	B	B	B	H	H	A	H	H	B
20	*Xpsm* 409.1	4	B	A	H	A	B	H	B	A	A	A	H	A	H	H	A	H	H	H	H	A	-	H	-	A	D
21	*Xpsm* 648	4	B	A	H	H	H	H	H	H	H	H	B	A	H	C	H	H	H	H	H	H	A	A	B	H	H
22	*Xpsm* 344	4	B	A	H	H	H	H	H	H	B	H	B	A	B	H	H	H	H	H	H	A	H	A	B	H	H
23	*Xpsm* 84	4	B	A	D	H	H	H	H	H	B	H	B	A	B	H	H	H	H	H	H	-	-	-	B	H	H
24	*Xpsmp* 2084	4	B	-	A	H	H	H	H	H	A	A	H	H	H	H	H	H	A	H	H	H	H	-	-	H	H
25	*Xpsm* 837.2	4	B	A	-	H	H	H	H	H	A	H	H	A	H	A	H	H	A	H	H	A	H	A	B	H	H
26	*Xpsm* 416.3	4	B	A	H	H	H	H	H	H	-	H	-	A	B	H	H	H	H	H	H	A	H	A	B	H	H

...contd. Appendix 1. Genotypic data points scored from 172 F_2 mapping population progenies using 46 marker loci (including SSRs and RFLPs) polymorphic betwwen pearl millet parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer/probe	LG	[a] 60	61	62	63	64	65	66	67	69	70	72	73	74	75	76	78	79	80	82	83	85	86	87	88	89
			[b] 51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
27	*Xpsmp* 2202	5	B	A	-	H	H	A	H	H	H	A	H	H	H	H	H	B	B	H	H	H	A	H	H	H	H
28	*Xpsmp* 2274	5	H	A	H	H	H	A	A	H	H	A	H	H	H	H	H	H	B	H	H	H	A	A	H	H	H
29	*Xpsmp* 2220	5	H	A	H	H	H	A	A	H	H	A	H	A	-	-	H	H	C	-	H	H	A	A	H	-	D
30	*Xpsmp* 2078	5	H	A	H	H	H	A	A	A	H	A	H	A	H	H	H	H	B	H	H	H	A	A	H	H	H
31	*Xpsmp* 2208	5	H	A	-	H	H	A	A	A	-	H	A	H	H	H	H	H	B	H	H	H	A	A	-	H	H
32	*Xpsmp* 2276	5	H	A	H	H	H	A	A	A	H	H	D	H	H	H	A	H	B	-	H	H	A	A	H	H	H
33	*Xpsmp* 2277	5	H	A	H	-	H	A	A	A	H	H	A	H	H	C	A	H	B	H	H	H	A	A	H	H	H
34	*Xpsmp* 2261	5	H	A	H	H	H	A	A	A	H	H	H	H	H	H	A	H	B	H	H	H	A	A	H	H	-
35	*Xpsm* 588	6	B	H	B	H	B	H	H	H	B	H	H	B	-	C	B	H	-	D	H	A	B	B	B	H	B
36	*Xpsm* 696	6	B	H	B	H	B	H	H	H	B	H	H	H	H	B	H	H	H	A	H	H	H	B	B	H	B
37	*Xpsmp* 2018	6	B	H	B	H	B	H	H	H	H	H	A	B	A	B	B	H	H	A	H	H	H	H	B	H	B
38	*Xpsm* 575	6	B	A	B	H	B	H	H	H	H	H	H	B	A	B	B	H	H	A	H	H	B	H	B	H	B
39	*Xpsm* 718	7	B	A	H	H	H	H	H	H	H	H	B	A	H	B	H	H	H	H	H	H	D	A	B	H	H
40	*Xpsm* 269	7	B	H	C	H	B	H	H	H	-	H	A	-	H	B	H	H	H	A	H	H	H	-	B	H	B
41	*Xpsmp* 2224	7	B	A	A	B	B	A	H	-	A	-	A	A	B	H	H	H	-	H	B	H	B	A	H	H	H
42	*Xpsmp* 2074	7	-	-	-	-	B	A	H	A	A	H	-	H	B	H	A	H	B	D	B	C	B	A	H	D	H
43	*Xpsmp* 2263	7	B	A	A	B	B	A	A	A	A	H	A	H	C	H	A	H	B	H	H	H	-	H	D	H	H
44	*Xpsmp* 2203	7	B	A	H	B	B	A	H	A	A	H	-	A	B	H	A	H	B	H	B	B	B	H	H	D	H
45	*Xpsm* 160	7	H	A	A	B	H	A	H	H	-	B	H	H	B	H	H	H	H	H	B	B	D	B	B	H	C
46	*Xpsm* 190	7	B	A	H	H	H	H	H	H	B	H	B	A	B	H	H	H	H	H	H	A	B	A	B	H	H

[a]Original plant DNA sample number from mapping population: [b]Serial number given to them; LG-Linkage group

A = Homozygote for the allele a from parental strain P_1 (ICMB 89111-P6) at this locus

B = Homozygote for the allele b from parental strain P_2 (ICMB 90111-P6) at this locus

H = Heterozygote (F_1) carrying alleles from both P_1 and P_2 parental strains

C = Not a homozygote for allele A (i.e. either B or H)

D = Not a homozygote for allele B (i.e. either A or H)

- = Missing data for the individual at this locus

Xpsmp indicates SSR (microsatellite) primers and *Xpsm* indicates RFLP probes

...contd. Appendix 1. Genotypic data points scored from 172 F_2 mapping population progenies using 46 marker loci (including SSRs and RFLPs) polymorphic betwwen pearl millet parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer/probe	LG	[a] 90	91	92	93	94	95	96	98	99	100	101	102	103	104	105	107	108	109	110	111	113	114	115	117	118
			[b] 76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	*Xpsm* 280	1	H	A	B	H	A	H	H	H	H	H	-	-	-	A	A	-	-	-	-	H	B	-	-	A	-
2	*Xpsm* 492	1	A	A	H	H	H	B	H	H	H	H	H	H	A	H	A	H	B	H	H	A	H	A	H	H	H
3	*Xpsmp* 2273	1	A	A	-	H	B	B	B	B	H	B	H	H	H	A	-	B	B	H	H	H	H	H	H	B	H
4	*Xpsm* 17	1	-	-	-	-	-	-	-	-	-	-	H	H	H	A	A	B	B	H	H	H	B	H	H	B	H
5	*Xpsmp* 2080	1	H	H	H	H	B	B	B	B	H	H	B	B	B	A	H	H	B	H	H	H	H	H	-	B	H
6	*Xpsmp* 2030	1	H	H	H	H	B	B	B	H	-	-	-	B	H	H	H	H	H	B	B	B	H	H	H	H	H
7	*Xpsm* 196.1	1	H	H	H	H	H	H	B	H	H	H	-	B	H	H	B	H	B	B	B	H	H	A	H	B	H
8	*Xpsm* 708.1	2	B	A	C	H	H	-	-	-	-	-	H	A	A	B	A	H	H	H	H	H	H	H	H	B	H
9	*Xpsmp* 2237	2	B	H	A	A	H	H	H	A	H	A	H	A	H	A	H	H	A	H	H	H	B	D	D	H	H
10	*Xpsmp* 2072	2	H	A	A	-	H	H	H	A	H	A	H	A	H	A	H	H	A	A	H	H	B	H	A	H	H
11	*Xpsmp* 2077	2	-	A	A	H	A	H	H	A	H	H	A	A	-	D	H	H	A	H	A	H	B	H	H	H	H
12	*Xpsmp* 2201	2	H	A	A	B	D	H	H	A	H	H	A	A	H	A	H	H	A	A	H	H	B	A	H	H	H
13	*Xpsmp* 2232.2	2	A	A	-	B	B	B	A	-	H	B	A	A	H	H	H	H	A	A	A	H	H	A	H	B	-
14	*Xpsmp* 2225	2	H	H	H	-	B	B	-	H	H	B	-	C	-	H	H	-	A	A	A	H	H	A	A	B	H
15	*Xpsmp* 2089	2	H	B	H	B	H	H	H	H	H	B	H	B	H	B	H	A	H	A	H	B	A	A	H	H	-
16	*Xpsm* 37	3	H	B	B	A	H	H	H	H	A	H	A	B	B	H	A	H	H	H	B	A	B	H	H	B	D
17	*Xpsm* 18	3	-	B	B	A	D	H	H	H	A	D	D	C	B	H	C	D	H	H	H	H	B	H	H	H	B
18	*Xpsmp* 2070	3	H	H	B	A	H	H	H	H	H	H	A	H	B	H	B	A	H	H	B	H	B	H	-	H	H
19	*Xpsmp* 2267	3	H	H	B	A	H	H	H	-	H	H	A	-	-	-	H	A	H	H	B	H	H	H	H	H	A
20	*Xpsm* 409.1	4	-	A	H	H	A	H	H	H	H	A	A	A	H	-	-	A	B	H	H	H	A	H	A	H	H
21	*Xpsm* 648	4	A	-	H	H	B	H	H	H	H	H	H	A	H	H	H	H	B	H	H	H	H	A	H	H	H
22	*Xpsm* 344	4	A	A	H	H	H	H	H	H	H	H	H	H	A	H	A	H	B	H	H	H	H	A	H	H	H
23	*Xpsm* 84	4	H	A	A	H	H	H	H	H	H	D	H	H	A	H	A	H	B	H	H	A	D	A	H	H	H
24	*Xpsmp* 2084	4	H	A	A	H	H	H	H	H	H	H	H	H	A	H	A	C	B	H	H	A	H	A	H	H	-
25	*Xpsm* 837.2	4	H	A	H	H	H	H	C	H	H	D	H	H	A	H	A	H	H	H	H	A	D	H	D	H	H
26	*Xpsm* 416.3	4	A	A	H	H	A	H	H	H	H	H	H	H	A	H	A	H	B	H	H	A	H	A	H	H	H

...contd. Appendix 1. Genotypic data points scored from 172 F_2 mapping population progenies using 46 marker loci (including SSRs and RFLPs) polymorphic betwwen pearl millet parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer/probe	LG	[a] 90	91	92	93	94	95	96	98	99	100	101	102	103	104	105	107	108	109	110	111	113	114	115	117	118
			[b] 76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
27	*Xpsmp* 2202	5	A	B	H	H	H	H	H	B	B	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	B
28	*Xpsmp* 2274	5	A	B	H	H	-	H	H	H	B	H	H	H	H	H	H	H	H	H	H	H	H	-	A	H	B
29	*Xpsmp* 2220	5	A	B	H	H	H	H	C	H	B	-	H	H	H	H	H	H	H	H	H	H	H	H	A	H	B
30	*Xpsmp* 2078	5	A	B	H	H	H	H	B	B	-	H	H	H	H	H	H	H	H	H	H	H	H	H	A	B	B
31	*Xpsmp* 2208	5	A	B	H	H	H	H	H	B	H	H	-	-	H	-	-	A	-	C	H	-	-	B	-	-	B
32	*Xpsmp* 2276	5	-	B	H	H	H	H	H	-	B	B	B	H	B	H	H	A	H	-	A	H	H	-	A	B	H
33	*Xpsmp* 2277	5	A	B	H	H	H	H	H	-	B	B	B	H	B	H	H	H	H	A	A	H	H	H	A	B	H
34	*Xpsmp* 2261	5	A	B	H	H	H	H	H	B	B	H	H	H	H	H	H	H	A	A	H	H	A	H	-	B	B
35	*Xpsm* 588	6	B	B	H	H	H	A	H	B	C	A	H	H	A	B	B	H	B	H	H	A	H	A	H	H	H
36	*Xpsm* 696	6	H	B	H	H	H	A	H	B	B	A	H	H	D	B	B	C	H	H	H	A	H	D	H	H	H
37	*Xpsmp* 2018	6	H	B	H	H	H	H	H	H	H	A	H	H	H	B	B	H	H	H	H	A	H	H	A	H	B
38	*Xpsm* 575	6	H	B	H	H	H	H	H	B	H	A	H	H	H	B	B	H	H	H	H	A	H	H	A	B	H
39	*Xpsm* 718	7	A	-	H	H	B	A	H	H	H	H	H	A	H	H	H	H	B	H	H	H	H	A	H	H	H
40	*Xpsm* 269	7	H	-	H	H	H	A	H	-	-	A	-	H	A	-	B	-	H	H	H	A	H	A	H	H	H
41	*Xpsmp* 2224	7	B	A	H	H	A	H	D	A	H	A	H	-	-	A	B	-	B	H	-	-	B	H	H	H	B
42	*Xpsmp* 2074	7	B	-	H	H	A	H	H	A	H	H	H	H	H	-	H	H	B	H	H	A	C	C	H	H	B
43	*Xpsmp* 2263	7	B	A	H	B	A	H	H	A	H	H	H	H	H	A	H	H	B	H	H	A	B	H	H	H	B
44	*Xpsmp* 2203	7	B	A	H	B	A	H	-	A	A	H	H	H	H	A	H	H	B	H	H	A	B	B	H	H	B
45	*Xpsm* 160	7	A	A	C	B	B	H	A	A	H	A	-	D	H	D	A	B	H	B	H	A	B	B	H	B	B
46	*Xpsm* 190	7	A	A	H	H	H	H	H	H	H	H	H	H	A	H	A	H	B	H	H	A	H	A	H	H	H

[a]Original plant DNA sample number from mapping population; [b]Serial number given to them; LG-Linkage group

A = Homozygote for the allele a from parental strain P_1 (ICMB 89111-P6) at this locus

B = Homozygote for the allele b from parental strain P_2 (ICMB 90111-P6) at this locus

H = Heterozygote (F_1) carrying alleles from both P_1 and P_2 parental strains

C = Not a homozygote for allele A (i.e. either B or H)

D = Not a homozygote for allele B (i.e. either A or H)

- = Missing data for the individual at this locus

Xpsmp indicates SSR (microsatellite) primers and *Xpsm* indicates RFLP probes

...contd. Appendix 1. Genotypic data points scored from 172 F_2 mapping population progenies using 46 marker loci (including SSRs and RFLPs) polymorphic betwwen pearl millet parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer/probe	LG	[a] 119 / [b] 101	120 / 102	121 / 103	122 / 104	123 / 105	124 / 106	126 / 107	127 / 108	128 / 109	129 / 110	130 / 111	132 / 112	133 / 113	134 / 114	135 / 115	137 / 116	138 / 117	139 / 118	140 / 119	141 / 120	142 / 121	143 / 122	144 / 123	145 / 124	147 / 125
1	*Xpsm* 280	1	-	-	H	A	A	-	H	H	B	-	H	-	H	B	H	H	-	B	A	H	H	B	B	-	B
2	*Xpsm* 492	1	H	A	A	A	H	H	C	H	H	H	H	A	B	B	H	H	A	-	H	H	H	H	H	H	B
3	*Xpsmp* 2273	1	H	B	B	A	B	H	A	-	H	B	H	A	H	B	H	H	H	B	H	H	H	H	H	A	B
4	*Xpsm* 17	1	H	B	B	A	B	H	A	A	H	B	H	A	H	B	-	H	H	B	H	H	H	H	H	A	B
5	*Xpsmp* 2080	1	H	B	H	A	B	H	H	A	H	B	H	A	H	B	A	H	H	B	H	H	H	-	H	A	H
6	*Xpsmp* 2030	1	H	-	B	A	-	H	H	-	-	H	-	H	B	B	A	-	H	-	A	H	B	B	H	H	H
7	*Xpsm* 196.1	1	H	B	H	-	D	H	H	H	B	H	B	H	B	B	H	B	H	H	A	H	B	B	H	H	H
8	*Xpsm* 708.1	2	A	B	B	H	B	B	H	C	B	B	H	H	A	A	H	H	H	A	A	A	-	H	H	A	B
9	*Xpsmp* 2237	2	H	H	H	H	B	H	B	A	A	H	H	H	A	H	H	B	B	H	A	A	H	H	H	H	B
10	*Xpsmp* 2072	2	H	H	H	H	B	H	-	A	A	H	H	H	A	H	H	B	B	H	A	A	H	H	H	A	B
11	*Xpsmp* 2077	2	H	H	H	H	B	H	B	A	H	H	H	A	A	H	H	H	H	H	A	A	H	B	-	H	B
12	*Xpsmp* 2201	2	H	B	H	H	B	B	H	H	B	H	H	D	A	B	H	-	B	-	A	A	H	B	H	H	B
13	*Xpsmp* 2232.2	2	H	B	H	A	B	C	H	H	-	B	H	A	A	B	H	H	B	H	A	A	H	B	H	-	B
14	*Xpsmp* 2225	2	H	B	H	A	B	B	A	H	H	B	H	A	H	B	-	H	H	-	A	H	H	B	D	H	C
15	*Xpsmp* 2089	2	A	B	A	A	H	H	A	B	B	B	A	H	H	B	-	H	H	H	C	B	B	B	H	H	B
16	*Xpsm* 37	3	-	H	A	B	H	H	A	H	A	H	B	-	A	H	H	H	H	B	A	C	H	A	B	B	H
17	*Xpsm* 18	3	D	A	A	H	D	H	H	D	D	B	B	H	A	A	D	H	B	B	A	H	-	A	A	C	H
18	*Xpsmp* 2070	3	H	A	A	H	H	H	H	H	A	B	B	H	H	A	H	H	B	B	A	B	B	A	A	A	H
19	*Xpsmp* 2267	3	H	A	A	H	H	H	H	H	A	B	H	H	H	A	H	H	-	B	A	B	B	A	A	A	H
20	*Xpsm* 409.1	4	D	-	-	A	B	A	-	A	H	A	H	H	-	B	H	H	H	B	-	-	-	-	A	H	H
21	*Xpsm* 648	4	H	A	A	A	H	H	H	H	H	H	H	A	B	B	H	H	B	B	H	H	-	D	B	H	B
22	*Xpsm* 344	4	H	A	A	A	H	H	B	H	H	H	H	A	B	B	H	H	B	B	B	H	H	A	H	H	B
23	*Xpsm* 84	4	H	A	A	A	H	H	B	H	H	H	H	A	B	B	H	H	B	B	B	H	H	A	H	H	B
24	*Xpsmp* 2084	4	H	A	A	A	H	H	B	H	H	H	H	A	B	B	H	H	B	B	B	H	H	A	H	H	B
25	*Xpsm* 837.2	4	-	-	D	D	H	B	B	H	H	H	B	-	B	-	H	H	B	B	-	-	-	A	H	-	B
26	*Xpsm* 416.3	4	H	A	A	A	H	H	B	H	H	H	H	A	B	-	H	H	B	B	H	H	H	A	H	H	B

...contd. Appendix 1. Genotypic data points scored from 172 F_2 mapping population progenies using 46 marker loci (including SSRs and RFLPs) polymorphic betwwen pearl millet parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer/probe	LG	[a] 119	120	121	122	123	124	126	127	128	129	130	132	133	134	135	137	138	139	140	141	142	143	144	145	147
			[b] 101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125
27	*Xpsmp* 2202	5	B	B	H	H	A	H	H	H	B	H	H	H	-	H	B	H	H	A	H	H	H	A	B	H	H
28	*Xpsmp* 2274	5	B	B	-	-	A	H	B	-	B	H	H	H	H	H	B	H	H	D	H	H	H	A	B	A	H
29	*Xpsmp* 2220	5	B	B	H	H	H	H	B	H	B	H	H	H	A	-	B	H	H	D	H	A	H	A	A	A	H
30	*Xpsmp* 2078	5	B	B	H	H	H	H	C	-	B	H	H	H	-	H	B	-	H	H	-	A	H	-	A	A	-
31	*Xpsmp* 2208	5	-	B	H	B	H	H	B	H	-	-	H	H	B	H	B	H	H	A	H	A	B	A	-	B	H
32	*Xpsmp* 2276	5	-	-	H	B	H	H	B	H	B	H	H	H	B	H	B	H	H	D	H	A	B	A	B	B	H
33	*Xpsmp* 2277	5	B	B	H	H	H	H	B	H	B	H	H	H	B	H	B	H	H	A	H	A	B	A	B	B	-
34	*Xpsmp* 2261	5	H	H	H	H	B	H	B	B	H	A	H	H	B	-	H	A	H	H	B	A	B	-	B	H	H
35	*Xpsm* 588	6	H	B	A	A	H	H	B	H	H	H	H	B	B	A	H	H	B	A	H	H	H	B	H	C	B
36	*Xpsm* 696	6	H	B	H	H	H	B	H	C	H	C	B	B	H	A	H	H	B	A	H	H	H	B	B	B	D
37	*Xpsmp* 2018	6	B	B	B	C	-	B	B	B	H	B	B	B	B	A	A	C	B	-	-	A	A	B	B	B	B
38	*Xpsm* 575	6	B	B	B	H	H	B	H	B	H	H	B	B	B	A	H	A	B	H	H	A	A	B	B	B	B
39	*Xpsm* 718	7	H	A	A	A	H	H	H	H	H	H	H	A	B	B	H	H	B	-	H	H	A	A	B	H	B
40	*Xpsm* 269	7	H	-	H	H	H	-	H	H	H	-	-	B	H	A	H	H	B	A	H	H	H	B	B	B	H
41	*Xpsmp* 2224	7	-	H	H	H	-	H	B	A	H	H	H	H	-	H	H	B	-	-	-	A	H	B	A	H	
42	*Xpsmp* 2074	7	H	A	H	H	H	H	-	A	H	H	A	H	H	A	-	H	B	H	B	A	A	H	B	A	H
43	*Xpsmp* 2263	7	H	A	H	H	H	H	B	A	H	H	A	H	H	A	H	H	-	D	B	-	-	H	H	A	H
44	*Xpsmp* 2203	7	H	A	H	A	D	H	B	A	H	H	H	H	H	A	H	H	B	B	B	A	A	H	H	H	B
45	*Xpsm* 160	7	-	-	-	-	H	H	-	H	A	-	-	A	H	H	A	H	B	B	-	A	-	H	H	-	B
46	*Xpsm* 190	7	H	A	A	A	H	H	C	H	H	H	H	A	B	B	H	H	B	B	H	H	H	A	H	H	B

[a]Original plant DNA sample number from mapping population: [b]Serial number given to them; LG-Linkage group

A = Homozygote for the allele a from parental strain P_1 (ICMB 89111-P6) at this locus

B = Homozygote for the allele b from parental strain P_2 (ICMB 90111-P6) at this locus

H = Heterozygote (F_1) carrying alleles from both P_1 and P_2 parental strains

C = Not a homozygote for allele A (i.e. either B or H)

D = Not a homozygote for allele B (i.e. either A or H)

- = Missing data for the individual at this locus

Xpsmp indicates SSR (microsatellite) primers and *Xpsm* indicates RFLP probes

...contd. Appendix 1. Genotypic data points scored from 172 F_2 mapping population progenies using 46 marker loci (including SSRs and RFLPs) polymorphic betwwen pearl millet parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer/probe	LG	[a] 148	149	151	152	154	155	156	157	158	159	161	162	163	164	165	166	167	168	169	170	173	174	175	176	177
			[b] 126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150
1	*Xpsm* 280	1	H	D	H	-	H	B	H	A	H	H	H	H	H	H	B	A	A	A	B	H	H	C	B	A	A
2	*Xpsm* 492	1	B	B	H	H	-	B	H	H	B	H	H	B	B	A	-	A	H	C	B	B	-	A	H	H	A
3	*Xpsmp* 2273	1	B	B	B	H	H	B	H	H	B	H	H	B	B	H	B	H	H	H	-	H	H	H	B	H	H
4	*Xpsm* 17	1	H	B	B	H	H	B	H	H	B	B	H	B	B	H	B	H	A	H	B	H	H	H	B	H	H
5	*Xpsmp* 2080	1	B	H	B	H	H	B	H	H	B	H	H	H	B	H	H	H	A	H	B	H	H	H	H	H	B
6	*Xpsmp* 2030	1	B	H	B	H	H	B	H	H	H	H	B	H	B	H	H	-	H	H	B	H	H	H	H	H	B
7	*Xpsm* 196.1	1	B	H	B	A	H	B	H	C	H	H	B	H	B	H	H	H	H	H	B	A	H	H	H	A	-
8	*Xpsm* 708.1	2	H	H	B	A	-	-	-	H	A	-	-	H	B	-	-	H	H	A	H	A	-	H	H	B	H
9	*Xpsmp* 2237	2	B	H	C	H	H	H	H	A	A	H	H	H	B	H	A	H	B	H	H	B	B	A	A	A	H
10	*Xpsmp* 2072	2	B	A	H	H	C	H	H	A	A	H	H	H	H	H	H	H	-	H	H	B	B	A	A	A	H
11	*Xpsmp* 2077	2	B	-	H	H	H	A	A	H	H	H	H	H	B	H	A	H	B	H	H	B	B	A	A	A	H
12	*Xpsmp* 2201	2	B	H	H	B	H	H	H	A	D	H	A	H	B	H	A	H	B	H	H	B	B	A	A	A	H
13	*Xpsmp* 2232.2	2	H	D	B	B	H	H	H	A	B	B	-	B	B	H	B	H	H	H	H	C	H	A	-	A	H
14	*Xpsmp* 2225	2	H	H	B	H	H	-	H	H	B	B	A	B	B	H	C	H	D	H	-	H	H	H	H	H	B
15	*Xpsmp* 2089	2	D	H	H	A	H	A	H	H	H	B	C	A	H	B	B	H	A	H	B	A	B	H	A	A	B
16	*Xpsm* 37	3	H	H	H	H	B	A	H	H	A	H	B	H	H	B	D	B	H	A	H	A	D	H	H	B	H
17	*Xpsm* 18	3	H	H	H	A	-	-	-	H	A	-	-	H	B	C	H	H	H	A	H	A	-	H	H	B	D
18	*Xpsmp* 2070	3	H	D	H	A	B	A	B	H	A	H	B	H	B	B	A	H	H	A	-	A	B	H	H	B	H
19	*Xpsmp* 2267	3	H	H	H	A	C	A	B	-	A	H	H	C	B	B	A	A	H	A	-	-	B	A	H	B	A
20	*Xpsm* 409.1	4	H	B	D	A	B	H	A	B	B	A	H	-	H	H	A	A	H	A	H	H	H	-	A	A	-
21	*Xpsm* 648	4	B	B	H	A	C	-	H	H	B	-	-	B	B	D	D	H	H	H	H	B	-	A	H	C	A
22	*Xpsm* 344	4	B	B	H	A	H	B	H	H	B	H	H	B	B	A	H	A	H	H	H	B	B	A	H	H	A
23	*Xpsm* 84	4	B	B	H	A	H	B	H	H	B	H	H	B	B	A	H	A	H	H	H	B	B	A	H	H	A
24	*Xpsmp* 2084	4	B	B	H	A	H	B	H	H	B	H	H	B	B	A	H	A	H	H	H	B	B	A	H	H	A
25	*Xpsm* 837.2	4	B	-	A	A	-	-	-	-	-	-	-	-	-	-	-	-	H	-	-	-	-	-	-	-	-
26	*Xpsm* 416.3	4	-	B	H	A	H	B	H	H	-	H	H	B	B	-	H	A	H	H	H	B	B	A	H	H	A

...contd. Appendix 1. Genotypic data points scored from 172 F_2 mapping population progenies using 46 marker loci (including SSRs and RFLPs) polymorphic betwwen pearl millet parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer/probe	LG	[a] 148	149	151	152	154	155	156	157	158	159	161	162	163	164	165	166	167	168	169	170	173	174	175	176	177
			[b] 126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150
27	*Xpsmp* 2202	5	A	-	A	H	B	H	B	H	H	B	H	H	H	H	H	H	H	B	H	A	B	A	A	H	H
28	*Xpsmp* 2274	5	H	H	A	H	B	H	B	H	H	B	H	H	H	H	H	H	B	B	H	A	B	A	A	B	H
29	*Xpsmp* 2220	5	H	H	A	H	B	H	H	H	H	B	H	D	H	H	H	D	B	B	H	A	B	A	A	B	A
30	*Xpsmp* 2078	5	H	D	-	A	B	-	H	-	H	B	H	H	H	H	H	D	B	B	H	A	B	H	A	B	A
31	*Xpsmp* 2208	5	H	H	H	A	H	H	B	H	H	B	H	H	H	B	H	H	B	B	H	A	B	H	A	B	H
32	*Xpsmp* 2276	5	H	H	H	H	H	H	B	H	H	B	H	H	H	B	H	H	B	B	H	A	-	A	A	B	H
33	*Xpsmp* 2277	5	H	H	H	C	H	H	B	H	H	B	H	H	H	B	H	H	B	B	H	A	B	A	A	B	H
34	*Xpsmp* 2261	5	B	H	B	H	B	H	H	B	-	H	H	B	H	H	B	H	H	A	B	H	A	-	H	H	-
35	*Xpsm* 588	6	B	B	H	A	H	H	B	C	C	B	H	H	C	H	H	H	D	H	H	B	H	B	H	H	B
36	*Xpsm* 696	6	D	H	H	-	H	H	B	B	B	C	H	D	B	H	D	H	A	H	B	B	H	H	H	H	B
37	*Xpsmp* 2018	6	A	H	H	A	B	H	B	B	-	B	H	A	B	H	A	B	A	B	B	H	B	B	A	H	B
38	*Xpsm* 575	6	A	H	H	A	B	H	H	B	B	B	H	H	B	H	H	B	A	B	B	H	B	H	A	H	B
39	*Xpsm* 718	7	B	B	H	A	H	A	H	H	B	-	-	B	-	H	H	H	H	H	H	B	A	A	H	C	A
40	*Xpsm* 269	7	H	H	H	-	H	A	B	H	H	H	-	B	H	-	-	H	H	H	-	B	-	H	H	H	A
41	*Xpsmp* 2224	7	H	H	H	-	D	H	C	H	A	-	B	H	H	A	H	H	B	H	H	A	-	-	H	H	H
42	*Xpsmp* 2074	7	H	H	H	A	H	H	B	H	A	H	H	H	B	A	H	H	-	H	H	A	H	H	H	H	H
43	*Xpsmp* 2263	7	H	D	H	H	D	H	B	H	A	A	A	H	B	A	H	H	B	H	H	H	H	H	H	-	H
44	*Xpsmp* 2203	7	H	H	H	H	H	H	B	H	A	A	A	H	B	H	H	H	H	H	H	H	H	H	H	H	A
45	*Xpsm* 160	7	B	A	A	A	C	-	-	H	H	A	H	H	H	B	H	A	D	A	H	H	B	B	C	B	A
46	*Xpsm* 190	7	B	B	H	H	-	B	H	H	B	-	H	B	B	A	H	A	H	H	H	B	B	A	H	H	A

[a]Original plant DNA sample number from mapping population; [b]Serial number given to them; LG-Linkage group

A = Homozygote for the allele a from parental strain P_1 (ICMB 89111-P6) at this locus

B = Homozygote for the allele b from parental strain P_2 (ICMB 90111-P6) at this locus

H = Heterozygote (F_1) carrying alleles from both P_1 and P_2 parental strains

C = Not a homozygote for allele A (i.e. either B or H)

D = Not a homozygote for allele B (i.e. either A or H)

- = Missing data for the individual at this locus

Xpsmp indicates SSR (microsatellite) primers and *Xpsm* indicates RFLP probes

...contd. Appendix 1. Genotypic data points scored from 172 F_2 mapping population progenies using 46 marker loci (including SSRs and RFLPs) polymorphic betwwen pearl millet parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer/probe	LG	[a] 178 [b] 151	179 152	181 153	182 154	184 155	185 156	187 157	188 158	189 159	191 160	192 161	194 162	195 163	196 164	197 165	198 166	199 167	201 168	203 169	204 170	205 171	206 172
1	*Xpsm* 280	1	A	D	H	H	B	B	-	A	B	B	-	H	H	A	H	H	B	B	B	A	A	H
2	*Xpsm* 492	1	H	B	A	H	-	H	B	H	-	B	H	B	H	A	A	B	H	B	H	H	B	B
3	*Xpsmp* 2273	1	A	B	B	H	B	H	H	H	H	H	B	H	B	A	A	-	H	-	H	H	H	H
4	*Xpsm* 17	1	H	B	B	A	B	H	H	H	H	H	B	H	B	A	A	B	B	B	H	H	H	H
5	*Xpsmp* 2080	1	H	B	B	H	B	H	-	H	B	H	B	H	B	A	H	B	B	B	H	H	H	H
6	*Xpsmp* 2030	1	H	-	-	H	H	B	-	H	B	H	B	A	B	A	H	B	B	H	H	H	H	-
7	*Xpsm* 196.1	1	B	A	H	A	H	H	H	H	B	H	-	A	B	A	B	H	A	H	A	A	H	H
8	*Xpsm* 708.1	2	A	B	B	H	H	A	H	A	C	A	H	H	A	H	H	H	A	A	H	H	A	H
9	*Xpsmp* 2237	2	A	H	A	A	H	A	A	H	A	D	H	H	A	B	H	B	H	H	H	H	-	B
10	*Xpsmp* 2072	2	A	A	A	A	H	A	-	H	A	A	B	D	H	B	H	B	H	H	H	H	B	B
11	*Xpsmp* 2077	2	A	A	A	A	H	A	H	H	A	H	B	H	H	B	H	B	H	A	H	H	H	B
12	*Xpsmp* 2201	2	A	A	A	A	H	A	A	H	B	H	C	H	H	B	A	B	H	A	H	A	H	B
13	*Xpsmp* 2232.2	2	A	B	B	H	B	A	A	-	B	-	B	B	H	-	-	B	H	H	H	A	B	-
14	*Xpsmp* 2225	2	H	B	B	H	B	H	A	H	B	H	B	H	C	A	A	B	B	B	H	D	H	H
15	*Xpsmp* 2089	2	-	C	B	A	H	H	H	A	-	H	H	B	H	H	H	H	H	H	H	A	B	H
16	*Xpsm* 37	3	A	B	H	H	A	A	B	H	H	A	B	A	A	H	H	B	A	A	H	H	A	H
17	*Xpsm* 18	3	A	B	H	H	H	H	H	D	B	A	H	H	A	H	H	H	A	A	H	H	A	H
18	*Xpsmp* 2070	3	A	B	H	A	H	H	H	A	B	A	H	H	A	H	H	H	A	H	H	H	A	H
19	*Xpsmp* 2267	3	A	B	-	A	H	-	-	A	-	A	H	A	H	H	H	H	A	H	A	H	A	H
20	*Xpsm* 409.1	4	H	H	A	H	A	B	H	A	H	-	B	A	B	H	A	-	H	B	H	H	A	H
21	*Xpsm* 648	4	H	B	A	H	H	H	B	H	A	B	C	B	H	H	A	B	A	B	B	H	B	C
22	*Xpsm* 344	4	H	B	A	B	H	H	B	H	A	B	H	B	H	H	A	B	A	B	H	H	B	B
23	*Xpsm* 84	4	H	B	A	B	A	H	B	H	A	B	H	B	H	H	A	B	A	B	H	H	B	B
24	*Xpsmp* 2084	4	H	B	A	B	A	B	B	H	A	B	B	B	H	B	A	B	A	B	H	H	B	B
25	*Xpsm* 837.2	4	-	-	A	H	A	B	B	A	-	B	B	B	A	B	A	B	A	B	A	A	B	H
26	*Xpsm* 416.3	4	H	B	A	H	A	-	B	H	A	B	H	-	H	H	-	B	A	B	H	H	-	C

...contd. Appendix 1. Genotypic data points scored from 172 F_2 mapping population progenies using 46 marker loci (including SSRs and RFLPs) polymorphic betwwen pearl millet parental lines ICMB 89111-P6 and ICMB 90111-P6

Sr. No.	Primer/probe	LG	[a] 178	179	181	182	184	185	187	188	189	191	192	194	195	196	197	198	199	201	203	204	205	206
			[b] 151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172
27	*Xpsmp* 2202	5	H	A	A	B	H	A	B	A	B	A	H	A	H	H	A	H	B	B	-	A	A	H
28	*Xpsmp* 2274	5	A	A	H	B	A	A	B	D	B	A	-	H	D	H	H	B	D	B	A	H	H	H
29	*Xpsmp* 2220	5	-	A	H	B	A	A	B	A	B	A	H	H	H	-	H	B	H	B	-	H	H	H
30	*Xpsmp* 2078	5	A	-	H	B	H	A	B	-	B	A	H	H	H	H	H	B	H	B	A	H	H	H
31	*Xpsmp* 2208	5	H	A	A	B	H	A	B	A	B	A	H	H	H	H	H	-	H	H	A	H	H	H
32	*Xpsmp* 2276	5	H	A	A	B	H	A	B	B	B	A	H	A	H	H	H	H	H	H	-	H	H	H
33	*Xpsmp* 2277	5	H	A	A	B	H	A	B	B	B	A	H	A	H	H	H	-	H	H	A	C	H	H
34	*Xpsmp* 2261	5	H	A	-	H	-	-	B	H	B	H	B	H	H	H	H	H	H	H	A	H	H	H
35	*Xpsm* 588	6	H	B	A	H	H	H	B	H	H	H	H	H	H	D	B	B	B	C	H	H	B	B
36	*Xpsm* 696	6	D	-	A	H	H	H	H	H	D	H	D	H	H	H	H	D	D	H	H	H	C	C
37	*Xpsmp* 2018	6	H	B	H	H	H	C	B	B	H	H	A	H	-	B	H	H	H	B	H	B	B	B
38	*Xpsm* 575	6	H	H	D	H	H	B	B	H	A	H	D	H	H	C	H	H	A	B	H	C	B	B
39	*Xpsm* 718	7	H	B	A	H	H	H	B	H	A	B	B	B	H	H	A	B	A	B	B	B	B	B
40	*Xpsm* 269	7	B	H	A	H	H	-	B	A	B	H	B	A	B	A	-	H	A	H	C	B	B	H
41	*Xpsmp* 2224	7	H	H	A	H	H	H	H	-	H	B	H	H	B	H	A	H	H	A	B	H	H	H
42	*Xpsmp* 2074	7	D	H	D	H	H	H	H	H	H	B	H	-	H	H	A	C	C	A	H	H	-	H
43	*Xpsmp* 2263	7	H	H	H	H	H	D	D	C	D	H	H	H	D	C	H	B	H	A	H	H	H	B
44	*Xpsmp* 2203	7	H	H	A	H	H	H	B	H	H	H	H	H	H	H	H	H	H	A	H	H	H	-
45	*Xpsm* 160	7	B	C	A	H	A	A	B	-	H	H	H	H	B	B	A	H	C	A	H	B	B	B
46	*Xpsm* 190	7	B	B	A	H	A	H	B	H	A	B	H	B	H	H	A	B	A	-	H	H	B	B

[a]Original plant DNA sample number from mapping population; [b]Serial number given to them; LG-Linkage group

A = Homozygote for the allele a from parental strain P_1 (ICMB 89111-P6) at this locus

B = Homozygote for the allele b from parental strain P_2 (ICMB 90111-P6) at this locus

H = Heterozygote (F_1) carrying alleles from both P_1 and P_2 parental strains

C = Not a homozygote for allele A (i.e. either B or H)

D = Not a homozygote for allele B (i.e. either A or H)

- = Missing data for the individual at this locus

Xpsmp indicates SSR (microsatellite) primers and *Xpsm* indicates RFLP probes

Appendix 2. The mean downy mildew screening data points from 172 $F_{2:4}$ self-bulks based on cross ICMB 89111-P6 × ICMB 90111-P6, against six Indian and two African populations of *Sclerospora graminicola* under green house conditions at ICRISAT, Patancheru, India and University of Wales, Bangor, UK, respectively.

Sr. No.	Ent. No.	CAZRI, Jodhpur, India (Sg 139)			ICRISAT, Patancheru, India (Sg 153)			IARI, New Delhi, India (Sg 298)			GAU MRS, Jamnagar, India (Sg 200)			RAU ARS, Durgapura, India (Sg 151)			MAHYCO, Jalna, India (Sg 150)			Maiduguri, Nigeria UWB-screen 43			Bamako, Mali UWB-screen 45		
		TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI
1	2	28.3	4.7	18.3	27.7	2.7	8.1	37.3	4.7	14.2	33.7	9.3	28.9	30.7	8.3	26.8	28.7	4.7	16.7	33.7	0.3	1.0	35.0	0.7	1.9
2	3	24.7	3.7	17.1	22.0	0.7	3.0	24.0	1.3	5.4	28.7	2.7	9.9	25.3	2.7	13.2	25.7	1.3	4.8	22.3	1.0	4.3	22.7	0.3	1.4
3	4	36.0	13.0	38.2	32.3	5.7	18.4	31.0	8.0	24.8	38.3	15.0	39.7	35.3	9.0	26.1	36.3	3.0	8.2	37.3	4.3	11.7	40.3	21.0	52.1
4	5	20.3	4.7	22.7	19.7	1.7	8.3	21.3	3.3	15.6	18.0	5.3	31.1	18.3	5.0	27.1	15.0	1.0	6.3	23.0	2.7	12.1	24.7	4.0	16.1
5	6	26.0	3.3	12.2	20.7	0.7	3.7	26.3	2.7	9.2	24.3	3.7	14.3	30.0	6.7	23.5	24.3	4.3	17.5	29.7	0.3	1.3	30.7	0.0	0.0
6	7	30.3	26.3	86.2	27.7	25.7	92.7	28.3	15.3	54.5	32.7	29.7	91.5	35.0	29.7	84.7	31.0	9.7	33.6	36.0	27.7	77.2	31.7	19.7	62.6
7	8	35.0	18.0	51.6	35.0	14.0	42.7	35.7	10.3	31.5	36.3	23.0	64.3	33.0	17.0	52.4	33.3	7.7	23.0	36.0	13.3	37.0	34.7	14.3	40.1
8	10	20.0	16.0	80.7	17.0	9.0	50.6	14.3	7.7	60.5	20.7	16.7	79.1	14.7	10.7	72.7	20.0	4.0	20.4	16.7	4.7	27.5	17.3	13.7	81.4
9	11	21.7	2.7	12.7	18.0	1.0	5.3	16.3	1.3	8.3	18.3	0.3	2.0	17.3	2.0	11.9	17.7	1.7	9.2	*	*	*	*	*	*
10	12	10.0	1.7	27.8	7.7	3.7	54.8	11.3	1.3	11.8	8.7	5.0	65.5	10.0	4.7	53.6	10.3	1.7	17.3	*	*	*	*	*	*
11	13	36.7	21.0	56.9	37.3	9.7	26.3	40.0	12.0	30.7	38.3	12.7	33.6	39.0	13.0	32.8	30.3	8.0	28.0	40.0	11.7	29.0	39.7	21.7	54.5
12	15	43.0	11.7	27.9	34.3	6.7	19.6	34.3	8.0	23.9	41.7	10.3	24.4	37.7	10.3	27.6	32.7	2.7	8.8	38.7	5.0	12.9	37.3	17.0	45.9
13	16	31.7	6.3	20.2	37.0	1.3	4.4	32.3	2.3	6.9	34.7	6.0	17.3	32.3	7.3	23.0	37.3	2.7	7.4	36.3	1.7	4.6	36.3	10.0	27.8
14	17	29.7	3.0	10.9	28.7	2.3	8.2	25.3	2.3	8.5	31.7	7.7	24.2	29.7	6.7	22.9	30.0	6.7	22.8	27.0	4.3	16.5	31.7	13.0	41.0
15	18	18.0	7.3	40.5	25.3	8.3	32.9	20.7	8.7	39.2	26.3	15.3	58.1	29.7	11.7	40.6	26.0	4.0	15.6	24.7	4.0	16.0	23.7	11.0	46.1
16	19	26.3	4.0	18.4	21.3	1.0	5.6	28.3	2.7	10.7	27.7	2.3	7.5	25.0	4.0	15.9	24.3	2.0	8.2	26.7	0.0	0.0	26.3	14.3	53.2
17	20	39.3	14.3	36.6	32.3	19.0	55.5	32.7	6.0	17.8	35.3	13.3	37.4	34.7	12.3	35.8	35.7	4.7	13.4	38.0	7.0	18.7	37.3	19.3	51.7
18	21	34.7	4.7	13.7	34.7	3.7	10.7	31.0	1.7	5.0	40.3	6.0	15.0	42.3	8.0	18.9	38.0	3.0	7.4	38.0	2.7	7.1	42.0	9.3	22.2
19	22	34.7	7.3	21.8	36.0	6.0	16.0	28.0	8.0	26.0	31.7	7.0	22.3	41.0	7.7	19.1	34.3	0.3	1.0	34.0	2.0	6.2	37.3	18.3	49.4
20	24	17.7	10.0	53.7	21.0	10.3	50.0	16.0	7.7	48.5	27.7	12.7	43.7	19.3	13.3	67.6	15.3	2.3	13.1	21.0	2.0	10.5	22.3	9.0	36.4
21	25	15.0	1.0	5.6	18.3	1.7	9.1	18.0	0.7	2.9	18.3	1.7	9.2	13.3	3.0	22.4	15.7	1.0	6.3	21.0	0.0	0.0	21.3	0.3	1.5
22	26	29.3	1.3	4.9	29.3	1.7	5.9	32.0	1.3	3.8	29.0	1.7	5.8	32.3	0.0	0.0	30.7	1.7	5.4	31.7	0.0	0.0	35.0	0.3	1.0
23	27	30.0	12.3	39.8	37.7	0.0	0.0	30.7	4.0	13.7	33.0	7.0	23.4	34.7	4.0	11.1	33.7	4.0	11.7	37.7	0.0	0.0	40.0	25.0	62.3
24	28	34.3	2.7	6.8	34.7	1.7	6.0	29.0	2.3	7.9	36.0	3.3	9.2	34.7	0.3	1.1	40.0	0.0	0.0	33.3	0.3	1.0	32.0	0.7	2.3
25	30	19.7	9.0	45.9	23.3	1.0	4.0	20.7	6.3	31.1	22.3	3.3	15.9	20.3	8.0	40.6	22.7	2.3	10.4	*	*	*	*	*	*

...contd. Appendix 2. The mean downy mildew screening data from 172 $F_{2:4}$ self-bulks based on cross ICMB 89111-P6 × ICMB 90111-P6, against six Indian and two African populations of *Sclerospora graminicola* under green house conditions at ICRISAT, Patancheru, India and University of Wales, Bangor, UK, respectively.

Sr. No.	Ent. No.	CAZRI, Jodhpur, India (Sg 139)			ICRISAT, Patancheru, India (Sg 153)			IARI, New Delhi, India (Sg 298)			GAU MRS, Jamnagar, India (Sg 200)			RAU ARS, Durgapura, India (Sg 151)			MAHYCO, Jalna, India (Sg 150)			Maiduguri, Nigeria UWB-screen 43			Bamako, Mali UWB-screen 45		
		TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI
26	31	24.7	2.7	11.3	27.3	4.0	15.9	28.3	3.7	13.1	25.3	6.7	26.0	24.7	3.7	14.7	23.3	3.7	16.3	25.0	4.7	18.9	26.7	8.0	30.2
27	32	31.3	1.0	3.9	26.7	0.0	0.0	28.0	2.0	7.4	28.0	1.7	5.9	31.3	0.7	2.2	28.3	1.3	4.5	30.0	0.0	0.0	30.0	0.0	0.0
28	33	21.7	6.7	28.1	24.7	7.0	30.4	26.0	4.3	16.7	28.3	7.7	28.1	25.3	7.7	30.8	25.7	1.0	3.9	30.3	1.0	4.4	28.0	13.3	47.9
29	34	16.3	5.0	32.6	22.3	5.3	21.9	27.0	6.3	25.5	26.3	14.0	52.7	21.7	6.3	31.8	22.3	3.0	13.7	18.7	3.3	16.9	22.7	13.3	56.6
30	35	24.3	8.0	36.4	18.7	6.0	30.8	15.7	4.3	28.0	26.0	3.7	15.3	19.3	8.0	42.4	17.0	1.7	10.7	19.0	7.0	34.1	17.0	9.7	57.6
31	37	21.7	15.3	67.7	26.0	9.0	35.3	29.7	6.0	19.6	30.0	4.3	13.0	34.7	12.0	31.7	26.0	4.0	16.3	31.0	5.7	18.2	33.3	29.7	88.2
32	38	39.7	14.3	37.1	36.0	9.7	27.3	36.3	13.7	41.4	36.0	15.7	44.1	39.3	11.7	30.2	36.7	1.3	4.0	38.7	7.3	19.0	34.3	22.0	63.9
33	39	35.7	19.0	51.3	30.3	1.3	5.1	33.3	6.3	19.7	36.0	3.0	8.3	29.7	13.0	46.4	37.3	2.3	5.9	35.0	0.0	0.0	38.0	27.0	71.1
34	41	18.7	7.7	41.4	23.3	1.3	8.5	23.7	2.7	11.3	29.7	3.7	12.8	25.7	2.0	6.1	21.0	1.7	8.0	25.3	0.0	0.0	29.3	20.3	68.1
35	42	35.0	15.7	46.7	30.0	14.3	47.4	31.0	9.0	26.0	33.7	17.0	51.2	31.3	14.3	45.9	30.7	5.3	17.3	34.7	12.0	34.8	31.7	13.7	43.2
36	44	25.7	0.7	3.1	30.0	0.0	0.0	32.0	0.7	2.7	37.0	0.0	0.0	32.0	1.7	6.3	33.3	3.0	8.7	33.7	0.0	0.0	37.3	0.0	0.0
37	45	12.7	5.3	45.4	16.0	4.7	28.4	16.3	4.0	24.3	17.0	8.0	46.3	15.7	6.7	46.5	18.0	3.7	20.6	21.0	2.0	9.6	21.7	9.0	42.0
38	46	4.0	3.0	72.2	4.0	3.7	91.7	3.7	2.3	69.4	3.7	3.7	100.0	5.3	5.0	91.7	2.3	1.3	55.6	*	*	*	*	*	*
39	47	15.3	7.3	46.5	15.0	2.0	15.2	12.0	1.3	14.4	10.7	2.0	21.8	9.0	3.3	48.6	11.3	0.3	3.7	10.7	0.0	0.0	14.0	6.0	42.2
40	48	38.7	26.3	70.5	30.7	17.0	56.5	35.3	17.3	49.4	35.0	20.0	57.5	38.0	16.3	43.6	42.3	3.0	7.0	33.7	7.0	20.9	36.3	29.7	81.4
41	49	37.7	12.3	35.6	36.0	14.0	38.6	42.3	7.3	18.9	38.0	10.3	27.0	36.3	6.3	17.8	36.3	1.7	4.3	38.3	6.0	15.8	37.3	6.3	16.6
42	50	25.7	3.0	11.6	25.3	7.0	25.8	26.3	4.3	16.3	28.0	3.0	10.7	28.0	3.0	9.6	31.7	4.0	12.6	33.3	0.7	1.9	29.3	6.0	20.8
43	51	31.0	3.3	10.7	30.0	0.7	2.2	34.7	1.7	4.4	34.7	3.0	8.9	34.0	0.3	1.0	34.0	2.0	6.3	21.3	1.3	5.4	23.7	2.7	10.6
44	52	28.3	11.7	41.6	34.7	10.3	30.4	33.0	8.0	22.1	37.7	13.0	34.7	35.7	10.3	29.0	36.3	8.3	23.0	36.3	5.3	14.7	27.3	12.7	42.8
45	53	28.3	17.3	60.1	29.0	10.0	34.7	31.3	10.7	35.9	35.3	15.3	44.4	27.7	13.3	43.9	26.7	3.3	12.5	31.3	3.7	11.7	31.0	14.0	43.8
46	55	20.0	3.7	17.9	19.3	0.3	1.5	25.7	1.0	3.4	26.0	3.0	11.8	19.0	2.7	14.2	15.3	2.0	12.7	18.7	0.0	0.0	23.3	5.0	23.1
47	56	33.0	17.0	61.0	31.7	5.0	17.8	29.3	4.0	13.4	35.0	5.3	14.8	33.7	6.3	19.1	30.3	1.3	4.2	36.3	3.7	10.0	34.3	8.0	23.6
48	57	14.0	8.0	60.2	11.0	8.7	80.6	15.0	6.7	44.9	14.7	7.0	50.3	10.3	7.3	72.0	11.3	4.7	41.7	13.0	4.3	30.4	13.5	4.5	35.8
49	58	28.0	7.7	26.4	28.0	7.3	27.8	34.7	3.7	11.7	33.0	8.7	25.9	28.0	9.3	33.7	26.7	6.3	23.7	27.7	3.0	10.2	29.3	7.0	22.6
50	59	32.3	2.0	6.7	30.7	0.3	1.0	30.3	1.0	2.7	34.3	7.7	22.5	32.7	1.3	4.2	31.0	2.7	8.6	37.7	0.0	0.0	37.0	0.5	1.3

...contd. Appendix 2. The mean downy mildew screening data from 172 $F_{2:4}$ self-bulks based on cross ICMB 89111-P6 × ICMB 90111-P6, against six Indian and two African populations of *Sclerospora graminicola* under green house conditions at ICRISAT, Patancheru, India and University of Wales, Bangor, UK, respectively.

Sr. No.	Ent. No.	CAZRI, Jodhpur, India (Sg 139)			ICRISAT, Patancheru, India (Sg 153)			IARI, New Delhi, India (Sg 298)			GAU MRS, Jamnagar, India (Sg 200)			RAU ARS, Durgapura, India (Sg 151)			MAHYCO, Jalna, India (Sg 150)			Maiduguri, Nigeria UWB-screen 43			Bamako, Mali UWB-screen 45		
		TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI
51	60	27.3	7.0	30.0	36.3	0.0	0.0	41.3	0.3	0.8	38.3	0.3	0.9	34.3	4.7	14.1	37.3	1.3	3.5	37.7	0.0	0.0	39.3	8.3	21.1
52	61	32.0	11.7	37.4	33.7	5.0	15.3	32.0	4.7	14.9	35.3	7.0	19.8	32.7	6.3	19.9	35.0	2.0	6.1	33.3	1.7	5.3	34.5	9.0	26.2
53	62	14.3	7.0	48.1	17.7	4.7	26.9	19.7	3.3	16.0	16.0	4.3	27.8	17.0	5.0	28.2	16.3	1.7	10.0	17.7	1.3	7.1	18.0	5.3	29.5
54	63	32.3	14.3	44.7	40.3	8.7	21.7	36.3	7.7	26.9	36.0	9.0	25.5	38.3	13.0	32.1	40.3	5.3	13.3	38.0	10.3	27.2	39.0	15.7	40.2
55	64	33.3	5.7	17.0	37.0	2.3	6.3	36.3	1.7	4.5	40.3	5.7	14.0	39.0	5.7	14.9	35.3	6.3	18.2	38.0	0.3	0.9	34.7	0.0	0.0
56	65	25.7	7.3	31.8	29.7	4.7	15.7	31.7	3.0	9.3	33.0	3.3	10.3	33.0	5.3	16.4	33.7	1.0	3.3	33.7	1.7	4.7	35.3	7.7	21.8
57	66	31.3	13.3	41.6	28.7	9.3	33.8	29.0	5.0	17.8	32.7	10.7	32.7	31.0	7.0	23.7	28.7	1.0	3.7	29.7	5.3	18.8	32.0	9.7	30.1
58	67	21.3	13.7	63.8	22.7	19.7	87.4	23.3	11.7	51.7	25.3	19.3	75.9	21.7	14.7	67.4	26.0	3.7	16.1	22.7	14.0	62.2	23.3	14.0	60.9
59	69	24.0	5.0	23.9	21.0	1.0	4.3	26.0	2.7	10.4	26.3	2.0	7.4	24.0	5.7	25.7	26.7	4.0	15.9	27.0	2.3	8.7	29.0	6.3	22.3
60	70	28.3	9.0	30.7	28.0	3.3	11.6	28.0	3.3	12.0	29.0	2.7	9.4	27.0	4.3	16.2	27.3	3.0	11.2	35.3	2.0	5.4	31.0	5.0	16.3
61	72	34.0	7.3	22.9	31.0	5.0	15.1	32.7	2.3	7.2	34.7	4.3	13.4	34.0	9.3	28.5	37.7	5.0	12.9	36.7	0.7	1.8	31.3	7.7	25.0
62	73	13.3	8.0	53.0	15.0	1.0	6.7	19.3	3.3	17.6	24.3	5.3	22.1	16.7	6.0	32.6	22.0	5.0	28.2	19.7	0.0	0.0	19.0	12.3	64.7
63	74	17.3	9.7	55.9	18.7	8.7	46.5	17.7	6.7	36.9	17.7	6.3	34.9	15.3	9.3	61.2	14.0	6.3	46.8	19.0	2.0	11.7	20.5	7.5	36.5
64	75	35.3	9.7	28.1	30.3	0.7	2.0	35.0	1.0	3.1	37.7	1.7	5.0	39.0	7.3	19.5	38.0	1.3	3.5	40.3	0.0	0.0	39.3	8.3	21.3
65	76	33.0	3.7	12.1	26.7	0.7	2.9	32.3	1.0	3.7	32.7	2.0	6.2	30.0	4.7	15.9	32.7	3.0	9.0	29.7	0.0	0.0	30.7	5.0	16.1
66	78	40.7	9.3	22.9	38.0	7.0	19.8	38.0	6.7	17.6	43.0	6.0	13.9	31.7	7.0	22.0	37.3	3.0	8.4	37.3	9.7	25.9	35.3	13.3	37.6
67	79	34.7	11.7	33.8	21.7	4.3	20.5	22.7	6.3	25.9	24.3	10.0	41.0	30.0	12.0	39.7	23.7	2.3	11.3	20.3	3.0	14.9	20.0	6.0	28.3
68	80	25.7	7.3	39.8	25.3	2.0	11.1	33.0	4.3	13.7	29.3	1.0	3.5	29.0	6.0	21.1	29.7	6.0	21.1	30.7	1.0	3.2	25.3	8.0	30.1
69	82	17.7	7.0	41.7	21.7	4.7	23.7	23.7	2.3	9.8	23.0	8.3	36.0	23.7	7.7	32.5	23.3	4.3	18.1	22.3	0.7	2.9	25.0	7.0	26.5
70	83	25.7	7.7	41.0	30.3	1.7	5.8	32.0	2.0	6.9	36.3	4.3	12.2	30.3	6.3	22.1	31.0	2.7	9.4	35.3	0.3	0.9	33.7	7.3	21.9
71	85	35.7	8.0	22.6	30.3	4.7	15.2	36.0	3.7	9.7	37.0	10.0	27.0	34.7	3.3	9.5	35.0	5.7	15.6	36.7	1.3	3.5	39.0	3.0	7.8
72	86	21.0	7.7	36.1	18.3	0.0	0.0	23.0	2.7	11.9	23.7	2.7	12.1	27.7	11.0	39.8	28.3	3.7	12.5	21.0	0.3	2.0	24.0	17.0	71.6
73	87	22.0	4.0	18.7	22.7	4.0	16.9	23.7	2.0	8.9	21.7	2.0	9.6	19.3	3.0	16.5	26.0	1.3	5.1	21.3	1.7	8.1	32.0	7.0	21.7
74	88	29.7	10.0	33.5	34.3	10.3	30.5	38.0	13.7	36.0	33.7	13.0	38.9	34.0	8.3	24.6	33.7	1.7	4.7	32.3	2.7	8.3	37.0	13.3	35.9
75	89	31.7	7.7	24.4	31.7	6.3	18.1	31.7	2.7	8.6	31.0	1.7	5.5	34.3	5.0	15.0	33.3	1.3	4.2	33.0	0.0	0.0	24.0	5.7	20.7

...contd. Appendix 2. The mean downy mildew screening data from 172 $F_{2:4}$ self-bulks based on cross ICMB 89111-P6 × ICMB 90111-P6, against six Indian and two African populations of *Sclerospora graminicola* under green house conditions at ICRISAT, Patancheru, India and University of Wales, Bangor, UK, respectively.

Sr. No.	Ent. No.	CAZRI, Jodhpur, India (Sg 139)			ICRISAT, Patancheru, India (Sg 153)			IARI, New Delhi, India (Sg 298)			GAU MRS, Jamnagar, India (Sg 200)			RAU ARS, Durgapura, India (Sg 151)			MAHYCO, Jalna, India (Sg 150)			Maiduguri, Nigeria UWB-screen 43			Bamako, Mali UWB-screen 45		
		TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI
76	90	28.3	19.0	67.5	27.0	10.0	36.5	29.0	11.0	37.8	34.3	11.3	33.1	32.0	16.3	52.0	37.0	10.0	27.9	35.7	3.3	9.5	30.7	14.0	43.7
77	91	27.3	27.0	99.0	26.7	25.7	96.3	29.3	27.7	94.7	32.7	32.7	100.0	28.0	27.7	98.7	26.3	11.7	47.7	28.0	18.7	64.7	27.7	23.7	85.0
78	92	40.3	9.0	25.6	36.7	5.3	15.4	43.3	4.7	10.6	37.3	5.3	14.3	35.7	2.0	5.7	40.0	4.0	10.4	36.0	1.7	4.6	38.3	2.3	6.2
79	93	24.3	8.7	35.0	25.0	3.0	12.1	27.7	4.0	14.4	36.7	5.3	14.2	24.7	6.7	28.3	28.3	0.7	2.6	31.7	1.7	5.2	34.0	10.7	32.1
80	94	23.7	8.7	41.6	23.0	1.0	3.9	30.3	3.3	11.0	29.7	1.7	5.8	25.0	4.3	17.6	23.3	0.7	2.6	26.0	0.3	1.1	27.7	17.0	60.6
81	95	33.0	5.7	16.8	32.0	3.0	9.1	32.3	3.7	11.3	29.7	2.7	9.0	32.0	7.0	23.3	33.3	6.0	17.7	30.0	0.7	2.2	33.0	9.0	27.5
82	96	41.7	18.7	45.3	34.0	11.0	33.0	37.3	10.0	27.1	39.7	16.7	42.0	41.3	9.7	23.3	38.7	2.0	4.4	38.7	4.0	10.3	40.3	15.0	37.0
83	98	16.7	7.7	47.9	19.7	14.0	70.8	21.3	5.7	24.3	22.7	13.0	55.8	20.3	9.0	46.3	24.7	2.0	7.9	24.0	12.7	53.2	24.0	9.3	38.5
84	99	38.7	14.0	36.2	38.3	3.7	9.6	37.0	5.7	15.3	36.0	6.3	17.5	44.3	13.0	29.3	40.0	2.3	5.9	40.3	3.7	9.2	40.0	11.3	28.6
85	100	43.3	43.3	100.0	46.3	42.7	91.9	41.7	28.7	69.9	35.0	32.3	91.7	35.7	35.0	98.2	42.0	12.7	29.3	36.7	31.7	86.6	36.3	23.0	63.1
86	101	40.7	4.3	10.7	44.7	7.3	16.7	42.3	4.3	10.2	39.0	7.3	18.9	43.7	10.0	22.4	49.0	4.0	8.1	38.3	4.7	11.8	40.7	14.0	34.4
87	102	23.7	20.7	86.9	33.0	28.7	86.7	31.0	20.0	62.1	30.7	27.7	89.8	26.3	25.3	96.8	27.7	11.7	43.5	26.0	12.7	48.3	27.0	23.5	86.8
88	103	29.3	21.3	71.7	23.3	7.3	31.6	24.3	7.0	25.9	25.0	8.3	33.3	24.0	9.7	42.2	21.3	2.7	12.6	20.7	3.0	14.4	25.3	10.0	40.9
89	104	26.7	13.7	50.7	26.0	5.3	20.5	27.7	5.3	17.4	33.3	8.0	23.9	24.0	7.7	31.9	31.3	2.7	8.2	29.3	2.3	7.6	26.3	9.7	36.9
90	105	20.7	15.7	78.7	19.7	11.7	64.9	22.0	6.3	29.1	13.7	9.7	74.9	17.7	12.7	71.4	20.3	4.0	20.3	15.3	6.0	38.2	16.7	10.7	64.5
91	107	32.3	25.7	80.9	35.7	15.7	46.4	33.0	12.7	38.4	30.3	22.7	73.5	33.7	22.0	65.4	31.7	12.0	38.3	31.3	20.7	65.1	34.5	18.5	52.1
92	108	36.0	6.3	19.6	38.7	2.3	6.5	43.7	1.7	3.9	45.0	8.0	17.6	48.7	8.7	18.1	47.0	6.7	14.0	38.0	0.0	0.0	39.7	0.0	0.0
93	109	47.0	5.0	11.4	40.0	4.7	12.1	37.7	4.7	12.1	37.0	5.0	14.2	34.7	8.0	23.4	38.3	5.7	14.9	37.0	5.7	15.2	38.7	16.7	43.1
94	110	39.0	10.0	24.9	32.7	3.3	10.3	36.7	3.7	10.7	33.7	3.7	11.0	37.7	4.0	10.9	36.3	3.0	8.2	38.7	1.7	4.3	36.3	5.0	14.3
95	111	29.0	7.7	26.2	30.7	0.0	0.0	35.0	3.7	10.6	34.0	3.0	8.9	31.3	9.3	31.3	33.0	1.7	5.2	36.3	0.0	0.0	36.3	1.7	4.2
96	113	29.3	24.0	83.7	23.0	12.0	55.2	24.7	8.0	30.7	32.0	12.3	38.7	30.3	12.3	39.7	28.7	3.0	10.3	30.3	6.7	21.8	30.3	23.3	76.6
97	114	32.0	16.3	50.3	30.7	10.7	34.8	34.0	8.7	25.4	34.7	8.3	24.1	29.3	12.3	45.2	33.0	5.0	14.9	34.0	7.7	22.8	30.7	15.7	51.3
98	115	31.3	17.7	54.8	26.0	7.0	27.3	30.7	5.0	15.4	30.7	5.7	18.8	26.7	10.0	38.1	25.0	5.3	21.6	31.0	5.7	21.9	32.7	15.0	47.6
99	117	28.3	7.0	33.2	26.3	1.7	6.7	31.7	2.3	7.4	31.0	4.7	15.2	24.3	5.7	24.8	25.0	3.3	13.6	27.7	0.3	1.1	26.0	7.0	26.9
100	118	26.0	11.7	45.9	23.0	3.3	14.7	26.0	5.0	19.4	26.7	6.3	23.6	27.3	7.7	30.2	26.7	4.7	18.4	29.0	4.7	16.0	27.3	12.0	44.3

...contd. Appendix 2. The mean downy mildew screening data from 172 $F_{2:4}$ self-bulks based on cross ICMB 89111-P6 × ICMB 90111-P6, against six Indian and two African populations of *Sclerospora graminicola* under green house conditions at ICRISAT, Patancheru, India and University of Wales, Bangor, UK, respectively.

Sr. No.	Ent. No.	CAZRI, Jodhpur, India (Sg 139)			ICRISAT, Patancheru, India (Sg 153)			IARI, New Delhi, India (Sg 298)			GAU MRS, Jamnagar, India (Sg 200)			RAU ARS, Durgapura, India (Sg 151)			MAHYCO, Jalna, India (Sg 150)			Maiduguri, Nigeria UWB-screen 43			Bamako, Mali UWB-screen 45		
		TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI
101	119	8.0	8.0	100.0	4.7	1.0	19.4	12.3	5.3	41.4	13.7	8.7	62.2	6.0	3.0	63.5	8.0	3.3	35.7	*	*	*	*	*	*
102	120	14.7	2.0	13.4	13.3	2.0	15.6	16.3	3.0	20.0	14.3	1.3	9.1	15.7	3.0	20.8	16.0	0.3	1.4	15.7	2.3	15.5	17.7	1.0	6.3
103	121	28.0	7.7	28.2	29.0	0.7	2.0	35.3	3.3	9.0	35.3	3.3	9.9	34.0	3.3	9.6	36.7	3.7	10.0	36.3	0.0	0.0	35.7	15.0	42.1
104	122	32.7	24.7	76.2	34.3	11.0	31.9	35.0	11.7	32.9	37.7	12.3	33.1	35.0	14.3	40.0	32.0	2.7	8.7	35.0	3.7	10.6	31.3	26.7	84.2
105	123	32.0	6.3	20.1	33.7	0.7	2.2	33.7	5.3	16.4	36.7	3.7	10.4	35.3	4.0	11.1	31.0	3.3	10.3	37.7	0.0	0.0	37.0	1.0	2.5
106	124	32.7	11.0	35.7	35.7	0.0	0.0	35.3	2.0	5.4	31.7	2.3	8.3	32.7	6.0	18.7	32.7	2.0	6.7	38.3	0.7	1.7	36.3	22.7	62.8
107	126	22.7	2.3	10.4	20.7	1.3	15.9	23.3	5.0	23.8	35.7	4.7	12.9	31.7	8.0	24.5	32.0	6.0	18.9	37.0	7.0	18.8	35.3	6.0	16.6
108	127	22.3	10.3	46.4	24.7	6.0	23.9	28.3	5.7	20.5	27.3	6.3	23.2	24.3	8.3	33.4	26.3	2.7	10.1	24.3	1.3	5.9	27.7	11.7	42.6
109	128	33.7	16.3	46.5	33.7	3.7	11.5	34.3	14.3	45.4	30.7	7.3	26.8	33.0	5.7	17.6	30.3	2.7	8.3	33.3	4.7	13.6	34.0	16.7	49.2
110	129	29.0	10.7	39.1	32.3	1.7	6.0	33.7	2.3	6.7	39.3	2.3	5.9	33.0	7.3	20.3	34.0	2.3	6.9	34.0	0.3	1.0	34.3	22.7	64.8
111	130	15.0	6.7	45.5	15.3	4.7	31.7	18.3	3.7	19.4	15.3	4.3	28.5	14.7	6.0	46.3	13.7	0.3	3.0	23.7	1.3	5.9	15.7	3.3	22.1
112	132	19.3	6.0	32.5	18.0	1.0	4.7	27.7	4.0	14.8	25.0	9.7	38.2	25.3	6.3	24.5	27.0	2.3	9.2	*	*	*	*	*	*
113	133	34.3	3.7	10.7	38.7	5.0	13.1	37.0	1.3	3.7	40.0	3.3	8.5	30.3	3.0	10.0	29.7	2.3	8.1	34.0	1.7	4.9	35.3	1.0	2.7
114	134	31.0	2.7	8.6	33.3	0.0	0.0	37.3	0.7	1.9	35.0	0.3	1.0	33.0	2.7	8.4	32.0	1.3	4.1	38.3	0.3	1.0	38.0	0.0	0.0
115	135	32.7	11.0	33.5	32.7	3.7	12.7	35.0	3.3	9.6	39.7	7.7	19.3	39.7	6.7	16.9	40.0	3.0	7.7	38.0	4.0	10.5	37.3	14.7	39.1
116	137	29.0	9.7	36.0	34.0	4.3	12.7	39.0	4.3	11.1	39.7	7.7	20.5	35.3	7.3	20.8	38.0	1.3	4.3	40.3	2.0	4.9	39.7	4.3	10.9
117	138	21.0	8.3	43.4	19.7	5.7	32.0	29.3	2.7	9.5	26.0	5.3	19.7	29.7	10.0	34.4	25.3	4.3	17.5	32.3	3.0	9.4	28.3	6.0	20.0
118	139	31.3	5.3	16.1	28.7	1.3	4.8	30.7	1.3	4.3	31.7	2.7	8.8	32.3	2.0	6.3	32.7	1.7	4.6	34.0	0.0	0.0	33.0	0.3	1.0
119	140	34.3	10.3	30.0	35.7	6.0	17.6	39.7	9.3	22.8	42.0	8.0	19.9	37.7	9.0	22.7	34.3	10.0	30.6	38.0	0.3	0.9	36.3	0.3	1.0
120	141	32.7	23.3	71.5	32.0	19.0	59.9	36.3	11.3	31.3	39.0	20.7	51.0	38.7	17.7	45.0	34.3	4.0	11.7	37.7	9.3	24.8	37.3	9.0	23.8
121	142	6.7	6.7	100.0	5.0	2.0	70.0	8.3	6.0	71.3	6.7	5.3	85.2	5.0	4.3	90.5	5.7	1.3	24.4	*	*	*	*	*	*
122	143	30.3	7.3	24.5	28.7	1.7	7.1	35.7	1.7	4.7	29.0	9.7	32.9	35.3	10.7	30.5	33.0	4.0	11.8	36.3	3.3	9.3	38.0	10.3	27.1
123	144	37.0	3.3	8.8	32.7	1.0	2.9	39.0	1.0	2.5	37.7	4.0	10.7	37.0	3.3	9.0	36.0	3.0	8.4	37.0	0.3	0.9	36.0	6.0	16.4
124	145	25.0	16.7	71.6	31.0	4.0	13.1	29.3	6.7	22.2	35.7	10.3	29.4	37.3	8.7	23.8	29.0	5.0	17.2	36.0	3.3	9.1	37.0	12.0	32.5
125	147	30.7	3.0	8.7	29.3	1.0	3.2	31.3	1.7	4.7	30.3	1.7	5.7	26.7	3.0	11.4	33.3	2.3	7.5	30.0	0.0	0.0	28.7	7.0	24.3

...contd. Appendix 2. The mean downy mildew screening data from 172 $F_{2:4}$ self-bulks based on cross ICMB 89111-P6 × ICMB 90111-P6, against six Indian and two African populations of *Sclerospora graminicola* under green house conditions at ICRISAT, Patancheru, India and University of Wales, Bangor, UK, respectively.

Sr. No.	Ent. No.	CAZRI, Jodhpur, India (Sg 139)			ICRISAT, Patancheru, India (Sg 153)			IARI, New Delhi, India (Sg 298)			GAU MRS, Jamnagar, India (Sg 200)			RAU ARS, Durgapura, India (Sg 151)			MAHYCO, Jalna, India (Sg 150)			Maiduguri, Nigeria UWB-screen 43			Bamako, Mali UWB-screen 45		
		TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI
126	148	29.7	11.7	39.0	32.0	5.7	17.5	36.3	4.7	12.9	37.0	10.7	29.3	29.0	11.7	41.8	32.7	3.7	10.5	34.7	7.3	21.2	36.0	4.0	11.0
127	149	33.0	17.0	53.6	38.7	6.7	17.9	40.3	4.0	9.9	38.3	4.0	11.2	34.0	6.3	18.9	36.7	2.3	6.4	39.7	7.7	19.4	37.3	7.7	20.2
128	151	39.7	3.3	8.7	39.0	0.0	0.0	33.0	1.7	6.0	39.0	1.0	2.4	35.0	1.0	3.2	37.7	0.3	0.9	36.3	0.0	0.0	38.7	0.0	0.0
129	152	32.0	4.0	12.7	30.3	0.7	2.0	35.0	4.0	11.3	37.7	2.0	5.2	34.0	4.7	14.6	34.3	1.7	4.9	39.0	0.0	0.0	37.0	8.7	23.3
130	154	27.7	3.0	10.6	27.0	0.3	1.3	28.3	1.3	6.1	26.3	2.0	7.9	30.0	2.7	9.8	29.0	1.0	3.7	29.0	0.7	2.2	27.7	1.0	3.7
131	155	34.7	16.7	53.4	36.7	14.0	39.1	35.3	12.7	35.8	37.7	19.0	50.2	35.0	12.3	36.7	31.7	2.7	8.6	36.3	10.3	27.9	37.3	14.0	37.4
132	156	17.3	10.7	62.3	17.0	1.3	8.1	13.7	1.3	10.0	15.3	2.7	20.6	19.0	5.0	27.3	19.0	2.3	10.0	17.7	0.3	1.9	21.0	7.0	35.3
133	157	39.7	12.3	29.2	33.0	12.3	37.4	37.0	7.0	18.9	40.7	7.3	18.0	36.7	8.3	22.2	36.3	2.0	5.5	37.3	5.7	15.4	38.0	20.3	53.1
134	158	24.3	7.7	31.6	17.7	2.0	11.6	22.3	3.7	17.5	30.0	2.7	8.8	22.3	1.3	5.7	17.0	0.0	0.0	22.0	0.0	0.0	26.5	0.0	0.0
135	159	26.3	5.0	19.2	27.7	0.0	0.0	27.7	2.0	6.8	25.0	1.3	5.4	25.0	4.7	17.7	26.0	1.0	3.6	27.3	0.3	1.4	29.3	2.0	7.8
136	161	29.0	6.7	22.8	23.7	2.3	9.9	26.7	3.7	14.5	29.3	7.3	24.4	33.7	5.3	15.5	31.7	4.0	12.6	27.3	2.3	8.5	23.3	3.3	14.5
137	162	39.7	5.7	13.7	42.0	3.0	7.0	32.7	3.3	12.2	37.7	3.7	9.4	37.0	12.0	33.7	34.3	0.7	2.0	36.3	5.0	13.7	36.3	5.7	16.0
138	163	30.3	2.0	6.5	39.0	0.0	0.0	39.7	1.7	4.4	36.7	1.3	3.5	39.0	2.7	6.8	38.3	2.7	7.5	40.3	0.0	0.0	39.0	0.0	0.0
139	164	22.3	7.3	35.1	26.7	0.3	1.3	27.7	4.7	16.2	34.0	5.3	16.3	29.3	8.7	29.5	32.3	1.7	5.1	28.3	0.0	0.0	28.0	7.0	25.6
140	165	35.0	7.7	23.0	33.7	6.3	20.4	38.0	4.7	12.2	34.3	6.7	19.0	37.7	11.7	31.1	37.3	1.0	2.7	30.7	14.0	45.0	30.3	13.3	43.1
141	166	32.7	19.0	60.7	36.0	10.7	29.7	40.0	13.0	32.4	33.0	16.3	49.2	38.3	24.7	64.0	36.0	6.7	19.6	38.0	4.7	12.3	37.7	30.3	80.3
142	167	25.3	12.3	51.8	21.0	14.3	70.2	29.0	15.0	52.3	24.3	15.3	62.7	25.0	16.7	66.4	27.7	6.0	23.9	29.0	6.7	21.9	28.3	21.0	73.4
143	168	37.7	7.3	19.9	32.0	6.3	20.7	37.7	7.0	18.4	37.7	7.0	18.3	38.7	6.3	16.5	41.3	2.3	5.7	37.0	5.7	15.2	38.3	5.3	14.0
144	169	16.3	8.3	52.6	21.0	5.7	27.9	24.3	6.7	27.5	29.3	12.0	41.5	22.0	9.0	42.7	18.7	1.7	8.6	25.7	8.3	32.5	24.0	7.0	33.3
145	170	33.7	3.3	9.9	31.7	2.0	6.2	39.7	4.0	10.0	37.7	1.7	4.1	35.7	5.7	15.8	35.0	2.0	5.7	36.7	5.0	13.4	39.0	6.3	15.9
146	173	31.3	3.0	10.0	29.0	0.0	0.0	31.0	0.7	2.2	33.0	0.7	1.6	37.0	3.0	8.1	37.0	1.3	3.6	37.7	0.0	0.0	36.7	4.3	11.5
147	174	21.7	9.7	48.3	21.7	4.7	20.3	23.7	4.3	28.7	19.7	8.0	38.0	26.0	15.7	60.6	22.3	5.0	22.6	13.7	1.3	9.3	16.0	9.0	57.7
148	175	34.7	26.7	78.1	36.7	33.3	90.6	38.3	15.0	39.8	34.0	28.7	84.8	35.7	28.7	80.1	36.3	11.7	31.9	37.0	23.7	64.0	38.7	25.3	65.7
149	176	30.3	10.0	33.9	30.0	7.7	28.2	24.7	4.7	18.2	29.3	8.0	26.8	33.7	9.3	27.6	26.7	3.3	13.5	36.0	2.3	6.3	37.3	12.0	32.1
150	177	14.0	8.3	58.8	13.0	0.3	2.8	10.7	2.0	15.3	14.0	5.3	38.4	14.0	4.3	31.4	12.7	1.0	8.3	12.7	0.0	0.0	11.7	6.7	48.3

...contd. Appendix 2. The mean downy mildew screening data from 172 $F_{2:4}$ self-bulks based on cross ICMB 89111-P6 × ICMB 90111-P6, against six Indian and two African populations of *Sclerospora graminicola* under green house conditions at ICRISAT, Patancheru, India and University of Wales, Bangor, UK, respectively.

Sr. No.	Ent. No.	CAZRI, Jodhpur, India (Sg 139)			ICRISAT, Patancheru, India (Sg 153)			IARI, New Delhi, India (Sg 298)			GAU MRS, Jamnagar, India (Sg 200)			RAU ARS, Durgapura, India (Sg 151)			MAHYCO, Jalna, India (Sg 150)			Maiduguri, Nigeria UWB-screen 43			Bamako, Mali UWB-screen 45		
		TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI	TPC	DPC	DMI
151	178	33.7	10.3	30.6	36.0	22.7	62.0	34.7	14.0	41.9	34.0	24.0	67.9	34.0	20.0	60.2	33.3	3.0	8.1	31.7	18.3	55.5	32.0	22.0	67.6
152	179	22.0	6.0	26.4	25.0	5.0	19.5	20.3	2.7	16.3	23.0	4.3	19.0	19.0	6.0	36.6	17.7	1.3	7.2	*	*	*	*	*	*
153	181	42.7	41.0	96.0	32.3	31.7	97.7	41.7	27.3	67.0	34.3	34.3	100.0	38.0	34.7	90.6	41.7	10.3	24.5	32.0	22.3	69.2	34.7	25.7	74.7
154	182	28.3	15.7	52.0	28.7	9.3	32.9	32.3	5.0	15.5	34.3	10.7	31.5	30.7	10.3	33.2	32.3	1.0	3.0	35.3	3.7	10.1	36.3	9.7	26.6
155	184	27.7	15.3	61.4	22.0	8.0	41.6	19.0	6.3	31.1	22.0	10.7	48.7	19.3	10.7	55.1	22.7	3.7	16.2	19.0	6.0	30.1	21.7	10.3	47.9
156	185	40.0	1.7	4.5	30.0	1.0	3.2	37.3	1.7	4.3	35.0	3.7	10.9	34.0	3.0	9.0	32.0	2.0	6.7	36.0	0.0	0.0	34.7	0.0	0.0
157	187	24.0	6.7	28.5	26.7	3.0	11.2	30.7	2.7	8.7	25.7	5.0	21.2	30.3	6.3	20.0	26.3	2.0	9.8	26.7	3.3	11.3	29.3	8.7	29.5
158	188	33.0	8.3	25.5	37.3	7.3	20.2	33.7	6.0	18.2	35.7	8.7	24.8	38.0	13.0	36.1	39.3	8.7	21.2	34.0	3.0	8.7	38.7	30.7	79.4
159	189	28.3	18.0	58.7	26.3	19.3	70.8	27.0	9.7	38.2	22.7	15.7	69.9	26.7	15.3	58.3	47.7	18.0	37.7	25.3	9.7	37.8	23.0	12.5	54.2
160	191	15.0	11.0	76.4	23.0	5.7	24.4	26.0	8.7	32.8	24.3	18.0	71.3	28.0	19.0	66.4	22.0	7.3	32.2	23.0	18.0	80.9	20.3	14.3	70.0
161	192	33.3	4.3	13.0	38.0	1.3	3.4	34.0	2.7	7.7	33.3	4.3	13.0	33.3	7.7	23.1	36.3	2.7	7.5	33.0	0.3	1.0	35.7	0.7	1.9
162	194	27.0	7.3	26.5	22.7	1.0	4.6	30.0	2.0	6.7	28.7	3.7	12.0	39.7	4.7	12.5	33.3	1.3	3.3	28.3	0.0	0.0	33.0	10.7	32.4
163	195	41.0	9.0	22.7	34.7	7.0	20.3	32.0	2.3	7.9	34.7	8.3	25.1	33.7	14.3	41.7	32.3	0.7	2.0	37.3	6.7	18.0	37.7	10.3	27.3
164	196	30.0	3.3	10.8	31.3	2.0	6.5	31.7	2.0	6.3	28.7	5.0	17.3	31.0	6.7	23.6	35.3	6.0	17.9	35.7	1.3	3.7	30.3	0.3	1.0
165	197	35.0	11.0	32.3	36.3	1.0	3.1	35.0	5.0	13.3	37.7	5.3	15.0	37.3	13.0	35.0	36.0	3.3	9.3	36.3	0.3	0.9	36.3	25.3	69.9
166	198	32.7	2.0	6.9	33.0	0.0	0.0	28.3	0.7	2.3	35.7	1.7	4.4	37.0	5.3	13.9	31.3	2.0	6.4	33.7	0.7	1.9	38.3	0.0	0.0
167	199	15.0	5.7	42.1	20.7	6.3	31.3	25.0	5.0	19.0	29.0	14.0	48.2	25.3	13.3	44.3	20.7	2.7	14.7	19.0	3.3	18.7	16.3	7.0	42.9
168	201	20.0	5.3	27.9	21.0	2.3	10.6	19.0	2.0	10.8	22.3	6.3	28.2	14.3	7.0	51.0	21.0	1.0	6.3	35.3	0.0	0.0	35.7	0.0	0.0
169	203	27.0	7.7	28.8	28.7	2.7	9.5	28.7	2.0	6.2	28.0	4.3	15.1	23.3	1.7	6.6	27.0	1.0	3.6	28.7	1.3	5.0	27.7	8.0	28.0
170	204	36.7	22.7	61.9	33.7	18.3	56.8	35.0	11.0	30.0	40.0	13.7	34.0	35.0	13.7	38.6	37.3	3.3	9.0	36.7	7.3	19.8	41.3	20.7	50.2
171	205	42.7	2.7	5.9	32.7	1.3	4.3	34.0	3.0	8.9	38.0	1.7	4.4	36.0	2.7	7.9	35.3	1.3	3.8	38.0	2.3	6.2	39.0	9.3	24.1
172	206	28.0	2.3	11.2	22.7	0.7	3.2	27.7	2.0	7.1	32.3	1.0	3.0	32.3	3.7	10.5	26.3	3.3	12.7	29.7	0.0	0.0	28.7	5.0	17.7

Note: All values in table are means values of three replications; TPC = total plant count; DMC = diseased mildew count; DM% = downy mildew incidence; - = indicates non availability of the data

Appendix 3. The replicated mean data points of grain and stover yield, and their components from multilocation trials of HHB 94-like hybrids conducted at ten locations (Haryana, Andhra Pradesh and Rajasthan) in India during *kharif* 2002 and *kharif* 2003

Hybrid No.	HHB 94-like hybrids	GY (g/m^2)	TF (days)	EPS ($\#/m^2$)	PH (cm)	PL (cm)	PD (mm)	FSY (g/m^2)	DSY (g/m^2)	SMC (%)	ETN ($\#/m^2$)	PY (g/m^2)	TGM (g)	PGN (No.)	TAGBY (g/m^2)	HI (%)
1). CCS HAU RRS Bawal Haryana during *kharif* 2002																
1	HHB 94 P2A	320.6	46	9.8	196	23.4	23.5	1708	806	52.2	24	542	5.70	2352	1348	23.4
2	HHB 94 P5A	330.0	47	10.4	200	22.2	23.5	1725	853	50.4	24	511	6.50	2244	1363	24.2
3	HHB 94 P6A	365.6	46	11.1	194	22.3	24.2	1686	731	56.7	29	545	5.94	2153	1276	28.8
4	ICMH 2002	328.3	46	11.4	190	19.6	28.0	1797	889	51.0	26	482	6.54	1967	1371	24.0
5	ICMH 2005	283.9	47	9.7	199	18.8	26.3	1917	947	50.3	24	473	6.13	1928	1420	19.9
6	ICMH 2006	263.3	47	10.5	194	18.6	24.9	1736	864	50.4	25	455	5.88	1766	1319	19.8
7	HHB 94 P2B	308.3	46	11.9	193	19.7	27.1	1564	744	52.3	26	448	6.85	1726	1192	26.3
8	HHB 94 P5B	308.9	46	11.2	195	22.6	24.0	1586	700	56.1	24	494	6.21	2190	1194	26.4
9	HHB 94 P6B	271.1	46	11.2	186	22.1	21.8	1472	642	56.3	29	461	5.42	1759	1102	24.7
10	HHB 94-Ori	361.7	45	9.7	198	23.9	24.2	1761	794	55.0	27	555	6.83	1957	1349	26.7
11	HHB 181 (C)	367.2	44	8.5	190	20.6	22.0	1697	858	49.6	24	567	6.83	2469	1426	26.0
2). CCS HAU Hisar Haryana during *kharif* 2002																
1	HHB 94 P2A	383.8	50	12.6	213	21.4	21.4	1984	873	56.3	30	591	7.38	1711	1464	26.1
2	HHB 94 P5A	315.6	50	13.1	208	21.4	21.4	1872	794	57.9	29	536	7.17	1522	1330	23.4
3	HHB 94 P6A	357.8	50	12.8	205	21.1	21.1	1916	924	52.3	29	588	7.61	1612	1512	23.7
4	ICMH 2002	324.1	48	13.6	207	19.7	19.7	2011	991	50.9	26	584	7.99	1561	1576	20.6
5	ICMH 2005	297.8	50	10.7	210	18.1	18.1	2263	1021	54.5	30	550	7.57	1325	1572	18.8
6	ICMH 2006	453.1	49	13.7	213	18.4	18.4	2291	1320	44.1	30	627	8.40	1930	1947	26.5
7	HHB 94 P2B	294.7	49	13.9	204	18.4	18.4	1781	858	52.1	28	516	7.48	1227	1373	22.5
8	HHB 94 P5B	365.6	51	13.8	207	21.4	21.4	2094	1007	51.6	32	616	7.87	1436	1623	22.4
9	HHB 94 P6B	368.8	50	14.0	214	20.3	20.3	1838	850	53.9	27	555	7.87	1724	1405	26.0
10	HHB 94-Ori	329.7	50	14.5	207	20.7	20.7	1747	784	55.1	30	541	7.61	1434	1325	25.1
11	HHB 181 (C)	480.7	46	12.2	201	20.0	20.0	2564	1357	47.1	42	839	7.88	1389	2195	22.1

...contd. Appendix 3. The replicated mean data points of grain and stover yield, and their components from multilocation trials of HHB 94-like hybrids conducted at ten locations (Haryana, Andhra Pradesh and Rajasthan) in India during *kharif* 2002 and *kharif* 2003

Hybrid No.	HHB 94-like hybrids	GY (g/m^2)	TF (days)	EPS ($\#/m^2$)	PH (cm)	PL (cm)	PD (mm)	FSY (g/m^2)	DSY (g/m^2)	SMC (%)	ETN ($\#/m^2$)	PY (g/m^2)	TGM (g)	PGN (No.)	TAGBY (g/m^2)	HI (%)
3). RP 9A, ICRISAT Patancheru during *kharif* 2002																
1	HHB 94 P2A	370.1	50	9.0	194	17.3	24.6	1695	457	72.8	32	465	9.06	1299	922	40.1
2	HHB 94 P5A	420.4	50	10.0	182	17.1	23.5	1821	513	71.7	34	516	9.54	1295	1029	40.9
3	HHB 94 P6A	403.4	49	8.0	182	17.1	23.9	1726	471	72.5	33	499	9.25	1338	970	41.5
4	ICMH 2002	379.3	49	10.0	207	16.4	25.6	1902	592	68.9	30	472	8.63	1462	1064	35.8
5	ICMH 2005	321.6	52	9.0	200	16.2	25.0	1962	566	71.2	29	405	7.92	1395	971	33.1
6	ICMH 2006	334.4	51	9.0	200	16.6	24.0	1768	522	70.3	29	423	7.72	1552	946	35.6
7	HHB 94 P2B	398.7	49	10.0	202	15.9	25.6	1839	539	70.7	30	466	8.66	1579	1004	39.7
8	HHB 94 P5B	387.4	49	11.0	185	17.7	23.9	1875	528	71.9	37	489	9.05	1182	1016	38.1
9	HHB 94 P6B	376.1	49	11.0	186	16.9	23.9	1765	484	72.6	37	480	8.29	1241	964	39.0
10	HHB 94-Ori	396.6	49	9.0	183	17.4	24.5	1742	489	71.9	33	500	9.02	1355	989	40.2
11	HHB 181 (C)	420.6	45	9.0	190	16.6	24.1	1648	493	70.0	37	524	8.54	1337	1017	41.3
4). RCE 24C, ICRISAT Patancheru during kharif 2002																
1	HHB 94 P2A	488.3	51	12.0	193	18.10	24.38	2591	611	76.2	35	598	9.25	1508	1208	40.4
2	HHB 94 P5A	494.7	51	12.0	191	17.90	23.28	2473	618	75.0	38	596	9.32	1399	1214	40.8
3	HHB 94 P6A	488.3	51	12.0	188	18.30	23.96	2425	566	76.6	38	602	9.44	1357	1169	41.7
4	ICMH 2002	463.0	51	12.0	204	15.60	25.08	2919	805	72.4	35	565	8.22	1617	1369	34.0
5	ICMH 2005	397.4	52	12.0	207	16.00	24.56	2780	650	76.6	32	488	8.10	1567	1138	34.9
6	ICMH 2006	399.0	52	12.0	207	15.70	23.90	2825	687	75.7	32	496	7.27	1729	1183	33.7
7	HHB 94 P2B	458.5	51	12.0	198	16.50	25.88	2756	694	74.7	33	556	8.61	1626	1250	36.7
8	HHB 94 P5B	468.4	51	12.0	194	18.10	23.78	2826	640	77.3	41	633	8.86	1308	1273	36.9
9	HHB 94 P6B	427.8	51	12.0	190	18.50	24.08	2554	547	78.6	36	535	9.17	1291	1082	39.7
10	HHB 94-Ori	507.7	50	12.0	190	17.50	24.14	2500	589	76.5	39	622	9.19	1428	1211	42.0
11	HHB 181 (C)	519.7	48	12.0	188	17.50	24.48	2472	639	74.3	40	618	8.65	1513	1257	41.5

...contd. Appendix 3. The replicated mean data points of grain and stover yield, and their components from multilocation trials of HHB 94-like hybrids conducted at ten locations (Haryana, Andhra Pradesh and Rajasthan) in India during *kharif* 2002 and *kharif* 2003

Hybrid No.	HHB 94-like hybrids	GY (g/m^2)	TF (days)	EPS ($\#/m^2$)	PH (cm)	PL (cm)	PD (mm)	FSY (g/m^2)	DSY (g/m^2)	SMC (%)	ETN ($\#/m^2$)	PY (g/m^2)	TGM (g)	PGN (No.)	TAGBY (g/m^2)	HI (%)
5). RP 6B, ICRISAT Patancheru during *kharif* 2003																
1	HHB 94 P2A	439.8	40	13.0	169	20	24	1426	419	70.6	43	581	9.19	1124	1000	44.0
2	HHB 94 P5A	423.1	40	13.0	162	20	24	1357	402	70.2	44	544	8.74	1117	946	44.8
3	HHB 94 P6A	423.6	39	13.0	165	19	24	1382	391	71.7	48	573	9.47	926	965	43.8
4	ICMH 2002	358.8	44	13.0	183	18	26	1536	508	66.5	39	514	7.57	1226	1022	35.1
5	ICMH 2005	395.2	46	13.0	182	17	26	2000	549	72.5	36	522	7.63	1437	1071	37.1
6	ICMH 2006	372.8	47	13.0	190	17	25	1828	566	69.0	41	542	7.16	1326	1108	33.7
7	HHB 94 P2B	414.2	40	13.0	160	20	24	1509	434	71.2	39	547	8.99	1194	981	42.3
8	HHB 94 P5B	403.5	40	13.0	162	20	24	1499	418	72.1	46	532	9.02	995	951	42.5
9	HHB 94 P6B	392.3	40	12.0	161	19	24	1407	349	75.3	43	531	8.65	1056	880	45.1
10	HHB 94-Ori	417.2	40	13.0	166	19	24	1321	399	69.8	49	561	8.33	1031	960	43.5
11	HHB 181 (C)	429.4	37	12.0	165	19	24	1253	394	68.5	48	577	8.52	1084	971	44.2
6). RCE 24C, ICRISAT Patancheru during *kharif* 2003																
1	HHB 94 P2A	413.0	47	13.0	163	19.5	24.6	1291	443	65.7	36	550	8.23	1410	993	41.7
2	HHB 94 P5A	403.2	46	13.0	163	19.6	25.0	1349	465	65.6	35	536	8.72	1337	1001	40.5
3	HHB 94 P6A	376.9	46	14.0	150	18.7	24.1	1118	390	65.0	36	501	9.29	1160	891	42.3
4	ICMH 2002	388.1	49	14.0	170	17.7	25.6	1352	500	62.9	33	542	9.11	1346	1043	37.1
5	ICMH 2005	315.8	50	14.0	177	17.3	25.4	1424	494	65.1	27	448	8.27	1412	942	33.0
6	ICMH 2006	371.4	50	15.0	176	17.4	24.7	1391	547	60.6	30	505	7.76	1603	1052	35.5
7	HHB 94 P2B	391.3	47	14.0	164	19.2	24.4	1281	435	66.0	34	529	8.08	1455	964	40.7
8	HHB 94 P5B	351.5	48	13.0	158	18.7	24.0	1209	417	65.6	33	468	8.77	1206	885	39.8
9	HHB 94 P6B	407.2	46	14.0	158	18.9	24.0	1317	433	67.1	39	540	8.45	1251	973	41.9
10	HHB 94-Ori	382.3	46	15.0	159	18.7	25.2	1172	404	65.7	38	507	8.58	1209	911	42.1
11	HHB 181 (C)	445.7	43	11.0	161	19.2	25.0	1243	490	60.5	44	593	8.04	1261	1083	41.3

...contd. Appendix 3. The replicated mean data points of grain and stover yield, and their components from multilocation trials of HHB 94-like hybrids conducted at ten locations (Haryana, Andhra Pradesh and Rajasthan) in India during *kharif* 2002 and *kharif* 2003

Hybrid No.	HHB 94-like hybrids	GY (g/m^2)	TF (days)	EPS ($\#/m^2$)	PH (cm)	PL (cm)	PD (mm)	FSY (g/m^2)	DSY (g/m^2)	SMC (%)	ETN ($\#/m^2$)	PY (g/m^2)	TGM (g)	PGN (No.)	TAGBY (g/m^2)	HI (%)
7). RAU RRS Nagaur, Rajasthan during *kharif* 2003																
1	HHB 94 P2A	125.6	54	11.0	174	19.0	22.4	730	216	70.6	14	174	7.11	1320	390.0	32.6
2	HHB 94 P5A	130.0	52	12.0	162	18.1	22.2	716	206	71.2	15	180	7.02	1222	386.1	33.4
3	HHB 94 P6A	141.6	52	13.0	167	19.5	23.1	697	203	70.9	14	190	6.93	1398	393.7	35.5
4	ICMH 2002	102.0	55	11.0	168	17.8	26.9	717	215	70.3	13	161	6.44	1236	376.0	27.1
5	ICMH 2005	108.1	56	10.0	163	17.0	26.7	793	231	71.0	12	161	6.82	1273	392.2	26.9
6	ICMH 2006	89.3	57	11.0	168	16.0	24.7	696	221	68.3	13	140	5.77	1170	361.1	24.4
7	HHB 94 P2B	119.2	53	11.0	168	18.2	21.9	751	217	71.2	13	169	6.88	1274	385.9	30.2
8	HHB 94 P5B	131.7	54	12.0	170	18.1	21.7	793	240	69.9	13	185	7.54	1292	425.3	30.0
9	HHB 94 P6B	105.5	51	11.0	162	19.3	22.1	693	205	70.7	14	183	6.77	1049	387.4	27.5
10	HHB 94-Ori	118.1	52	12.0	157	18.2	22.0	632	182	71.3	15	167	6.86	1122	348.7	32.8
11	HHB 181 (C)	134.6	47	12.0	158	17.2	20.2	633	194	69.3	14	175	6.88	1366	368.8	36.4
8). CCS HAU RRS Bawal, Haryana during *kharif* 2003																
1	HHB 94 P2A	247.5	55	13.0	184	22.0	24.8	1615	648	59.3	26	414	8.16	1150	1062	23.1
2	HHB 94 P5A	303.3	56	13.0	188	21.6	25.3	1940	837	57.1	27	462	8.73	1293	1299	22.6
3	HHB 94 P6A	247.7	54	13.0	179	22.0	25.1	1547	667	56.7	24	401	7.80	1288	1067	22.8
4	ICMH 2002	213.4	58	13.0	184	18.7	28.0	1708	775	54.5	23	341	7.92	1158	1116	19.4
5	ICMH 2005	213.9	58	13.0	185	18.1	29.7	2196	959	55.9	27	408	8.45	879	1332	15.5
6	ICMH 2006	206.0	55	13.0	187	18.5	27.8	1636	758	53.7	22	349	6.95	1380	1108	18.7
7	HHB 94 P2B	266.5	57	12.0	185	22.1	25.3	1675	733	55.9	30	409	8.37	1049	1143	23.3
8	HHB 94 P5B	267.2	57	13.0	182	22.6	24.6	1853	728	61.0	33	437	8.78	914	1165	23.3
9	HHB 94 P6B	278.6	54	14.0	178	22.1	24.6	1767	769	55.3	33	444	8.19	1043	1213	22.9
10	HHB 94-Ori	296.7	54	13.0	178	21.1	24.4	1592	725	54.4	32	449	8.06	1148	1174	25.4
11	HHB 181 (C)	235.8	50	12.0	172	19.7	25.0	1361	544	59.8	24	388	8.17	1292	933	25.1

...contd. Appendix 3. The replicated mean data points of grain and stover yield, and their components from multilocation trials of HHB 94-like hybrids conducted at ten locations (Haryana, Andhra Pradesh and Rajasthan) in India during *kharif* 2002 and *kharif* 2003

Hybrid No.	HHB 94-like hybrids	GY (g/m^2)	TF (days)	EPS ($\#/m^2$)	PH (cm)	PL (cm)	PD (mm)	FSY (g/m^2)	DSY (g/m^2)	SMC (%)	ETN ($\#/m^2$)	PY (g/m^2)	TGM (g)	PGN (No.)	TAGBY (g/m^2)	HI (%)
9). CCS HAU Hisar, Haryana during *kharif* 2003																
1	HHB 94 P2A	270	54	11.0	211	22.6	25.8	1878.6	780.8	58.3	34	442	7.50	1055	1222.6	22.2
2	HHB 94 P5A	278	56	13.0	208	22.3	25.6	1780.0	816.0	54.0	32	420	7.14	1209	1236.2	22.2
3	HHB 94 P6A	221	54	12.0	205	23.2	25.8	1500.0	704.3	53.2	34	384	6.83	933	1088.2	20.0
4	ICMH 2002	243	54	15.0	212	20.0	29.5	2087.5	877.5	57.8	31	444	7.13	1118	1321.1	18.4
5	ICMH 2005	198	55	10.0	202	20.5	28.9	1663.1	770.6	54.0	28	360	6.76	1047	1130.5	17.3
6	ICMH 2006	209	55	13.0	203	20.1	27.4	1842.7	915.0	50.4	31	380	6.30	1071	1295.2	16.0
7	HHB 94 P2B	274	55	12.0	211	22.7	25.1	1946.9	864.0	55.8	40	498	6.49	1068	1362.5	20.1
8	HHB 94 P5B	235	55	14.0	207	22.9	25.0	1743.8	828.0	52.1	33	416	6.69	1081	1243.7	19.3
9	HHB 94 P6B	206	54	12.0	198	22.3	24.4	1518.8	652.5	57.1	32	384	6.68	989	1036.9	20.1
10	HHB 94-Ori	249	55	13.0	204	22.9	25.7	1695.0	769.5	54.9	35	413	6.74	1030	1182.3	20.8
11	HHB 181 (C)	179	51	10.0	184	19.0	23.6	1071.9	532.5	49.6	30	356	6.52	974	888.2	20.8
10). RAU RRS Durgapura, Rajasthan during *kharif* 2003																
1	HHB 94 P2A	200.8	47	11.0	203	21.7	24.7	908	419	52.9	16	265	7.44	1627	683.7	29.3
2	HHB 94 P5A	178.4	46	11.0	197	22.0	25.0	1051	455	55.7	15	260	7.86	1474	714.9	25.0
3	HHB 94 P6A	228.0	47	11.0	198	21.4	25.0	1033	446	56.9	17	300	8.54	1548	745.8	30.5
4	ICMH 2002	240.6	49	11.0	205	18.3	27.3	940	436	53.4	17	287	8.22	1727	723.9	33.7
5	ICMH 2005	257.1	49	9.0	207	17.6	27.2	1128	521	52.7	19	335	8.08	1716	855.2	30.9
6	ICMH 2006	271.6	49	11.0	198	17.5	26.0	1065	460	56.6	17	308	8.12	2006	768.8	35.5
7	HHB 94 P2B	276.2	47	11.0	199	21.9	24.9	1010	467	53.1	18	323	8.00	1914	790.1	35.4
8	HHB 94 P5B	256.7	47	10.0	201	21.8	24.9	1077	513	52.1	17	317	8.56	1771	829.2	31.1
9	HHB 94 P6B	260.1	46	10.0	198	21.3	24.4	1043	459	56.1	19	341	8.28	1687	800.6	32.7
10	HHB 94-Ori	278.8	46	11.0	195	21.0	24.7	1129	549	51.8	18	335	8.14	1873	883.6	31.9
11	HHB 181 (C)	295.2	42	9.0	190	20.1	23.9	1058	446	57.4	19	355	8.00	1951	800.8	36.7

Note: All values in this appendix are replicated mean; GY - grain yield; TF - Time to 50 % flowering; EPS - effective plant stand; PH - plant height; PL panicle length; PD - panicle diameter; FSY - fresh straw yield; DSY - dry straw yield; SMC - straw moisture content; ETN - effective tiller number; PY - panicle yield; TGM - 1000 -grain mass; PGN - panicle grain number; TAGBY - total above-ground biomass yield; HI - harvest index; and (c) - check

Appendix 4. Preparation of stock solutions used during the present study

CTAB (Cety Trimethyl Ammonium Bromide) (2%) buffer

CTAB	20 g
1 M Tris	200 mL
5 M NaCl	280 mL
0.5 M EDTA	40 mL
Na_2SO_3	2.5 g
Distilled Water	460 mL

Make up volume to 1 litre and add Mercaptoethanol (0.17%) while using CTAB (2%) solution only.

Rnase (10 mg/mL)

Dissolve RNase in double distilled water/ or RNase buffer (kit), place in a tube in boiling water bath for 10 minutes. Allow this to cool on a bench and store at -20°C.

Chloroform: isoamyl alcohol (24:1)

Chloroform	240 mL
Isoamyl alcohol	10 mL

Make up required volume. Shake well and dispense the solution in a fumed chamber. Store in dark at room temprature.

Ethanol (70%)

Absolute alcohol	70 mL
Distilled Water	30 mL

NaCl (5 M)

NaCl	292.2 g
dH_2O	750 mL

Make up volume to 1 litre, filter and autoclave.

Phenol/chloroform

Mix equal volumes of the buffered phenol and chloroform: isamyl alchohol (24: 1). Store at 4°C. Give gentle shake to mix properly just before use.

Sodium acetate (2.5 M. pH 5.2)

Sodium acetate	340.2 g
dH_2O	500 mL

Adjust pH to 5.2 with glacial acetic acid. Make up volume to 1 litre and autoclave.

Tris HCl (1 M, pH 8.0)

Tris	121.1 g
dH_2O	800 mL

Dissolve in water. Adjust pH to 8.0 with conc. HCl. Make up volume to 1 liter and autoclave.

...contd. Appendix 4. Preparation of stock solutions used during the present study

EDTA (0.5 M, pH 8.0)

Na_2 EDTA	186.1 g
dH_2O	800 mL

Dissolve properly. Adjust pH to 8.0 with sodium hydroxide pellets. Make up volume to 1 liter and autoclave.

$T_{10}E_1$ buffer

0.5 M Tris HCl pH 8.0	10 mL
0.5 M EDTA pH 8.0	1 mL

And make up volume to 1 liter with sterile distilled water.

$T_{50}E_{10}$ buffer

0.5 M Tris HCl pH 8.0	50 mL
0.5 M EDTA pH 8.0	20 mL

Make up volume to 500 mL with sterile distilled water.

10X Tris-Borate Buffer (TBE) (per liter)

Tris base	108 g
Boric acid	55 g
EDTA (0.5 M) pH 8.0	40 mL
dH_2O	Up to 1000 mL

Mix well and store at 4°C.

1% Agarose

Agarose	2.5 g
TAE/TBE	250 mL
Ethidium bromide	8 µL

Take TAE or TBE based on the tank buffer.

6X Gel loading buffer (0.25% Bromophenol blue, 40% sucrose)

Sucrose	4 g
Bromophenol Blue	2.5 mL
dH_2O	Upto 10 mL

Store at 4°C.

Ethidium bromide (10 mg/mL)

Ethidium bromide	100 mg
dH_2O	10 mL

Dissolve in distilled water gently, wrap tube in aluminium foil and store at 4°C.

Caution: Ethidium bromide is extremely mutagenic.

...contd. Appendix 4. Preparation of stock solutions used during the present study

Acrylamide/biacrylamide 29: 1 (w/w)	
Acrylamide	29 g
Bisacrylamide	1 g
Water (deionised distilled)	Up to 100 mL
Store at 4°C.	
10% (w/v) Ammonium per sulphate	
Ammonium per sulphate	1 g
Water (deionised distilled)	10 mL
Make fresh stock every week and store at 4°C.	
TEMED (N, N, N', N'-tetremethyl dehylene diamine	
Store at 4°C.	
Loading buffer for non-denaturing PAGE, 5X	
EDTA (0.5 M, pH 8.0)	10 mL
NaCl (5 M)	1 mL
Glycerol	50 mL
Distilled Water	39 mL
Binding silane buffer	
Binding silane buffer	1.5 µL
Acetic acid	5 mL
Ethanol	993.5 mL
Store at 4°C.	
100 base pairs ladder (50 ng/mL)	
100 bp ladder (stock conc. 1µg/µL)	50 µL
Blue (6X dye)	165 µL
$T_{10}E_1$ buffer	785 µL
Hybridization solution (For New England bio-lab kit)	
dNTP mixture (excluding dATP)	6 µL
α-^{32}P dATP	5 µL
Klenow fragments	1 µL
7.5 Ammonium acetate	
Ammonium acetate	57.75 g
Sterile dH_2O	75 mL
Make up volume to 100 mL with dH_2O.	
LB medium 500 mL	
NaCl	5 g
Trypton	5 g
Yeast extract	2.5 g
Make up volume to 500 mL, adjust pH to 7.2 with 1 N NaOH and autoclave.	

...contd. Appendix 4. Preparation of stock solutions used during the present study

LB + Agar medium 500 mL	
NaCl	5 g
Trypton	5 g
Yeast extract	2.5 g
Agar	7.5 g
Make up volume to 500 mL, adjust pH to 7.2 with 1 N NaOH and autoclave.	
GTE solution	
Glucose 0.5 M pH 8.0	2 mL
Tris 0.5 M pH 8.0	5 mL
Make up volume to 100 mL, filter it and store at 4°C.	
dNTP mixture (For New England bio-lab kit)	
dCTP	50 µL
dTTP	50 µL
dGTP	50 µL
Sol. A (for plasmid extraction)	
Lysozyme	4 mg
GTE solution	1 mL
Everytime use freshly prepared and well dissolved solution and take 200 µL/reaction.	
Ampicillin 50 mg/mL (5 mL)	
Ampicillin	250 mg
Sterile dH_2O	5 mL
Make up required volume.	
Sol. B (for plasmid extraction)	
10% SDS	1 mL
1 N NaOH	2 mL
Sterile dH_2O	7 mL
Everytime use freshly prepared and well dissolved solution and take 300 µL/reaction.	
Developer	
Developer A powder	172.5 g
Developer B powser	10.0 g
Warm sterile dH_2O upto 52°C	700 mL
Slowly add A and B make up volume to 1 liter with SDW.	
Stop bath (3% of Hac)	
Hac (acetic acid)	30 mL
dH_2O	970 mL
Rapid fixer	
Rapid fixer powder	264.4 g
dH_2O	700 mL
Make up volume to 1 liter with sterile dH_2O.	

...contd. Appendix 4. Preparation of stock solutions used during the present study

S Buffer for DNA extraction

1M Tris ph 8.5	100 mL
5M NaCl	20 mL
0.5 M EDTA pH 8.0	100 mL
SDS	20 g

Make up volume to 1 litre and autoclave it.

20 X SSC

NaCl	175.32 g
Sodium citrate	88.23 g

Disolve in distiiled water gently and make up volume to 1litre.

20% SDS

SDS	200 g
dH_2O	700 mL

Dissolve gently and make up volume to 1litre.

Pre-hybridisation solution

Sodium Phosphate Dibase	71 g
dH_2O	800 mL
SDS	70 g

Dissolve SDW and adjust pH 7.2 with orthophosphoric acid.

Add BSA	10 g

Make up volume to 1 litre.

Salmon DNA (5 mg/mL)

Salmon DNA powder	500 mg
ddH_2O	100 mL

Dissolve it gently and properly and store in -20°C.

^{32}P Blot washing (stripping) solution after hybridisation

Solution 1		
	20 X SSC	100 mL
	20% SDS	25 mL
Solution 2		
	20 X SSC	10 mL
	20% SDS	25 mL
Stripping off solution		
	20 X SSC	5 mL
	20% SDS	25 mL

Proteinase K (10 mg/mL stock)

Proteinase K powder	100 mg
ddH_2O	10 mL

Make up the required volume.

Appendix 5. Field plan of HHB 94-like hybrid trials conducted across ten locations in AndhraPradesh, Haryana and Rajasthan during kharif 2002 and 2003.

Sr. No.	Test environment/location	State/cluster	Conditions	Seasons/Years	Plot Dimensions
1	RCE24B, Patancheru	AP	Irrigated Normal Fertility	Kharif 2002	1-m row x 4-rows x 60 cm x 15 cm
2	RP9A, Patancheru	AP	Irrigated Normal Fertility	Kharif 2002	1-m row x 4-rows x 75 cm x 15 cm
3	CCS HAU Hisar	Haryana	Irrigated Normal Fertility	Kharif 2002	1-m row x 4-rows x 50 cm x 15 cm
4	HAU RRS Bawal	Haryana	Rainfed Normal Fertility	Kharif 2002	1-m row x 4-rows x 45 cm x 15 cm
5	RCE24C, Patancheru	AP	Irrigated Normal Fertility	Kharif 2003	1-m row x 4-rows x 60 cm x 15 cm
6	RP6B, Patancheru	AP	Irrigated Normal Fertility	Kharif 2003	1-m row x 4-rows x 75 cm x 15 cm
7	CCS HAU Hisar	Haryana	Irrigated Normal Fertility	Kharif 2003	1-m row x 4-rows x 50 cm x 15 cm
8	HAU RRS Bawal	Haryana	Rainfed Low Fertility	Kharif 2003	1-m row x 4-rows x 45 cm x 15 cm
9	RAU RRS Durgapura	Rajasthan	Rainfed Low Fertility	Kharif 2003	1-m row x 4-rows x 50 cm x 15 cm
10	RAU RRS Nagaur	Rajasthan	Rainfed Normal Fertility	Kharif 2003	1-m row x 4-rows x 60 cm x 15 cm

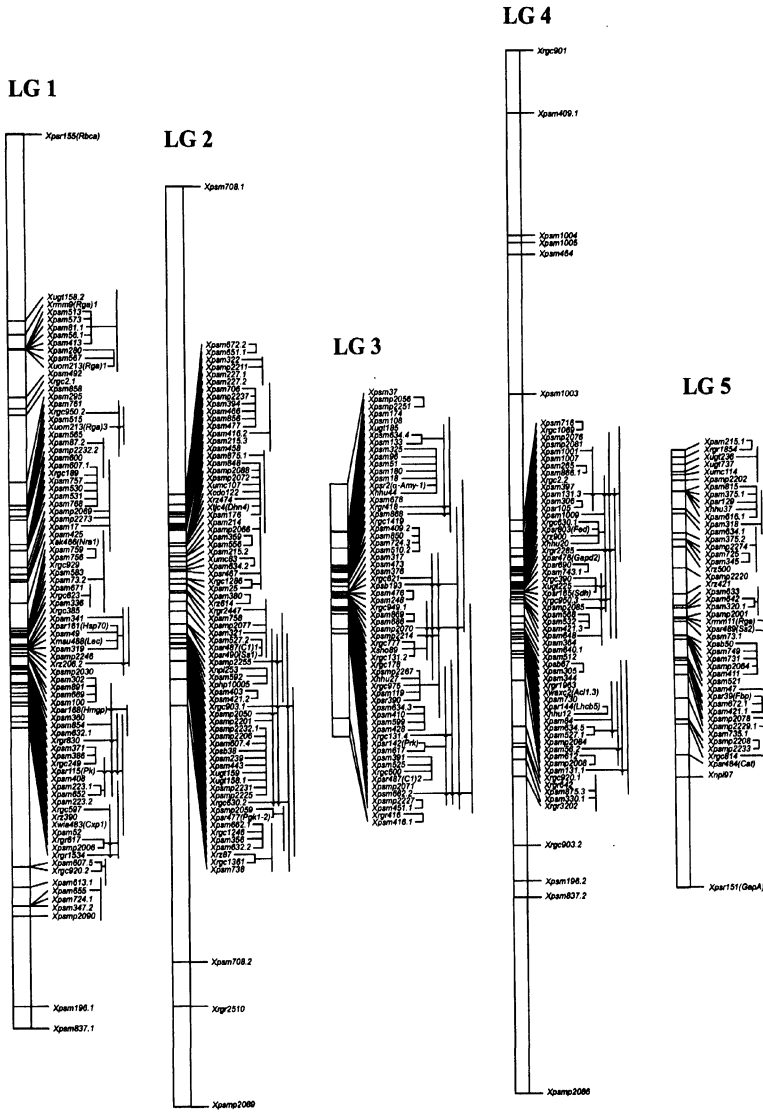
Appendix 6. The List of DNA markers techniques that have been developed over the years. Courtesy: Mohan *et al.* (1997) and Gupta and Varshney (2000)

Acronym	Technique	Reference
AFLP	Amplified Fragment Length Polymorphism	Vos *et al.*, 1995. *Nucl. Acids Res.*, ***23***: 4407-4414.
ALP	Amplicon Length Polymorphism	Ghareyazie *et al.*, 1995. *Theor. Appl. Genet.*, ***91***: 218-227.
AP-PCR	Arbitrarily primed PCR	Welsh and McClelland, 1990. *Nucl. Acids Res.*, ***8***: 7213-7218.
AS-PCR	Allele Specific PCR	Sarkar *et al.*, 1990. *Anal. Biochem.*, ***186***: 64-84.
CAPS	Cleaved Amplified Polymorphic Sequence	Lyamichev *et al.*, 1993. *Science*, ***260***: 778-783.
DAF	DNA Amplification Fingerprinting	Caetano-Anolles *et al.*, 1991. *Bio Technology.*, ***9***: 553-557.
IMP	Inter-Mite (Miniature Inverted-repeat Transposable Elements) Polymorphism	Chang *et al.*, 2001. *Theor. Appl. Genet.*, ***102(5)***: 773-781.
ISSA=ISSR	Inter-SSR Amplification = Inter Simple Sequence Repeat	Zietkiewiez *et al.*, 1994. *Genomics*, ***20***: 176-183.
MP-PCR	Microsatellite Primed PCR	Meyer *et al.*, 1993.. *J. Clinical Biol.*, ***31***: 2274-2280.
RAMS	Randomly Amplified Microsatellite	Ender *et al.*, 1996. *Mol. Ecol.*, ***5***: 437-447.
RAPD	Restricted Amplified Polymorphic DNA	Williams *et al.*, 1990. *Nucl. Acid Res.*, ***18***: 6531-6535.

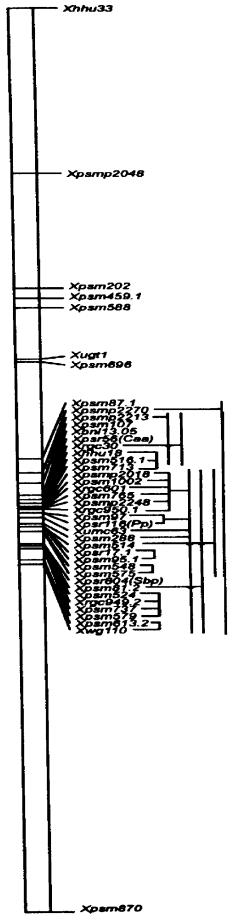
...contd. Appendix 6. The List of DNA markers techniques that have been developed over the years

RMAP	Retrotransposon-Microsatellite Amplified Polymorphism	Kalender *et al.*, 1999. *Theor. Appl. Genet.*, ***98***: 704-711.
RFLP	Restricted Fragment Length Polymorphism	Botstein *et al.*, 1980. *American J. Hum. Genet.*, ***32***: 314-331.
SAP	Specific Amplicon Polymorphism	Williams *et al.*, 1991. *Theor. Appl. Genet.*, ***82***: 489-498.
SCAR	Sequence Characterized Amplified Region	Williams *et al.*, 1991. *Theor. Appl. Genet.*, ***82***: 489-498.
SNP	Single Nucleotide polymorphism	Nikiforov *et al.*, 1994. *Nucl. Acids. Res.*, ***22***: 4167-4175.
SSCP	Single Strand Conformation Polymorphism	Orita *et al.*, 1989. *Proc. Natl. Acad Sci. USA*, ***86***: 2766-2770.
MSCP	Microsatellite Simple Sequence Length Polymorphism	Rongwen *et al.*, 1995. *Theor. Appl. Genet.*, ***90***: 43-48.
SSLP	Microsatellite Simple Sequence Length Polymorphism	Jarman and Wells, 1989. Trends Genet., ***5***: 367-371.
SSR	Simple Sequence Repeat	Hearne *et al.*, 1992. *Trends Genet.*, ***8***: 288-294.
STMS	Sequence Tagged Microsatellite Sites	Beckmann and Soller, 1990. *Bio Technology*, ***8***: 930-932.
STS	Sequence Tagged Sires	Fukuoka *et al.*, 1994. *DNA Res.*, ***1***: 271-277.

Pearl millet consensus maps



LG 6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Xpsm1061
Xbrw13.05
Xpsr58(Cae)
Xpc30
Xhhu16
Xpsm516.1
Xpsm715
Xpsmp2018
Xpsm1002
Xpsc601
Xpsm765
Xpsmp2248
Xpc950.1
Xpsm91
Xpsr118(Pp)
Xumc53
Xpsm268
Xpsm514
Xpsr17.1
Xpsm95.1
Xpsm548
Xpsm575
Xpsr904(Sbp)
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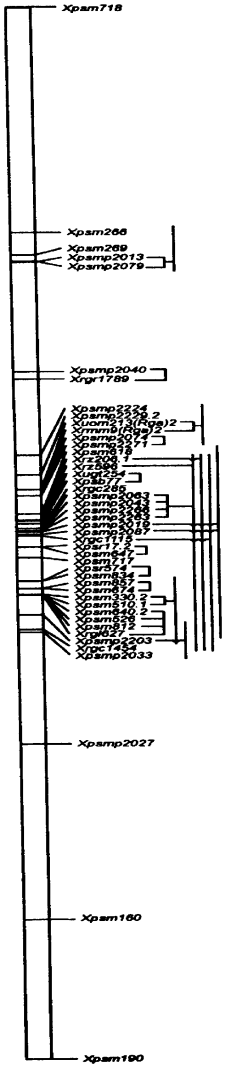
Xpsm813.2
Xwg110
Xpsm870

LG 7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Xmms1(Rpg)2
Xpsmp2074
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Xz2208.1
Xz2583
Xugt54
Xpsm577
Xpc246
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Xpsmp2241
Xpsmp2244
Xpsmp2316
Xpsmp2387
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Xpsr112.2
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Xpsm717
Xpsr574
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Xpsm857
Xpsm674
Xpsm1303
Xpsm5107
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Xpsm598
Xpsm812
Xgwm27
Xpsmp2203
Xpc1454
Xpsmp2033
Xpsmp2027
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Xpsm190

Title of thesis	:	QTL mapping for improvement of downy mildew [*Sclerospora graminicola* (Sacc.) J. Schroet.] resistance (DMR) in pearl millet [*Pennisetum glaucum* (L.) R. Br.] hybrid parental line ICMB 89111.
Full name of the degree holder	:	**SURINDER KUMAR GULIA**
Admission No.	:	99A56D
Title of degree	:	Doctor of Philosophy in Plant Breeding
Name and address of the major advisor	:	**Dr. R.S. SANGWAN** Head, Oilseed section, Department of Plant Breeding CCS Haryana Agricultural University Hisar – 125 004 (India)
Degree awarding university/Institute	:	CCS Haryana Agricultural University Hisar – 125 004 (India)
Major subject	:	Plant Breeding
Total number of pages in thesis	:	291 + 36
Number of words in the abstract	:	Approx. 900

Thesis abstract

Pearl millet downy mildew, caused by pseudo-fungus *Sclerospora graminicola,* is the most devastating disease of pearl millet (*Pennisetum glaucum* L.) causing huge grain and straw production losses on single-cross hybrids in India. The allogamous and highly variable natures of both the host and pathogen are great hindrances to breeding for host plant resistance to this disease. DNA markers (especially co-dominant markers) and QTL mapping provide insights into facets of quantitative inheritance patterns in breeding disease resistant crop cultivars. The present study was undertaken at ICRISAT-Patancheru to construct a skeleton genetic linkage map for a pearl millet mapping population of 172 F_2-derived F_4 progenies derived from a single F_1 plant from a plant × plant cross, ICMB 89111-P6 (susceptible) × ICMB 90111-P6 (resistant), to identify and map QTLs controlling downy mildew resistance (DMR), to study inheritance of DMR and finally, to assess the agronomic performance in multilocation trials of different HHB 94-like hybrids produced on sub-selections of ICMA/B 89111 and ICMB 90111. Bulks of each of 172 F_2–derived F_3 progenies were used to isolate nuclear DNA that was genotyped for 46 polymorphic SSR and RFLP marker loci. These 172 progenies were selfed to produce $F_{2:4}$ self-bulks for seedling greenhouse DM screens against eight pathogen populations from India (six) and Africa (two). The pooled ANOVA from screens of these 172 $F_{2:4}$ self-bulks against six Indian (Asian) and two African pathogen populations revealed significant intracontinental pathogenic variability while variance component analysis revealed significant intercontinental variability in downy mildew mapping population progenies. ANOVA for all screens against individual pathogen populations, as well as across all eight pathogen populations, revealed high operational heritabilities, which were sufficient to permit the use of phenotypic data from these screens in QTL mapping. Mendelian segregation patterns among the 172 $F_{2:4}$ self-bulks screened against these eight pathogen populations showed that at least one to four genes were controlling resistance to this range of pathogen populations. The pathogen populations from Jodhpur (India) and Bamako (Mali, Africa) were observed to be the most highly virulent among the eight pathogen populations used in this study. Spearman rank correlations revealed a general

trend of stronger similarities among pathogen populations from India (northern India > southern India) and between Indian and African populations than between the two African pathogen populations themselves. Cluster analysis demonstrated that virulence of the pathogen population from Jalna was dissimilar to that of all other pathogen populations used in this study and virulence of the pathogen population from Jamnagar was closely related with that from Durgapura, and that virulence of the pathogen population from Patancheru with that from New Delhi.

About 60% of polymorphic marker loci showed segregation distortion with higher than expected abundance of heterozygotes and homozygotes for alleles of the resistant male parent (ICMB 90111-P6). A skeleton genetic linkage map of 748 cM (Haldane) was constructed using Mapmaker/Exp ver. 3.0. Simple interval mapping (MapMaker/QTL) and composite interval mapping (PlabQTL) were used to detect QTLs. Of nine major putative DMR QTLs detected, one mapping on LG4 was common for all pathogen populations except that from Bamako, Mali. A majority of the DMR QTLs detected exhibited over-dominant inheritance, and the more resistant alleles were nearly all from resistant parent ICMB 90111-P6. At least one DMR QTL was detected and mapped for each of the eight pathogen populations used. Marker-assisted selection (MAS) and backcrossing can now be used for improving DMR of elite pearl millet hybrid parental line ICMB 89111, as putative QTLs controlling resistance against a diverse range of DM pathogen populations have been identified, along with polymorphic flanking markers that can be used to expedite transfer of these resistance QTLs from donor parent ICMB 90111-P6.

HHB 94-like hybrids showed highly significant differences among genotypes (HHB 94-like hybrids), environments and genotype × environment interactions for grain, and stover yield and most of their component traits across all ten field test environments and across three multiple-field test environment clusters in Haryana, Rajasthan and Andhra Pradesh. Environments represented the major source of variation followed by genotypes and genotype × environment interactions. The HHB 94-like hybrids changed the ranks for entry mean performances for most traits in the different individual test environments. Test hybrids based on sub-selections of ICMA 89111 exhibited superior performance of most of the grain and stover yield components compared to those based on sub-selections of ICMB 89111 and ICMB 90111, and their mean performance was better in the Andhra Pradesh multiple-field test environment cluster among the three clusters. Grain yield showed strong positive correlation with effective tiller number, panicle yield, 1000-grain mass, panicle grain number, and harvest index; but negative correlations with time to 50% flowering and plant height. Heritability estimates for a majority of traits ranged from high to very high across individual as well as across clusters of field trial environments.

These results indicated that ICMB 90111 has genes for high yield potential and QTLs for DMR that could be used to improve HHB 94. Use of ICMB 90111 as a parent in hybrids breeding program and as a parent in developing breeding populations based on its cross with ICMB 89111, could enhance the possibility of improving HHB 94 by marker-assisted selection to introgress QTLs for both DMR and yield from ICMB 90111 into the background of ICMB 89111 and its male-sterile counterpart ICMA 89111, which is the seed parent of the original HHB 94.

Surinder Kumar Gulia	(R.S. Sangwan)	Head of Department
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