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LINKAGE MAP CONSTRUCTION AND IDENTIFICATION OF QTLs FOR DOWNY MILDEW (*Sclerospora graminicola*) RESISTANCE IN PEARL MILLET (*Pennisetum glaucum* (L.) R. Br.)

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Thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Agriculture) in Plant Breeding and Genetics to the Tamil Nadu Agricultural University, Madurai

DEPARTMENT OF AGRICULTURAL BOTANY
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2001

Certificate

This is to certify that the thesis entitled "Linkage map construction and identification of QTLs for downy mildew (*Sclerospora graminicola*) resistance in Pearl millet (*Pennisetum glaucum* (L.) R. Br.)" submitted in part fulfillment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY** (Agriculture) in **Plant Breeding and Genetics** to the Tamil Nadu Agricultural University, Coimbatore is a record of bona fide research work carried out by **Mr. P. AZHAGUVEL,** ID Number 98-810-011 under my supervision and the guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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DEDICATED TO

World's Semi-Arid poorest among poor farmers.

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ABSTRACT

LINKAGE MAP CONSTRUCTION AND IDENTIFICATION OF QTLs FOR DOWNY MILDEW (*Sclerospora graminicola*) RESISTANCE IN PEARL MILLET (*Pennisetum glaucum* (L.) R. Br.)

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Pearl millet (*Pennisetum glaucum*) is the sixth important cereal crop. It is the staple food crop for poor farmers in the semi-arid tropical regions of India and West Africa. The worst disease of pearl millet is downy mildew caused by a pseudo-fungal pathogen (*Sclerospora graminicola*). The genetics of this host-pathogen system is poorly understood. The study of resistance has been hindered by both the host and the pathogen being outbreeding and highly variable and the segregation for host-plant resistance showing continuous variation. Earlier reports suggested that resistance to downy mildew is a quantitative trait controlled by polygenes. In this study a new pearl millet mapping population was developed from a resistant x susceptible cross. A genetic linkage map with a length of 561.8 cM was constructed using RFLP markers along with three morphological markers: d_1 and d_2 dwarf plant height, and *P* purple foliage colour. The downy mildew segregation pattern of F_2:F_4 progenies from cross (IP 18293 x Tift 238D1) was studied against six Indian (Patancheru, Jalna, Jamnagar, Jodhpur, Durgapura and New Delhi) and two African pathogen populations (Niger and Mali). Pathogen population variability with reference to the resistance behaviour among the

segregating families was studied and the best fit Mendelian segregation ratios were discussed. QTL mapping was performed using interval mapping methods (Mapmaker/QTL) and composite interval mapping methods (QTL Cartographer). Seven different host-plant resistance QTLs are identified against these eight pathogen populations. There was a common resistance QTL for the Indian pathogen populations from Patancheru, Jodhpur, Jalna and Jamnagar on linkage group 2. Over-dominance of resistance was the inheritance pattern most commonly observed in the different screens. All of the identified resistance QTLs were from the resistant parental line IP 18293. Strategies for utilizing the identified resistance QTLs for improving downy mildew resistance of elite pearl millet hybrid parental lines were discussed.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

1. INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is widely grown as a food crop of subsistence agriculture in sub-Saharan Africa and the Indian subcontinent on a total of about 27 million ha (Rachie and Majmudar, 1980; FAO and ICRISAT, 1996) having average grain yield 500-600 kg ha^{-1}. In addition ca. 4 million hectares is grown mostly as a forage crop (Gates *et al.*, 1999) or a mulch component of minimum tillage systems (Bonamigo, 1999) in commercial agriculture on other continents. Pearl millet has a number of advantages that have made it the traditional staple cereal crop in subsistence or low-resource agriculture in hot semi-arid regions. These advantages include tolerance to drought, heat and leached acid sandy soils with very inherent fertility due to low clay and organic matter contents. Pearl millet also has the ability to grow rapidly in response to brief periods of favourable conditions and has one of the highest growth rates of all the cereals (Kassam and Kowal, 1975; Craufurd and Bidinger, 1989).

The pearl millet downy mildew pathogen [*Sclerospora graminicola* (Sacc.) J. Schröt.] is an obligate biotropic oomycete, which reproduces asexually by means of sporangia that germinate to release motile zoospores and sexually through soil-borne oospores. Although usually thought of as fungi, these motile life stages of oomycetes indicate that they are actually more closely related to the brown algae (Hess *et al.*, in press). The downy mildew disease of pearl millet develops after colonization of undifferentiated tissue, which results in symptoms resembling those of systemic virus diseases, with growth disturbance and chlorosis. The white mass of asexual sporangia that are produced on the surface of infected leaves superficially resemble powdery mildews of temperate cereals (which are caused by true fungi), and can spread rapidly through a crop (Singh and Williams, 1980). 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 Majmudar, 1980). Following the release and widespread adoption of genetically uniform pearl millet single-

cross hybrids 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). Due to its systemic nature and its ability to flourish under a wide range of environmental conditions, the disease can cause considerable losses in grain yield and is particularly destructive to genetically uniform single-cross F_1 hybrids of pearl millet. 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. However, resistance should be used as a component (albeit a major one) of an integrated disease management system. Breeding for resistance to diseases of current and potential economic importance contributes to increased 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. Resistance genes have been widely used in disease control for a hundred years or more and are very actively used in modern crop breeding (Crute and Pink, 1996; Agrios, 1997). They can be moved between plant varieties of the same species or related genera by conventional crossing procedures.

For any crop 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.*, 1981; Shinde *et al.*, 1984), or in some cases can be rather simply inherited (Deswal and Govila, 1994; Singh and Talukdar, 1998). 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. However, regional variability in the pathogen is now recognized as a major hindrance to effective resistance breeding. Genetic variability between populations of the downy mildew pathogen has required expensive, time-consuming multi-locational field trials to study the patterns of inheritance of resistance (Deswal and Govila, 1994), or some combination of field and greenhouse screening (Jones *et al.*, 1995). There is clear evidence of the existence of physiological specialization within *S. graminicola* (Ahmad *et al.*, 1978; ICRISAT, 1989; Jones *et al.*, 1995; Thakur and Rao 1997; Thakur *et al.*, 1992, 1998). High heterogeneity in pathogen populations has been observed between seasons at individual locations and among single-oospore isolates of *S. graminicola* in India (Thakur *et al.*, 1992). At least six populations of this pathogen with distinct virulence differences have now been identified in India (Thakur *et al.*, 1998).

A potential revolution in the understanding of, and ability to manipulate, oligogenic and quantitative traits is offered to plant breeders by recent advances in genetic marker technology (Young, 1999). Molecular biology can be used to guide traditional plant breeding. Without the introduction of transgenic DNA, it offers an incredible diversity of opportunities for improvement of disease resistance in plants. DNA sequences that match specific chromosomal loci can be used to detect restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD) microsatellite polymorphisms, amplified fragment length polymorphisms (AFLP) or other molecular genetic marker loci (Jones *et al.*, 1997; Mohan *et al.*, 1997; Prioul *et al.*, 1997). Once linkage between a marker locus and the gene for an agronomic trait of interest has been established, DNA diagnostic tests can be used to guide plant breeding as a substitute for other phenotypic tests such as assays for disease resistance. Untapped natural variation for agronomically desirable traits is present in existing sexually compatible breeding material and molecular genetic tools such as quantitative trait locus analysis and guided introgression can aid tremendously in harnessing this genetic potential. The development and availability of abundant, naturally occurring, molecular genetic markers (RFLP, RAPD, isozymes, etc) during last two decades has generated renewed interest in counting, locating and measuring the effects of genes (polygenes or QTLs) controlling quantitative traits.

The main advantages of using molecular markers for the introgression of resistance genes into cultivars a gain in time (Tanksley *et al.*, 1989; Melchinger, 1990). By applying new analytical methods, the genetic dissection of quantitative inheritance of physiological, morphological and behavioral traits in any plant or animal species is now possible. 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). 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). Pyramiding of the resistance genes in a breeding program could be very well possible using molecular-marker-based genotyping, when the phenotypes are epistatic. Without molecular marker-based methods, genotyping individual plants for their resistance gene complement requires two generations of testcrosses and screening against multiple pathogen isolates. Thus resistance gene pyramiding becomes much more practical once marker-assisted selection methods for an individual resistance gene is possible.

Morphological variants with distinct phenotypic expression are being used to establish linkage studies. In pearl millet such variations were observed in height and colour. Purple pigmentation in different plant parts like nodes, internodes, leaf blades, anthers and glumes is observed in pearl millet. Five sources of genetic dwarfism are known in pearl millet (Burton and Fortson, 1966; Koduru and Krishna Rao, 1983; Appa Rao *et al.*, 1986; Anand Kumar and Andrews, 1993). Converting tall pearl millet varieties to dwarf by utilizing these dwarfing genes enabled breeders to develop lodging resistant grain and forage cultivars, and facilitated hybrid seed multiplication.

The current study was planned with the following objectives:

Objectives:

I. To construct a skeleton linkage map in a pearl millet mapping population of a downy mildew resistant × downy mildew susceptible cross

II. To determine the inheritance of downy mildew resistance segregating in this mapping population in screens against Indian and African pathogen populations of *Sclerospora graminicola*

III. To identify QTLs controlling downy mildew resistance effective against these Indian and African pathogen populations

IV. To locate the dwarfing genes d_1 and d_2, and the purple foliage colour gene *P*, on the molecular marker-based skeleton linkage map of pearl millet

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 10 million ha in India, producing annually 10.5 and 4.5 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, 1999). To keep pace with the food demand of growing human and livestock populations in the semi-arid regions, increasing the productivity of pearl millet is a gigantic task requiring concerted efforts from national and international research and development organizations including both their public and private sectors (Rai and Anand Kumar, 1994).

2.1.1. AS A FOOD GRAIN

Pearl millet grain has a high nutritional value and a higher protein content than maize and sorghum (Maiti and Bidinger, 1981). The amino acid profile of pearl millet grain is better than that of normal sorghum and 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, that reduce protein availability. As with other cereals, the phytic and nicotinic acids contained in pearl millet grain are found mainly 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). Rats fed 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 weanling food (Klopfenstein *et al.*, 1985). Autoclaving the grain appears to alleviate the symptoms.

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).

2.1.2. AS A FEED GRAIN AND FODDER CROP

In Tamil Nadu the demand for pearl millet as a food grain has been sharply diminishing, whereas demand for it as a raw material in the poultry and animal feed industry and to a lesser extent in the food-processing industry has increased over the years (Ramasamy *et al.*, 2000).

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 stages (Tabosa *et al*., 1999).

Hanna *et al.* (1991) suggested that pearl millet has a good potential to be used as a high quality grain like corn and sorghum in rations of chickens, beef cattle and swine. Costa (1992) reported that pearl millet has demonstrated great potential as forage, 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. 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.

Studies conducted by several workers (French, 1948; Singh and Barsaul, 1976; Sharma *et al.*, 1979; Stringhini and Franca, 1999) showed that millets compared favourably with maize in poultry diets. Fancher *et al.* (1987) reported that the metabolizable energy (ME_n) content of ground pearl millet grain varied from 2.9 to 3.2 kcal g^{-1} dry matter. Lloyd (1964) observed that broilers fed on pearl millet rations were heavier and had better feed conversion than those fed on maize rations.

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 pearl millet consistently provided higher quality pasture than permanent pastures.

Collins *et al.* (1997) noted that 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 favourable to human health. Gelaye *et al.* (1997) found that pearl millet increased the neutral detergent fiber content in the rations, with an increase of lignin and cellulose concentrations in the diets, but the levels of calcium, phosphorous and crude protein were also higher than those of maize.

In studies with fish, Silva *et al.* (1995) tested the use of whole-grain pearl millet for feeding *tambaqui* and carp fish species in a study lasting two production cycles, and concluded that supplementary feeding with pearl millet grains resulted in a 26% increase in fish production compared to full grain maize.

2.1.3. As a crop for harsh areas

Pearl millet is a hot climate plant that is xerophilous and has efficient drought escape and tolerance mechanisms. Pearl millet area covers 26 million hectares in sub-Saharan Africa and South Asia, where low input farming is the main activity for the approximately 400 million people living in these regions (Tabosa *et al.*, 1999). It is almost the only cereal crop that can be grown in parts of tropical and subtropical Asia and Africa with an 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 exceptional ability to tolerate drought, pearl millet extends food grain production into regions too arid for sorghum (Burton, 1983). It tolerates drought, low soil fertility, and low soil pH and responds well to water and good management (Anand Kumar, 1989).

Among all the cereals, pearl millet grows the best in sandy soils and under poor fertility conditions and has the greatest drought tolerance (Maciel and Tabosa, 1982). Its better adaptation than other cereals to low-fertility soils, based on its superior ability to extract nutrients due to a deep fibrous root system, ultimately endows it with good 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 IN MORE INTENSIVELY FARMED AREAS

Saturnino and Landers (1997) advocated zero tillage seeding of pearl millet following the soybean crop in Brazil, 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). Lower 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 genetical studies. It has a small diploid genome with a haploid DNA content of 2.36 to 2.5 pg (Bennett, 1976; Martel *et al.*, 1997), with a small number (seven) of large chromosome pairs with two distinctive nucleolar organizers, making it a highly suitable organism for genetical studies (Jauhar and Hanna, 1998). 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 for studying domestication and crop/wild complex evolution (Poncet *et al.*, 1998, 2000).

2. 2. DWARFING GENES and PURPLE COLORATION

2.2.1. DWARFING GENES

Dwarfing genes have been successfully utilized in the breeding of short-statured cultivars of wheat, rice, barley, sorghum and pearl millet with improved lodging resistance Dwarf grain cultivars are better able than their counterparts to respond positively to high levels of nitrogen application due to their reduced lodging vulnerability Therefore, under well-managed conditions dwarf cereal genotypes will frequently yield more harvestable grain than their tall counterparts because they are less vulnerable to lodging under conditions of high soil fertility and ample moisture availability Dwarf cereals also tend to have an improved grain harvest index, better leaf-to-stem ratios and easier manual and mechanical harvesting characteristics than their tall counterparts Dwarf plants in pearl millet were discovered almost simultaneously in the USA and India Burton and Fortson (1966) reported the inheritance of reduced plant height from five different sources, named D_1 to D_5, in pearl millet Dwarfness in source lines D_1 and D_2 is controlled largely by one or two recessive genes When transferred to near-isogenic backgrounds, dwarfness in D_1 and D_2 is controlled by single but different recessive genes, designated as d_1 and d_2 The d_2 dwarf gene in pearl millet has several pleiotropic effects on plant phenotype Principally, it reduces plant height by 50% through a reduction in the length of all stem internodes except the peduncle (Burton and Fortson, 1966), leading to a higher proportion of leaves (Rai and Hanna, 1990) Based on the composition and the digestibility of dwarf pearl millet forage, Johnson *et al* (1968) concluded that the d_2 gene could be used in improving the nutritive value of pearl millet forage

Forage from d_2 dwarf plants had significantly higher IVDMD (*in vitro* dry matter digestibility) than forage from tall plants (Hanna *et al* , 1979) A similar response reported previously (Burton *et al* , 1969) for the *d* gene was attributed to increased leaf percentage and improved nutritional quality of the stem fraction of pearl millet forage

2.2.2. PURPLE COLORATION

Pigmented plant parts constitute easily recognizable morphological genetic markers for use in several aspects of studies on higher plants Because of this distinct advantage, genetics of pigmentation has been worked out in several model plant systems like maize, rice, and *Petunia* The purple coloration is due to three

anthocyanidins—cyanidins, delphinidin and pelargonidin (Raju *et al*, 1985) Inheritance of purple pigmentation of the coleoptilar leaf in pearl millet was reported to be controlled by a single dominant gene (Yadav, 1976) Koduru and Krishna Rao (1979) reported that purple seedling base, which is recognizable from the 1-leaf or 2-leaf stage, is controlled by the complementary interaction of two dominant genes Pb_1 and Pb_2 Gill (1969) reported that purple foliage pigmentation is controlled by a single dominant gene *Rp* whereas Minocha *et al* (1980) reported that it is controlled by two complementary genes Appa Rao *et al* (1988) concluded that purple pigmentation on all plant parts is controlled by a single dominant gene They observed that purple coloration of leaf sheath, leaf blades, internodes, bristles, and glumes are inherited as a single unit indicating pleiotropic effect of a single gene They proposed the symbol *P* for this trait Mode of inheritance and linkage relationships of genes responsible for purple coloration of seven plant parts in pearl millet was discussed by Manga *et al* (1988) They reported linkage between genes for purple node, auricle and purple leaf midrib, margin and leaf sheath, purple internode and purple apicule

Singh *et al* (1967) reported that the purple colour of lemma, palea and bristles (scored together) is a monogenic dominant trait Gill (1969) reported that purple bristling (Bep_1 and Bep_2) and purple glume (Pg_1 and Pg_2) traits are controlled by two sets of dominant complementary genes

2. 3. PEARL MILLET DOWNY MILDEW AND ITS IMPORTANCE

2.3.1. THE HISTORY

The millet downy mildew pathogen was first described as *Protomyces graminicola* on *Setaria verticillata* by Saccardo in 1876 Schroter in 1879 renamed it as *Sclerospora graminicola* (Ullstrup, 1973) Downy mildew was first reported on *Setaria viridis* (L) P Beauv by Farlaw (1884), and later reported on pearl millet and other hosts (Bhat, 1973) This disease is of great economic importance in India but also causes 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 *Sclerospora graminicola* (Sacc) J Schrot] is a highly destructive and widespread 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 has been the disease downy mildew caused by an oomycetic pseudo-fungus Downy mildew is the most devastating disease of pearl millet in India and a major epidemic occurred there in the early 1970s 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)

2.3.2. Pearl millet Downy mildew resistance inheritance studies

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) 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

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 goodness of fit test of F segregation data to a 3 1 ratio Among 1165 segregating inbred lines the proportion of disease free plants was far more than that of diseased plants

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 inheritance pattern was rather complex and could involve genic interactions Gene symbols *DM1DM2 DM1dm2* and *dm1DM2* were proposed for the resistant genotypes and *dm1dm2* for the susceptible genotypes by Gill *et al* (1978) The F_2 and the testcross data revealed the presence of duplicate dominant factors in the inheritance of resistance to this disease in ten crosses Singh *et al* (1978) using inbreds and a sick plot observed resistant and susceptible reactions in the F_1 and F_2 generations of crosses between resistant and susceptibility pearl millet inbreds In the

F_2, the frequency of resistant plants was higher in R × R crosses than in other crosses such as R × S and S × S suggesting polygenic inheritance

The inheritance of resistance to downy mildew in pearl millet was studied by Basavaraju *et al* (1981) 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 cross IP 18292 × Tift 23DB for inheritance of downy mildew resistance Resistance was controlled by a single dominant gene from IP 18292 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 With the availability of precise inoculation techniques, and highly homozygous resistant and susceptible parental lines, more precise information on the genetics of resistance will soon become available (Singh, 1995)

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) 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, 1999, Witcombe and Hash, 2000)

2.3.3. Downy mildew – Screening

The life cycle of *Sclerospora graminicola* (Sacc) J Schrot is comprised of both sexual and asexual phases The sexual stage produces oospores, which are soil or seed borne and 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 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 oospore-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 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 cultivars three weeks before sowing test material

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 given 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 sporangia mL^{-1})

Singh *et al* (1997) explained all screening techniques available for this disease including dip inoculation spray inoculation drop inoculation injection inoculation settling 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)

2.3.4. 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 Ahmed (1981) reported two races for Gulberga and Mysore 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

Ball and Pike (1983) showed that host cultivars responded differently 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)

A number of downy mildew resistant pearl millet inbred lines have been developed by backcrossing programs, but the effectiveness of resistance in these may 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 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 basis 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

2.4. MOLECULAR MARKER 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 DNA base sequence Thus, there is a tremendous amount of DNA variation present in natural populations of plants These variations have been detected in restricted (ie , enzymatically digested) genomic DNA of plants and 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 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 screen for traits that are extremely difficult, expensive, or time consuming to score phenotypically

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 simultaneous, 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 connection of phenotypic characters with the genomic loci responsible for them However, the real advantage of using molecular markers is to permit backcross transfer 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 marker maps (Jones *et al* , 1997)

The following is a list of DNA marker techniques that have been developed over the years (Mohan *et al* 1997 Gupta and Varshney 2000)

Acronym	Technique	Reference
AFLP	Amplified Fragment Length Polymorphism	Vos *et al* 1995
ALP	Amplicon Length Polymorphism	Ghareyazie *et al* 1995
AP-PCR	Arbitrarily Primed PCR	Welsh and McClelland 1990
AS-PCR	Allele Specific PCR	Sarkar *et al* 1990
CAPS	Cleaved Amplified Polymorphic Sequence	Lyamichev *et al* 1993
DAF	DNA Amplification Fingerprinting	Caetano Anolles *et al* 1991
IMP	Inter MITE (Miniature Inverted-repeat Transposable Elements) Polymorphism	Chang *et al* 2001
ISA=ISSR	Inter SSR Amplification = Inter Simple Sequence Repeat	Zietkiewiez *et al* 1994
MP-PCR	Microsatellite Primed PCR	Meyer *et al* 1993
RAMS	Randomly Amplified Microsatellite	Ender *et al* 1996
RAPD	Random Amplified Polymorphic DNA	Williams *et al* 1990
REMAP	Retrotransposon Microsatellite Amplified Polymorphism	Kalendar *et al* 1999
RFLP	Restriction Fragment Length Polymorphism	Botstein *et al* 1980
SAP	Specific Amplicon Polymorphism	Williams *et al* 1991
SCAR	Sequence Characterized Amplified Region	Williams *et al* 1991
SNP	Single Nucleotide Polymorphism	Nikiforov *et al* 1994
SSCP	Single Strand Conformation Polymorphism	Orita *et al* 1989
SSLP	Microsatellite Simple Seque ce Length Polymorphism	Rongwen *et al* 1995
SSLP	Minisatellite Simple Sequence Length Polymorphism	Jarman and Wells 1989
SSR	Simple Sequence Repeat	Hearne *et al* 1992
STMS	Sequence Tagged Microsatellite Sites	Beckmann and Soller 1990
STS	Sequence Tagged Site	Fukuoka *et al* 1994

2.5. 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; Helentjaris, 1987; Paterson *et al.*, 1988 and Weber and Helentjaris, 1989). RFLP is the most reliable DNA polymorphism that can be used for accurate scoring of genotypes. It has provided a relatively rapid 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). Since RFLP markers have no known effect on the phenotype of the plant, they are ideal for studying quantitative traits (Stuber *et al.*, 1992).

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). In plants, RFLPs were first been used in maize, tomato and rice to saturate their already extensive genetic maps (Bernatzky and Tanksley, 1986; Helentjaris *et al.*, 1986a; McCouch *et al.*, 1988).

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 development 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 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 linkage maps indicate the location of specific restriction fragments of chromosomal DNA relative to one another. Ellis (1986) reported that simple consideration of RFLP mapping as a method of

analyzing the inheritance of quantitative characters suggests that there are several limitations to the utility of this approach.

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 (Mohan *et al.*, 1997).

Costs of applying RFLPs to genetic improvement were assessed by Beckmann and Soller (1983) in terms of individuals and number 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 resource 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 RFLP in plant breeding with particular reference to downy mildew resistance in pearl millet. A number of recent papers suggest that the use of RFLPs as markers offers a clear advantage in breeding for important qualitative and quantitative traits (Edwards *et al.*, 1987; Melchinger, 1990; Paterson *et al.*, 1991; Arunachalam and Chandrashekaran, 1993; Mohan *et al.*, 1997; Young, 1999).

2.6. 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 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 available, and the traits for which they might be used for QTL mapping.

2.6.1. DNA POLYMORPHISMS AMONG PARENTS

Sufficient detectable DNA sequence polymorphisms between parents must be present. This cannot be over-emphasized, for in the absence of detectable DNA polymorphism, segregation analysis and linkage mapping are impossible. Naturally outcrossing species tend to have high levels of DNA polymorphisms and virtually 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 variation are generally lower and finding suitable DNA polymorphisms 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 changes (Riedel *et al.*, 1990) provide better methods for uncovering polymorphisms within narrow-based crosses. Probes based on minisatellites (Dallas, 1988) or simple repeated tetranucleotide motifs (Weising *et al.*, 1989) can uncover polymorphism 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)

2.6.2. 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 true F_1 hybrids, 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 Beckman (1990) described 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 a permanent mapping resource (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 advantages 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.

2.7. LINKAGE MAPPING

Mapping is putting marker loci (and QTLs) in order, indicating the relative distances among them, and assigning them to their linkage group on the basis of their recombination values from all pair-wise and three-point combinations. The first 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.

2.7.1. THE BASIS

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), located genes governing quantitative characters—often in great detail—and taken the first steps towards 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 (Tanksley, 1993; Beckmann and Soller, 1986; Landry and Michelmore, 1987).

2.7.2. SUCCESS STORIES

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 plant species have been constructed (Helentjaris, 1987; McCouch *et al.*, 1988; Heun *et al.*, 1991; Tanksley, 1993; Mohan *et al.*, 1997)

A detailed map of lettuce was constructed by Landry *et al.* (1987) 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 *PstI* genomic library covering 1,389 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 of known function. 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.

2.7.3. 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 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 χ^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 "HyperGene" converts genotypic data into a "graphical genotype" (Young and Tanksley, 1989a,b), in this a 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 Joinmap (Stam 1993; Stam and Van Ooijen, 1995) analyses 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 Mapmanager, with different versions such as QTX, QTXP and QTX-Classic for Macintosh- and IBM compatible computers (Manly, 1993; Manly and Olsen, 1999), can be used to analyse the results of genetic mapping experiments using backcrosses or recombinant inbred lines.

2.7.4. GENETIC MAP OF 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 × ICMP 85410 and Tift $23D_2B_1$ × IP 18292) were employed. The total length of this map, which comprised seven linkage groups, was 303 cM and the average distance between loci was about 2 cM. The individual linkage groups (LG) varied from 90 cM for LG1 to only 30 cM for LG6 (Devos *et al.*, 1995). This pearl millet genetic linkage map was unusual among grass genomes in that it was particularly short, but this

BR 62865

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)

2.8. QUANTITATIVE TRAIT LOCI

2.8.1. THE BASIS

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 colour Everson and Schaller (1955) found morphological markers that flanked a chromosomal region affecting yield in barley (*Hordeum vulgare* L)

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 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

2.8.2. QTL MAPPING AND 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 by 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 *Pseudomonas solanacerarum* in tomato was reported by Danesh *et al* (1994) using DNA marker genotypes and disease

resistance 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 in disease resistance have been mapped in tomato for resistance against insects (Nieuhuis *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), and in maize for northern corn leaf blight (Freymark *et al.*, 1993) and stalk and ear rot (Pê *et al.*, 1993).

Manzanares-Daulex *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 were localized for the leaf rust (*Puccinia hordei*) resistance in barley, which explained 96.1% of the segregating genetic variation (Kicherer *et al.*, 2000). In sugar beet, four QTLs associated with *Cercospora* resistance on chromosomes III, IV, VII and IX were revealed using composite interval mapping (Setiawan *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).

2.8.3. 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; Geldermann, 1975; Hill, 1975; Jenson, 1989; Knapp *et al.*, 1990; Lander and Bostein, 1989; Soller and Beckmann, 1983, 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 markers bracketing a QTL, a procedure termed 'Interval Mapping' (Jensen, 1989; Knapp *et al.*, 1990; Lander and Botstein, 1989, Weller, 1987), 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 in a "straight forward" fashion 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.

Prioul *et al.* (1997) described the genetical 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 currently in use by 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 by Knapp *et al.* (1990) and Haley and Knott (1992) for QTL analysis using regression. 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 uses 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 differences.

Estimating the location and the size of the 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 a confidence interval 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 constructing 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.

Kearsay 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 the genotype 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.

2.8.4. 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 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, 1995; 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 software 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 resolution 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/)*. A similar composite interval mapping method has been developed by Jansen and co-workers (Jansen, 1993; Jansen and Stam, 1994) called multiple QTL modeling (MQM).

Multimapper (Sillanpaa and Arjas, 1998), 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 and Liu and Knapp, 1992).

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

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

2.8.5. Selective genotyping and QTL reliability

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 detecting QTL, 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 the 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 per cent error rates in genotyping can double estimates of genetic map distance (Brzustowicz *et al.*, 1993). The quality of the 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).

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 mapping population size 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 spacings 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), seed hardness (Keim *et al* , 1990), seed size (Fatokun *et al* , 1992), maturity and plant height (Lin *et al* 1995) disease resistance (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) 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

2.8.6. 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 RFLP maps exist, a skeleton map needs to be transferred. In pearl millet less than 40 single-copy probe-enzyme combinations will produce such a map, with an average map distance of less than 15 cM between marker loci (Liu *et al.*, 1994).

Howarth *et al.* (unpublished) identified QTLs for downy mildew resistance and seedling heat tolerance from pearl millet mapping populations produced from crosses ICMP 451 × H77/833-2 and H 77/833-2 × PRLT 2/89-33. Hash *et al.* (unpublished) worked with mapping populations from crosses PT 732B × P 1449-2, 81B × ICMP 451 and 841B × 863B to locate QTLs for resistance to pearl millet downy mildew. QTLs for host-plant resistance effective against downy mildew African and Indian pathogen populations were identified in a new mapping population based on cross W 504 × P 310 (Maria Kolesnikova-Allen, unpublished).

Materials and Methods

3. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. MAPPING POPULATION PARENTAL LINES

Two pearl millet inbred lines, IP 18293 and Tift 238D1, were used as parental lines. The bleached-leaf mutant IP 18293 was isolated from a segregating population of a cross between IP 10399 (India) × IP 10729 (Sudan). Bleached-leaf colour distinguishable ten days after emergence, is controlled by a single recessive gene b_1 (Appa Rao *et al.*, 1990). IP 18293 is a highly downy mildew resistant, *P* purple foliaged, d_2 dwarf genetic stock developed by the Genetic Resources Division, ICRISAT, Patancheru, India (Appa Rao *et al.*, 1996). Tift 238D1 is a downy mildew susceptible, green foliaged, d_1 dwarf line, reported to carry a single dominant gene for fertility restoration in the A_1 cytoplasmic-genetic male sterility system, that was developed at the Coastal Plain Experiment Station, Tifton, GA, USA (Burton, 1966) (Fig. 1).

Parental lines have been selfed continuously for more than five generations to ensure homozygosity at essentially all loci.

3.1.2. DOWNY MILDEW PATHOGEN POPULATIONS

Indian Isolates

1. ICRISAT, Patancheru Sg153
2. MAHYCO, Jalna Sg150
3. GAU, MRS Jamnagar Sg140
4. CAZRI, Jodhpur Sg139
5. RAU, ARS Durgapura, Jaipur Sg151
6. IARI, New Delhi Sg201

African Isolates

1. Sadore, Niger
2. Bamako, Mali

3.2. PATHOGEN INOCULUM

3.2.1. COLLECTION

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 and every 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, spores were removed from the leaves and the spore suspension was made with de-ionized water and collecting the run-off

3.2.2. MAINTENANCE

A wide diversity of populations of this pathogen have been identified from India and samples of these are being maintained at ICRISAT, Patancheru The pathogen populations were 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 (>80% infection under heavy inoculum pressure) The susceptible plants were 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 the sporangial suspensions particularly in the vicinity of the seedling growing points The pots were then covered with polythene bags and incubated at 20 C to promote infection After 12 hours, the bags were removed and pots were maintained at 20-25 C on benches in the greenhouse

One day before the actual inoculation of the screening materials, leaves from two-three month-old infected plants were collected in the late afternoon, cleaned of old sporangia and incubated overnight at 20 C in a covered box lined with moist blotting paper Resulting sporangia were collected into ice cold water (below 2 C) and the concentration of the spore suspension adjusted to approximately 1 5 10^5 sporangia mL^{-1}

FIGURE 1. PARENTAL LINES FOR THE NEW MAPPING POPULATION BASED ON CROSS IP 18293 X TIFT 238D1

IP 18293, downy mildew resistant, purple foliaged line, with d_2 dwarfing gene; developed at the Genetic Resources Unit, ICRISAT, Patancheru, India

Tift 238D1, downy mildew susceptible, green foliaged line with d_1 dwarfing gene; developed at Tifton, Georgia, USA

3.3. SCREENING MAPPING POPULATION PROGENIES

3.3.1. SCREENING AT ICRISAT, PATANCHERU

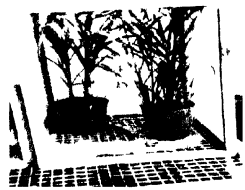
A mapping population (from IP 18293 × Tift 238D1) of 142 F_2F_4 entries along with their two parental lines and control entries such as 7042(S), 7042(R) = ICML 22 (Singh *et al*., 1994), 700651 = ICML 16, and HB 3, was evaluated under greenhouse conditions during *kharif* 2000, at ICRISAT, Patancheru 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 to thirty-five seeds were sown at a uniform distance on a well-leveled soil surface and covered with a 2 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 4-5 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 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 The same inoculation procedures were followed with three time replications for each of the six pathogen populations (a total of 18 different screens) 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 15 days after inoculation (Fig 2)

3.3.2. SCREENING AT BANGOR, UK (Wendy A Breese, pers comm)

Seeds of two parental lines appropriate control entries, and each of 142 skeleton-mapped F_2F_4 pearl millet progenies from the (IP 18293 · Tift 238D1)-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 and each screen had a variable number of replicates For each pathogen population a single inoculation date with three (Niger) or four (Mali) pots per entry was used, with pots arranged in a randomized complete block design prior to inoculation Pots were placed

FIGURE 2. SCREENING PROCEDURE FOLLOWED TO EVALUATE DISEASE REACTIONS OF SEGREGATING F_2-F_4 PROGENIES OF THE (IP 18293 × TIFT 238D1)-BASED POPULATION AGAINST A RANGE OF DOWNY MILDEW PATHOGEN POPULATIONS

Maintenance of inoculum separately for each pathogen population



Harvest sporangia

Sowing of F_{2-4} progeny seeds for screening

Optimal stage for spraying inoculum and counting total number of seedlings

Post-infection counting diseased plants 15 days after inoculation

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

Post-inoculation incubation cover under polythene sheet at 20^0C for 16 h.

on flood benching in a controlled environment greenhouse providing a 16 hour daylength (0600-2200 h) with a light intensity of between 500 and 1200 μE 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 lines with moist laboratory roll for 8 h at 20 C in the dark The resulting sporangia were collected into chilled (below 2 C) distilled water and the 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 The pots were covered with a polythene sheet to maintain a high level of humidity and incubated in the greenhouse at 20 C for 15 hours 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

3.3.3. Mapping population-Generation advancement

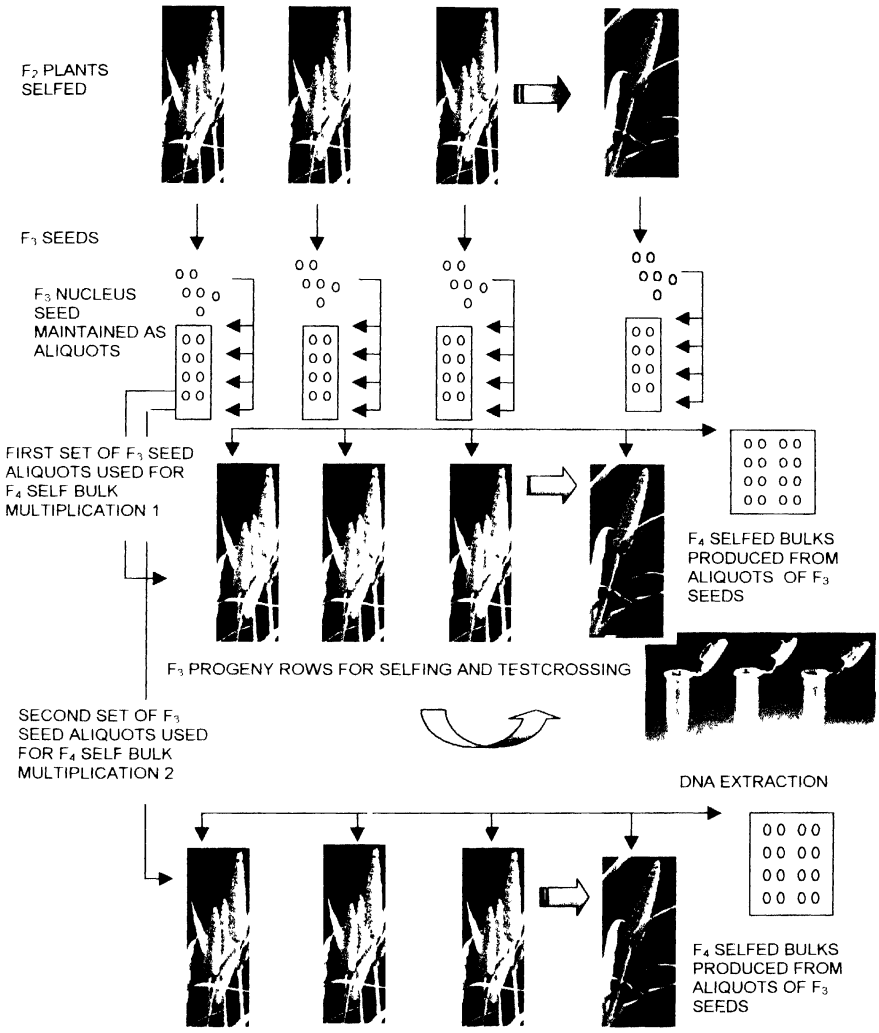
A mapping population was produced at ICRISAT, Patancheru, from the cross IP 18293 Tift 238D1 by selfing ten F_1 plants derived from a plant · plant cross The single F_1 plant that produced the largest number of selfed seed was selected to provide F_2 seeds from which to produce the mapping population F_2 plants were raised with their parental lines and green-foliaged, d_2 dwarf, A_1 cytoplasmic male-sterile line 81A (Anand Kumar *et al* , 1984) at the Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai during summer season of 1999 (February sowing) Each of the individual F_2 plants was selfed to produce F_3 seed Single-plant testcrosses were also made on to 81A using the individual F_2 plants as male parents Among the segregating F_2 plants, purple-foliaged individuals were selected, and pollen collected from these was dusted on to the stigma of d_1 dwarf, green-foliaged parental line Tift 238D1 Observations were recorded on the segregating F_2 plants for traits like plant height (both quantitative and qualitative), purple colour variations and panicle length

Approximately 30 selfed seeds harvested from each of 142 individual F_2 plants were sown in pots (one pot per F_2 plant) to produce unselected seedlings for transplanting and the resulting plants raised under field conditions as F_2F_3 (F_2 derived F_3) progenies in plot RL 18, at ICRISAT, Patancheru, during the late *kharif* season of 1999 (August sowing) Before the actual transplanting of the F_3 plants, bulk leaf samples were collected from all F_3 seedlings in the progeny of an individual F_2 plant for use in DNA extraction The remaining F_3 seed is being maintained as a nucleus stock for use in further multiplication of F_4 self bulk (F_2F_4) seed (Fig 3) as described by Hash and Witcombe (1994) In the progeny rows, observations were recorded for plant height (both quantitative and qualitative) uniformity in foliage colour, uniformity in height, and downy mildew incidence (affected plants and green ears) Testcrosses were made on male-sterile line 81A using bulk pollen collected from all of the F_3 individual plants in a progeny row

3.3.4. TESTCROSSES EVALUATION

Testcross trials were conducted at ICRISAT, Patancheru (field numbers RP 11C and RP 8A) Testcrosses were sown along with the parental lines and 81A in a single replication augmented design trial Testcross Trial I, including the crosses of 81A all segregating F_2 plants and Tift 238D1 - purple-foliaged F_2 plants, was evaluated during the *rabi* season of 1999-2000 (January sowing) in field RP 11C Testcross Trial II of crosses produced on green-foliaged *d*, dwarf male-sterile line 81A using bulk pollen from the F_2 F_3 progeny rows, were evaluated in the *kharif* season of 2000 (June sowing) in field RP 8A Each entry was sown as a single row with 75 cm distance between ridges, thinning was done 2-3 weeks after sowing to maintain a plant-to-plant distance of approximately 15 cm Basal dose of DAP (100 kg/ha) was applied and the top dressing of urea (100 kg/ha) was done 30 days after sowing Observations were recorded in both trials on traits such as plant height (qualitative and quantitative), leaf colour, uniformity, and pollen fertility/sterility reaction For taking fertility percentage, at least 10 plants were selfed to assess the selfed seed set Data from these three morphological traits were used to score individual F_2 plants as heterozygous or homozygous for alleles of one of the two parental lines and these scores used as marker loci in genetic linkage map development

FIGURE 3. PROCEDURE FOLLOWED FOR THE MULTIPLICATION OF SEED USED IN PHENOTYPING FOR QTL ANALYSIS OF A PEARL MILLET MAPPING POPULATION. TISSUE SAMPLES FOR DNA EXTRACTION WERE COLLECTED FROM BULK OF F_3 SEEDLINGS PRODUCED FROM INDIVIDUAL F_2 PLANTS DERIVED FROM A SINGLE F_1



3.4. 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 given by Sharp *et al* (1988) was used for pearl millet genomic DNA isolation in this study Dark-grown, young seedlings or soft, non-green, stem internode tissues are generally used to isolate genomic DNA as they yield better 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

3.4.1. GRINDING

Normally two types of methods are used to grind the leaf tissues The process of DNA isolation must be able to lyse the cell wall and cell membrane and release the DNA into an extraction buffer having SDS (sodium dodecyl sulphate) EDTA (ethylene diamine tetra acetic acid) and proteinase K This is followed by differential centrifugation to isolate genomic DNA from cell debris, precipitation of SDS-protein-carbohydrate complexes with a acetate-isopropanol solution followed by phenol and phenol-chloroform extractions, and a second precipitation of DNA with absolute alcohol

3.4.1.1. METHOD I

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

3.4.1.2. METHOD II

A small coffee grinder can serve the purpose and will be very much useful, particularly when preparing tissue samples for DNA to be extracted from a population of more than 150 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 are then kept at 4 C overnight to permit evaporation/sublimation of any remaining dry ice

In this present study, method II was followed DNA was isolated from 5 grams of etiolated F_1 seedlings produced from selfed seed of a given F_2 plant, 10-14 days after emergence These seedlings were quick-frozen in liquid nitrogen and ground to a fine

powder using a coffee grinder with dry ice The ground tissue was transferred to a 50 mL plastic centrifuge tube and kept overnight to allow evaporation/sublimation of any remaining dry ice The following morning, 20 mL of extraction buffer containing 100 mM Tris-HCl pH 8 0, 50 mM EDTA pH 8 0, 100 mM NaCL, 2% SDS was added to the centrifuge tube The tubes were then incubated at 65 C in a water bath for 30 minutes, with occasional gentle inversion for proper mixing, before 50 µL of proteinase K (10 mg mL^{-1}) was added Samples were again mixed and incubated for an hour in a 55 C water bath Samples were then extracted with equal volumes of phenol-chloroform-isoamyl alcohol (25 24 1 v/v) mixture and the emulsion was separated by centrifugation at 5,000 rpm for 20 minutes at 4 C in a Sorvall HB7 rotor The upper aqueous phase was re-extracted with equal volumes of chloroform-isoamyl alcohol (24 1 v/v) and the emulsion separated by centrifugation at 5,000 rpm for 20 minutes at 4 C To the aqueous phase, an equal volume of isopropanol was added and gently mixed and the sample then incubated at -20 C for 30 minutes Precipitated DNA was spooled with a hook and dissolved in 2 mL of RNase-$T_{50}E_{10}$ (50 mM Tris-HCl pH 8 0 and 10 mM EDTA pH 8 0) buffer and incubated overnight at room temperature To inactivate RNase, samples were re-extracted with equal volumes of phenol-chloroform as described above To the aqueous phase, 2 5 volumes of ethanol was added and held at -20 C for 1 hour to precipitate DNA DNA was spooled and washed with 70% ethanol (twice) and pellets were air-dried The DNA samples were finally resuspended in appropriate volumes of $T_{10}E_1$ (10 mM Tris-HCl and 1 mM EDTA pH 8 0), according to the size of the individual pellets and stored at 4 C

3.4.2. DNA QUANTIFICATION AND PURITY CHECK

DNA was quantified based on 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 analysed in an 0 8% TAE-agarose gel to test its integrity as described by 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 formula was used

$$\text{DNA concentration (20 µg)} = \frac{\text{(No of gels to be made x 20)}}{\text{[(OD value (260 nm) x 50]/µl of DNA used for taking OD 260nm)}}$$

3.5. RESTRICTION ENZYME DIGESTION

For each mapped F_2 plant from the population twenty 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 carried out in a total volume of 30 µL and incubated overnight at 37 C The reaction was terminated by addition of 3 µL of loading buffer (25% sucrose 0 1% bromophenol-blue and 20 mM EDTA) in each 30 µL sample

3.6. 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™) 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 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 (Fig 4)

Figure 4. Image of an ethidium bromide stained 1.0% agarose gel separation of genomic DNA fragments digested with the restriction enzyme *Hind*III

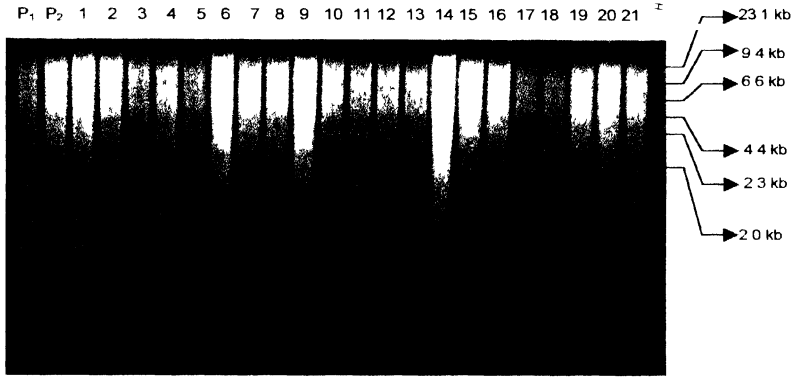
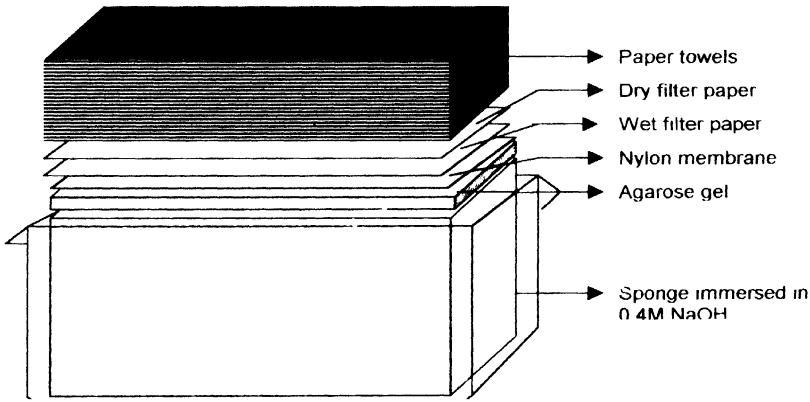


Figure 5. Southern blot technique to transfer the restricted DNA fragments from agarose gel to the nylon membrane



3.7. SOUTHERN BLOT HYBRIDIZATION

3.7.1. PREPARATION OF SOUTHERN BLOTS

DNA fragments separated electrophoretically after digestion were transferred from agarose gel on to nucleic acid nylon transfer membranes (Hybond-N$^+$ Amersham Pharmacia Biotech Ltd) following the procedure of Southern (1975) An exploded view of this procedure is given in Figure 5 The sponge was partially dipped in an alkali solution of 0 4 M NaOH This solution served as a denaturing agent and vehicle for a capillary transfer of DNA fragments As the alkali solution passes through the gel on its way to being 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 and wrapped with cling film and stored at -20 C for future use

3.7.2. PROBES USED

The majority of 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 100 clones identified as detecting single copy or low copy loci were used

3.7.2.1. *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 carbenicillin (10 mg mL^{-1}) to produce stab cultures (Liu *et al* 1994)

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

3.7.2.2. PLASMID DNA EXTRACTION

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 a speed of 6000 rpm 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. Again centrifugation was performed at 10,000 rpm for 10 minutes. To the supernatant, 30 µL of RNAase (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 $T_{10}E_1$ and stored at –20°C.

3.7.2.3. 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 minigel 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 $T_{10}E_1$

3.7.2.4. 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 was used in a PCR reaction using M13 universal and M13 reversal primers The following recipe was used to make the PCR reaction

PCR components		PCR conditions	
Water	32 5 µL	94 C 1 minute	
10X PCR buffer	5 0 µL	40 C 1 minute	32 cycles
2 5 mM dNTP	2 0 µL	70 C 2 minutes	
Universal primer	1 0 µL	72 C 5 minutes	
Reversal primer	1 0 µL	4 C Storage	
Taq poymerase	0 5 µL		
Template DNA	5 0 µL		
Total	50 0 µL		

After amplification the amplified insert was purified using the DNA purification kit Geneclean II 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 (Fig 6)

3.7.3. 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 4 µL was denatured by heating at 95 C for 10 minutes, then quenched on ice for 5 minutes before the labeling reaction mixture was added and incubated at 37 C for 3 hours The reaction was terminated by adding 2 5 µL of 3 M NaOH to use in the hybridization step

Labeling reaction mixture 5 µL of oligo-labelling buffer (Amersham Pharmacia Biotech), 2 µL equimolar concentrations of dCTP, dGTP and dTTP, 2 µL (10 mg ml^{-1}) acetylated BSA, 5 µL of 50 µCi 32P-dCTP, and 2 units of Klenow enzyme

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

3.7.4. HYBRIDIZATION TO LABELED PROBE

3.7.4.1. PRE-HYBRIDIZATION

Southern blots were pre-hybridized at 65 C with 5 mL of pre-hybridization solution (3 mL of 5x HSB, 1 5 mL of denatured salmon sperm DNA, 1 5 mL of Denhardt's solution and sterile distilled water to 15 mL) for 6 hours in case of new blots and one hour for stripped blots Pre-hybridization was performed in a Techne Hybridizer (HB-1D)

3.7.4.2. HYBRIDIZATION

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

3.7.4.3. WASHING OF BLOTS

Following hybridization, the blots were washed using four changes of 50 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 liter 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 liter with distilled water) Membranes were air dried and enclosed in cling films

3.7.5. AUTORADIOGRAPHY

Autoradiography was conducted at -70 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 (Fig 7) 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 were photographed using Kodak 100 ASA color print films

3.8. SCORING RFLP BANDS AND GENOTYPING

The banding patterns obtained from RFLP procedure were scored as follows

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

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

H = Heterozygote carrying alleles from both a and b parental strains

C = Not a homozygote for allele a (ie either B or H)

D = Not a homozygote for allele b (ie either A or H)

- = Missing data for the individual at this locus

After scoring the individual progeny genotypic data were typed into a Microsoft® Excel spreadsheet in a format suitable for analysis by MapMaker (i e rows =genotype score at a given locus columns = individual F_2 plants from the mapping population)

3.9. PROBE-ENZYME DNA POLYMORPHISM BETWEEN PARENTS

To identify polymorphic combinations of probes and restriction enzymes initial screening of parental lines was carried out before the actual genotyping of individuals in the F_2 mapping population For this, the parental DNA from IP 18293 and Tift 238D1 was restricted with four endonuclease restriction enzymes and probed against the available

Figure 6. Image of an electrophoresed agarose gel of amplified and purified inserts of *Pst*I pearl millet probes. The 0.8% agarose gel was stained with ethidium bromide. Sizes of the relevant inserts:
1 = *Psm*108 (0.5 kb), 2 = *Psm*618 (1.2 kb), 3 = *Psm*464 (0.4 kb),
4 = *Psm*735 (1.6 kb), 5 = *Psm*087 (0.7 kb), 6 = *Psm*196 (2.2 kb),
7 = *Psm*214 (0.4 kb), 8 = *Psm*280 (1.1 kb), 9 = *Psm*515 (2.1 kb),
10 = *Psm*473 (0.7 kb), 11 = *Psm*425, (2.0 kb), 12 = *Psm*459 (0.9 kb),
13 = *Psm*458 (0.7 kb), 14 = *Psm*321 (1.0 kb) and 15 = *Psm*718 (0.7 kb).

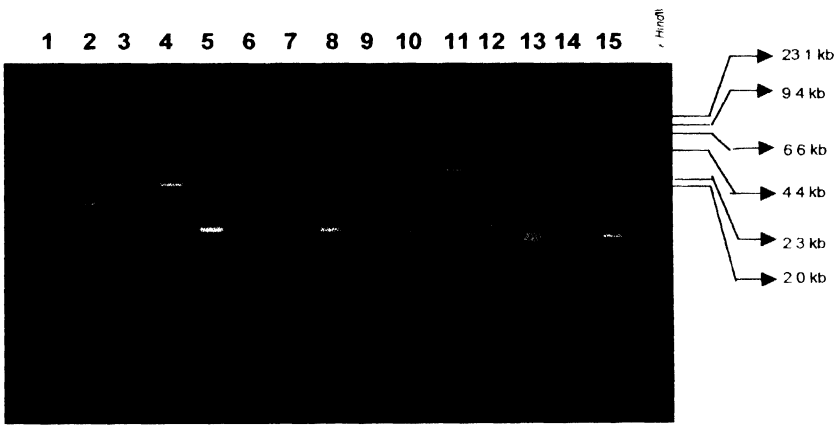
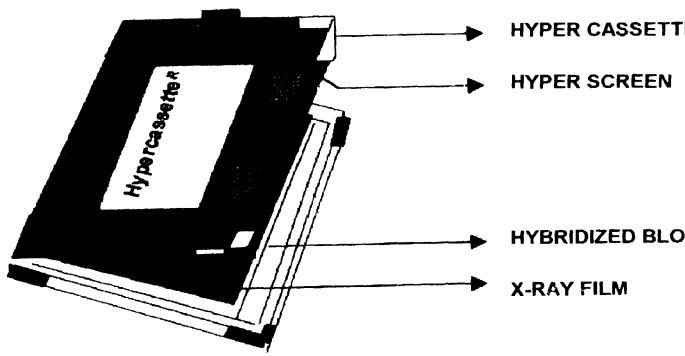


Figure 7. Autoradiography to expose the hybridized blot



pearl millet PgPSM probes From this screening, the polymorphic combinations were noted down and used for further screening of the segregating F_2 population

3.10. STATISTICAL ANALYSIS

3.10.1. FREQUENCY DISTRIBUTION

To identify clear breakpoints for resistance and susceptibility to downy mildew disease incidence, frequency distribution with class values of 0-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 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 reaction of the pearl millet mapping population progenies screened against each pathogen populations

3.10.2. CHI-SQUARE TEST

Chi-square goodness of fit tests are 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

$$\chi^2 = \Sigma (O-E)^2 / E$$

where

O = observed frequencies

E = expected frequencies

Σ = summation over all the classes

n = number of classes in the hypothetical distribution

All possible Mendelian ratios for monogenic digenic and trigenic segregations and their epsitatic interactions were taken as a expected frequencies and compared against the observed frequencies of downy mildew incidence susceptible and resistance behaviour using distribution breakpoints in the disease incidence frequency histograms

to assign groups to "susceptible" or "resistant" classes for estimation of observed frequencies

3.10.3. PEARSON RANK CORRELATION COEFFICIENTS

Pearson rank correlation coefficients were calculated for disease incidence of the mapping progenies 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 mean downy mildew incidence (%) values of each mapping population progeny against each of a pair of pathogen isolates as the x and y inputs, and summing across all progenies in the population

Pearson rank correlation coefficient r = cov (x y) / (σ_x , σ_y)

where

$$\text{cov}(x\ y) = \overline{\Sigma x_i\ y_i - \bar{x}\bar{y} / N}$$

$$\sigma_x = \overline{\Sigma x_i^2 - \bar{x}^2 / N}$$

$$\sigma_y = \overline{\Sigma y_i^2 - \bar{y}^2 / N}$$

r = correlation coefficient

σ_x = standard deviation of x

σ_y = standard deviation of y

x = rank for variable 1

y = rank for variable 2

N = total number of mappir
aga nst the two pathogen populations

= mapping population progeny i N

3.10.4. CLUSTERING ANALYSIS

Clustering analysis was done with a hierarchical clustering technique—Euclidean distance with average link analysis—using Genstat version 5 from Rothamsted UK (Genstat, 1993) to group the pathogen populations from India and Africa again using the

mean disease reactions of the 142 progenies from the (IP 18293 × Tift 238D1)-based mapping population in screens against each of the eight pathogen populations as the input data.

3.11. LINKAGE MAPPING

Linkage analysis was accomplished using the program Mapmaker/Exp version 3.0b supplied by E.S. Lander, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts (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 "Build" command was performed to place new markers from the phenotypic data set in the appropriate position within the identified linkage group.

3.12. QTL MAPPING

3.12.1. USING MAPMAKER/QTL

Trait data from F_2, F_2F_3, F_2F_4 and testcrosses were averaged for each entry and sorted to correspond with the progeny order of the genotype. The total number of progeny individuals from the cross IP 18293 · Tift 238D1 with both trait and genotype information was 142. QTL mapping was carried out using the program MapMaker/QTL version 1.1b (Lander and Botstein, 1989. Lincoln *et al*, 1992b). MapMaker/QTL calculates weight and dominance from the change in phenotype resulting from the substitution of B parent alleles for A parent alleles. In this cross, female parent IP 18293 (scored as 'B') was resistant and the male parent Tift 238D1 (scored as 'A') was susceptible to downy mildew.

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

$$F_2\ Trait_i = Mean + (Weight \cdot Num_i) + (Dominance \cdot Het_i) + Noise$$

Where

Mean = the mean value of the components of the trait 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 individual number i , either 0, 1, or 2

Dominance = the dominance component of the B allele effect at this locus

Het_i = 1 if individual number i is an A/B heterozygote, and 0 otherwise

Noise = variation in the trait not controlled by this locus (a normal random variable)

3.12.2. Using QTL Cartographer

Output data files from the Mapmaker/Exp were used as an input file for QTL Cartographer (Basten *et al.*, 1994; Basten *et al.*, 1997) to check the results of identified QTLs by the Mapmaker/QTL. The analyses performed were based on composite interval mapping (CIM) (Zeng, 1993, 1994). Composite interval mapping is an extension of interval mapping with some selected markers also fitted in the model as cofactors to control the genetic variation of other possible markers or unlinked QTLs

Results

4. RESULTS

4.1. DOWNY MILDEW INHERITANCE STUDIES

4.1.1. PARENTAL AND CHECK ENTRIES REACTION

Parental line IP 18293 was highly resistant and exhibited no symptoms of infection against four pathogen populations from India (from Jalna, Jodhpur, Patancheru and Jamnagar), but was moderately susceptible to those from Bamako, Mali (31% incidence), Durgapura, India (40% incidence), Sadore, Niger (41% incidence) and New Delhi, India (70% incidence) Parental line Tift 238D1 recorded 100% disease incidence against Patancheru, New Delhi and Duragapura pathogen populations A moderate disease incidence was observed in the susceptible parental line Tift 238D1, in screens against the Jodhpur (57%) and Jalna (74%) pathogen populations Disease incidence on Tift 238D1 was very high in screens against both the African pathogen populations (Niger and Mali)

Among the various control entries 7042(S) showed complete susceptibility to all pathogen populations except that from New Delhi (23% incidence) The resistant control entry 700651 was reasonably resistant against all Indian pathogen populations except that from Patancheru (80% incidence)

4.1.2. PATANCHERU PATHOGEN POPULATION

Segregation among the F_2F_4 families for disease reaction against the ICRISAT Patancheru pathogen population is depicted in the Figure 8 At the break point of 20% DMI, it gave the best fit to a ratio of 9 7 (resistant susceptible) indicating complementary epistatic interaction of two dominant resistance genes (Tables 2 and 3) At the break point of 60% DMI, a segregation ratio of 15 1 (indicating duplicate dominant resistance) was observed to fit best, whereas at 75% DMI, the observed segregation pattern best fit the trigenic ratio of 63 1

4.1.3. JALNA PATHOGEN POPULATION

Disease reactions of F_2F_4 families from the segregating population against the pathogen population from MAHYCO, Jalna showed that two to three resistance genes for resistance genes (Fig 9) At a break point of 30% DMI, the trigenic ratio of 37 27

Table 1. Reactions of pearl millet mapping population parental lines IP 18293 and Tift 238D1, and four resistant and susceptible inbred control genotypes, against six Indian and two African populations of *Sclerospora graminicola* under greenhouse conditions at Patancheru, India (June 2000) and Bangor, UK (June 2000)

Host genotypes	Pathogen population	Number of plants		Disease incidence (%)
		Disease-free	Diseased	
IP 18293	MAHYCO, Jalna, India	43	0	0
	RAU, ARS Durgapura, India	33	22	40
	CAZRI-Jodhpur, India	50	0	0
	ICRISAT- Patancheru, India	41	0	0
	GAU, MRS Jamnagar, India	40	0	0
	IARI, New Delhi, India	11	27	71
	Bamako, Mali	154	72	31
	Sadore, Niger	67	46	41
Tift 238D1	MAHYCO, Jalna, India	27	75	74
	RAU, ARS Durgapura, India	15	92	86
	CAZRI-Jodhpur India	50	66	57
	ICRISAT- Patancheru, India	0	106	100
	GAU, MRS Jamnagar India	16	81	83
	IARI, New Delhi India	0	118	100
	Bamako, Mali	14	270	95
	Sadore, Niger	2	152	99
7042S	MAHYCO Jalna, India	49	149	75
	RAU, ARS Durgapura, India	26	170	87
	CAZRI-Jodhpur, India	22	158	87
	ICRISAT- Patancheru, India	1	168	99
	GAU MRS Jamnagar, India	5	177	97
	IARI, New Delhi, India	43	13	23
	Bamako Mali	29	294	92
	Sadore, Niger	47	192	80
7042R = ICML 22	MAHYCO, Jalna, India	136	76	36
	RAU, ARS Durgapura, India	135	77	36
	CAZRI-Jodhpur, India	178	39	18
	ICRISAT- Patancheru, India	112	103	52
	GAU, MRS Jamnagar, India	115	110	49
	IARI, New Delhi India	59	10	14
700651 = ICML 16	MAHYCO, Jalna, India	211	1	0
	RAU, ARS Durgapura, India	204	21	9
	CAZRI-Jodhpur, India	193	6	3
	ICRISAT- Patancheru, India	40	156	80
	GAU, MRS Jamnagar, India	194	25	11
	IARI, New Delhi, India	38	0	0
HB 3	MAHYCO, Jalna, India	196	15	7
	RAU, ARS Durgapura, India	113	93	45
	CAZRI-Jodhpur, India	40	125	76
	ICRISAT- Patancheru, India	5	176	97
	GAU, MRS Jamnagar, India	32	178	85
	IARI, New Delhi, India	23	35	60
	Bamako, Mali	67	265	80
	Sadore, Niger	154	97	61

Table 2. Chi-square estimates for goodness of fit to a range of classical Mendelian segregation ratios for greenhouse screen downy mildew reaction against eight pearl millet downy mildew populations among 142 F_2 F_4 families derived from a single F_1 plant of the pearl millet cross IP 18293 × Tift 238D1

Source of pathogen	Mendelian ratios													
	1 63	1 15	9 55	3 13	1 3	27 37	7 9	9 7	37 27	3 1	13 3	55 9	15 1	63 1
Patancheru														
Break point 20	18 00	3 92	1 33	0 82	0 45	0 05	0 04	**0 00**	0 01	0 23	0 48	0 83	2 66	12 70
Break point 60	54 30	12 83	5 15	3 61	2 46	1 05	0 98	0 54	0 50	0 17	0 09	0 04	**0 00**	0 19
Break point 75	59 40	14 11	5 71	4 03	2 77	1 24	1 16	0 68	0 63	0 26	0 17	0 10	0 02	**0 01**
Jalna														
Break point 30	25 40	5 70	2 07	1 35	0 81	0 19	0 17	0 02	**0 01**	0 06	0 19	0 36	1 50	7 67
Break point 45	44 70	10 45	4 10	2 84	1 89	0 73	0 67	0 32	0 29	0 04	0 01	**0 00**	0 14	1 26
Break point 80	62 10	14 77	6 01	4 26	2 94	1 33	1 25	0 75	0 70	0 31	0 21	0 14	0 05	**0 00**
Jamnagar														
Break point 15	4 85	0 88	0 18	0 07	**0 00**	0 07	0 09	0 30	0 34	1 13	1 80	2 69	7 18	31 46
Break point 35	19 50	4 28	1 48	0 92	0 52	0 08	0 06	**0 00**	0 00	0 18	0 40	0 72	2 38	11 52
Break point 55	40 28	9 35	3 63	2 48	1 63	0 59	0 54	0 23	0 20	0 01	**0 00**	0 02	0 31	2 14
Break point 90	61 21	14 55	5 91	4 18	2 88	1 30	1 22	0 72	0 68	0 29	0 19	0 13	0 04	**0 00**
Jodhpur														
Break point 20	34 72	7 98	3 03	2 05	1 31	0 43	0 38	0 13	0 11	**0 00**	0 02	0 11	0 62	3 68
Break point 65	60 32	14 33	5 81	4 11	2 83	1 27	1 19	0 70	0 65	0 28	0 18	0 19	0 03	**0 00**
Break point 80	62 10	14 77	6 01	4 26	2 94	1 34	1 25	0 75	0 70	0 31	0 21	0 15	0 05	**0 00**
Durgapura														
Break point 40	0 11	**0 00**	0 06	0 11	0 20	0 55	0 59	1 04	1 11	2 56	3 75	5 33	13 25	55 99
Break point 50	1 02	0 11	**0 00**	0 01	0 06	0 32	0 35	0 72	0 78	1 97	2 96	4 27	10 83	46 26
Break point 80	19 50	4 28	1 48	0 92	0 52	0 08	0 06	**0 00**	0 01	0 18	0 40	0 72	2 38	11 52
Break point 95	41 01	9 53	3 70	2 54	1 67	0 62	0 56	0 25	0 22	0 01	**0 00**	0 02	0 27	1 98
New Delhi														
Break point 65	0 19	**0 00**	0 04	0 09	0 17	0 50	0 55	0 98	1 05	2 46	3 61	5 15	12 83	54 30
Break point 75	1 26	0 15	**0 00**	0 01	0 04	0 29	0 32	0 68	0 73	1 89	2 83	4 10	10 45	44 73
Mali														
Break point 40	**0 00**	0 04	0 13	0 19	0 29	0 68	0 73	1 22	1 30	2 88	4 18	5 91	14 54	61 18
Break point 65	1 05	0 11	**0 00**	0 01	0 06	0 32	0 35	0 71	0 77	1 96	2 94	4 24	10 77	46 04
Break point 85	26 16	5 89	2 15	1 40	0 85	0 21	0 18	0 03	**0 02**	0 05	0 17	0 36	1 41	7 26
Niger														
Break point 60	0 02	**0 01**	0 09	0 15	0 24	0 61	0 66	1 13	1 20	2 72	3 96	5 62	13 94	58 57
Break point 85	3 46	0 58	0 09	0 02	**0 00**	0 12	0 15	0 40	0 45	1 35	2 10	3 10	8 15	35 40

(Values in the bold letter are the best for the Mendelian ratios)

Table 3. Reactions of 142 segregating F_2 F_4 families derived from a single F_1 plant of the cross (IP 18293 × Tift 238D1) against six pearl millet downy mildew pathogen populations of Indian origin and two of African origin

Origin of pathogen population	Mendelian ratios giving best fit
ICRISAT-Patancheru Andhra Pradesh India	9 7 15 1 63 1
MAHYCO Jalna Maharashtra India	37 27 55 9 63 1
GAU MRS Jamnagar Gujarat India	1 3 9 7 13 3 63 1
CAZRI-Jodhpur Rajasthan India	3 1 63 1
RAU ARS Durgapura Rajasthan India	1 15 9 55 9 7 13 3
IARI New Delhi Delhi India	1 15 9 55
Sadore Niger Africa	1 15 1 3
Bamako Mali Africa	1 63 9 55 37 27

Table 4. Pearson rank correlation coefficients for greenhouse seedling downy mildew reaction among 142 F_2 F_4 families derived from a single F_1 plant of the pearl millet cross (IP 18293 × Tift 238D1) when screened against six Indian populations and two African pearl millet downy mildew populations

	Patancheru	Jalna	Jamnagar	Jodhpur	Durgapura	New Delhi	Niger	Mali
Patancheru	-	0 661**	0 709**	0 482**	0 264**	0 185*	0 186*	0 121
Jalna		-	0 786**	0 461**	0 198*	0 183*	0 117	0 108
Jamnagar			-	0 454**	0 134	0 167	0 119	0 096
Jodhpur				-	0 320**	0 189*	0 119	0 077
Durgapura					-	0 592**	0 379**	0 433**
New Delhi						-	0 328**	0 286**
Niger							-	0 621**
Mali								-

** Significant at $P<0.01$
* Significant at $P<0.05$

Figure 8. Frequency distribution of disease incidence (%) among F_2 F_4 progenies from the pearl millet cross (IP 18293 x Tift 238D1) when screened under greenhouse conditions in Patancheru against a *Sclerospora graminicola* population from ICRISAT, Patancheru, Andra Pradesh, India

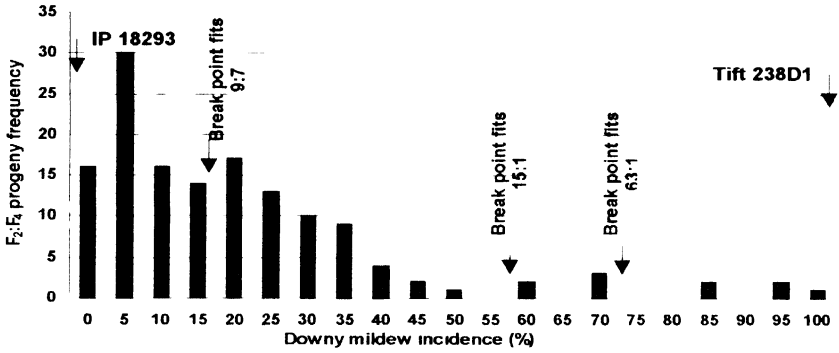
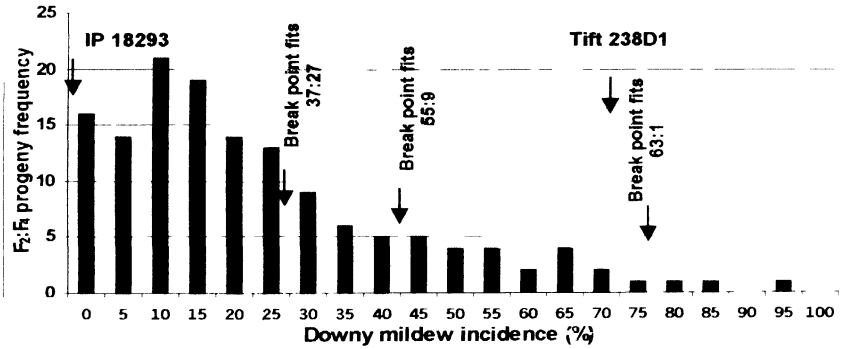


Figure 9. Frequency distribution of disease incidence (%) among F_2 F_4 progenies from the pearl millet cross (IP 18293 x Tift 238D1) when screened under greenhouse conditions in Patancheru against a *Sclerospora graminicola* population from MAHYCO, Jalna, Maharastra, India



(resistant susceptible) best fits the observed distribution (Tables 2 and 3) If we consider break point at 45% DMI, this segregation fit well with another trigenic ratio of 55 9 (resistant susceptible) (two complementary resistance genes and one inhibitory gene) It also fit a 63 1 genetic interaction also fits when we use a break point of 70% DMI

4.1.4. JAMNAGAR PATHOGEN POPULATION

Segregation of F_2F_4 mapping population families from pearl millet cross IP 18293 × Tift 238D1 against a downy mildew pathogen population from GAU MRS Jamnagar is presented as a frequency diagram in Figure 10 At a break point of 35% DMI the observed segregation fits a digenic complementary gene ratio of 9 7 It also fit at the break point of 45% DMI The digenic ratio of 13 3 (one basic gene and one inhibitor gene) was observed to provide the best fit at a break point of 55% DMI

4.1.5. JODHPUR PATHOGEN POPULATION

When screened against the downy mildew pathogen population from CAZRI-Jodhpur, segregation among the F_2F_4 families was observed to best fit a Mendelian segregation ratio of 3 1 (resistant susceptible) at a break point of 20% DMI (Fig 11) However, it also fit trigenic ratios of 63 resistant to 1 susceptible at a break points of 65 and 80% DMI (Tables 2 and 3)

4.1.6. DURGAPURA PATHOGEN POPULATION

From the screening results it is evident that the RAU ARS Durgapura pathogen population from Jaipur, Rajastan, India is highly virulent against this mapping population as nearly all F_2F_4 families screened against it recorded very heavy downy mildew disease incidence (Fig 12) Two to three genes appear to be controlling the limited degree of resistance and a digenic ratio of 1 15 (resistant susceptible) fits well at the break point of 35% DMI, whereas a trigenic 9 55 was observed to give the best fit at the break point of 45% DMI We also observed fits to a 9 7 atio (complementary dominant gene interaction) and a 13 3 ratio (one basic gene and one inhibitor gene) at break points of 75% and 85%, DMI respectively (Tables 2 and 3)

4.1.7. NEW DELHI PATHOGEN POPULATION

The IARI, New Delhi pathogen population also provided very high disease incidence in screens against this set of segregating F_2F_4 families (Fig 13) The

Figure 10. Frequency distribution of disease incidence (%) among F_2 F_4 progenies from the pearl millet cross (IP 18293 x Tift 238D1) when screened under greenhouse conditions in Patancheru against a *Sclerospora graminicola* population from GAU, MRS Jamnagar, Gujarat, India

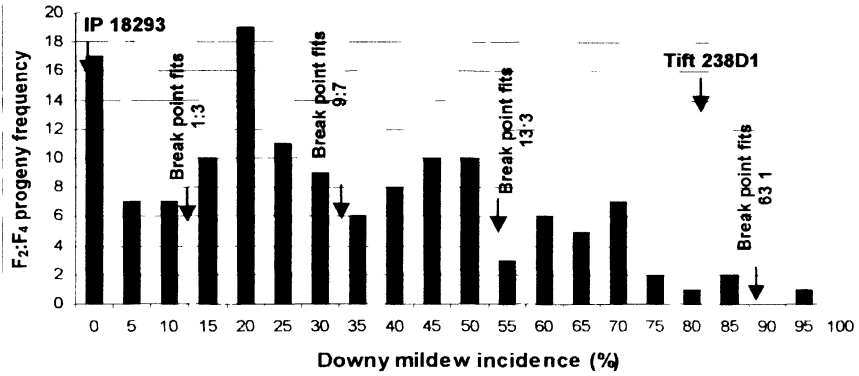


Figure 11. Frequency distribution of disease incidence (%) among F_2 F_4 progenies from the pearl millet cross (IP 18293 x Tift 238D1) when screened under greenhouse conditions in Patancheru against a *Sclerospora graminicola* population from CAZRI-Jodhpur, Rajastan, India

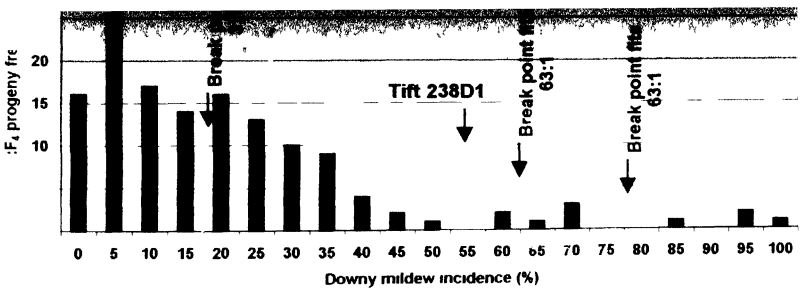


Figure 12. Frequency distribution of disease incidence (%) among F_2 F_4 progenies from the pearl millet cross (IP 18293 x Tift 238D1) when screened under greenhouse conditions in Patancheru against a *Sclerospora graminicola* population from RAU, ARS Duragapura, Jaipur, Rajastan, India

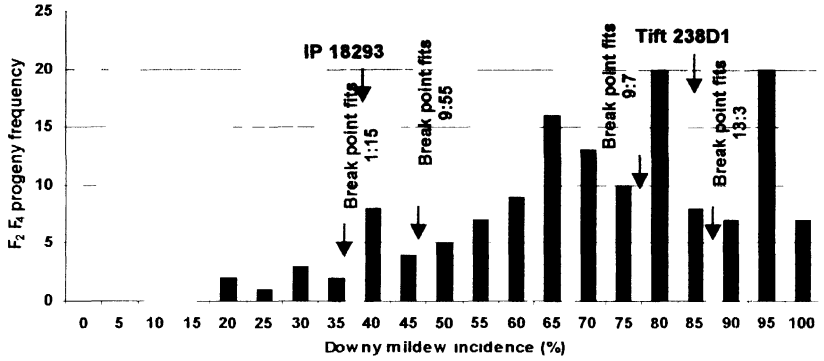
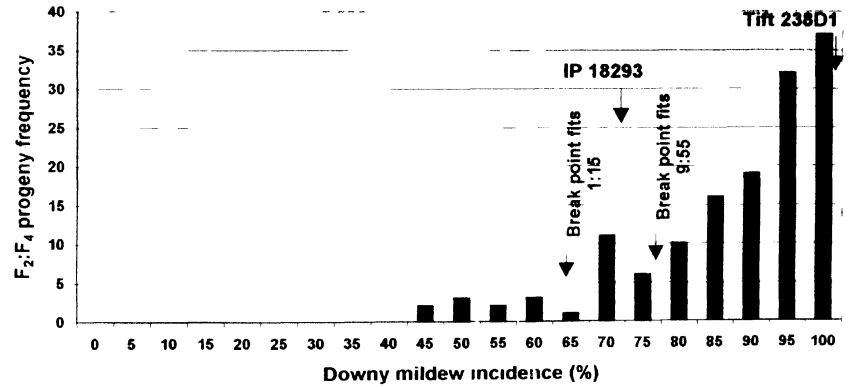


Figure 13. Frequency distribution of disease incidence (%) among F_2 F_4 progenies from the pearl millet cross (IP 18293 x Tift 238D1) when screened under greenhouse conditions in Patancheru against a *Sclerospora graminicola* population from IARI, New Delhi, India



observed segregation clearly showed the digenic interaction ratio of 1:15 (resistant:susceptible) indicating duplicate dominant epistatic interactions with dominant susceptibility, when we considered a break point at 65% DMI. At a break point of 75% DMI, the observed segregation best fit the trigenic ratio of 9:55 (resistant:susceptible) (Tables 2 and 3).

4.1.8. SADORE, NIGER PATHOGEN POPULATION

The disease reactions against the pathogen population from Sadore, Niger showed that one to two host-plant resistance genes of relatively modest effects are governing downy mildew disease reaction in this set of F_2F_4 progenies (Fig. 14). The ratio of 1:15 (resistant:susceptible) fits the observed frequency distribution well at the natural break point of 60% DMI. However, the observed distribution gave the best fit to a 1:3 (resistant:susceptible) ratio when a break point of 85% DMI was considered (Tables 2 and 3).

4.1.9. BAMAKO, MALI PATHOGEN POPULATION

Frequency distribution of downy mildew incidence among the F_2F_4 progenies when screened against a pathogen population from Bamako, Mali suggested that three host-plant resistance genes of relatively modest effects are responsible for the observed variation in disease reaction (Fig. 15). The trigenic ratios of 1:63, 9:55, 37:27 resistant:susceptible gave the best fits to observed segregations at the natural break points of 40, 65 and 85% DMI respectively (Tables 2 and 3).

4.1.10. PEARSON RANK CORRELATION COEFFICIENTS

Pearson correlation coefficient values (significant at $P<0.05$ and $P<0.01$ level) based on disease reactions of the 142 F_2F_4 progenies used in this study are presented in the Table 4. Strong positive correlations were observed between mapping progeny disease reactions to pathogen populations from Jalna and Jamnagar (r=0.79), Patancheru and Jamnagar (r=0.71) and Jalna and Patancheru (r=0.66). Between the two African pathogen populations, a significant correlation (r=0.62) was also recorded. Finally for the two most highly virulent Indian pathogen populations, from New Delhi and Durgapura, a positive significant correlation of r=0.59 was observed

Figure 14. Frequency distribution of disease incidence (%) among F_2:F_4 progenies from the pearl millet cross (IP 18293 x Tift 238D1) when screened under greenhouse conditions in Bangor, UK against a *Sclerospora graminicola* population from Sadore, Niger

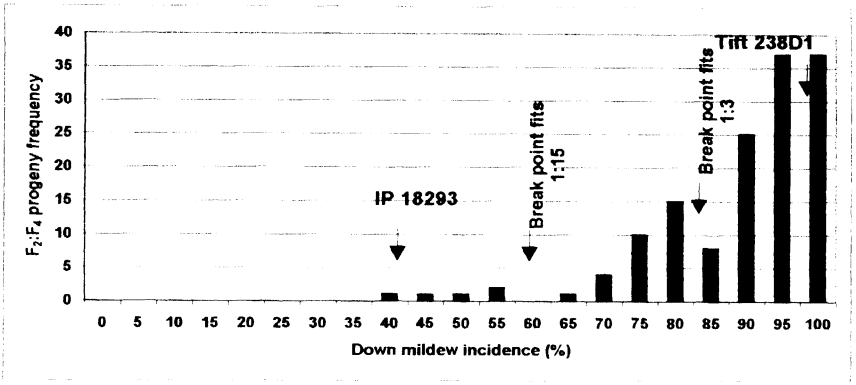
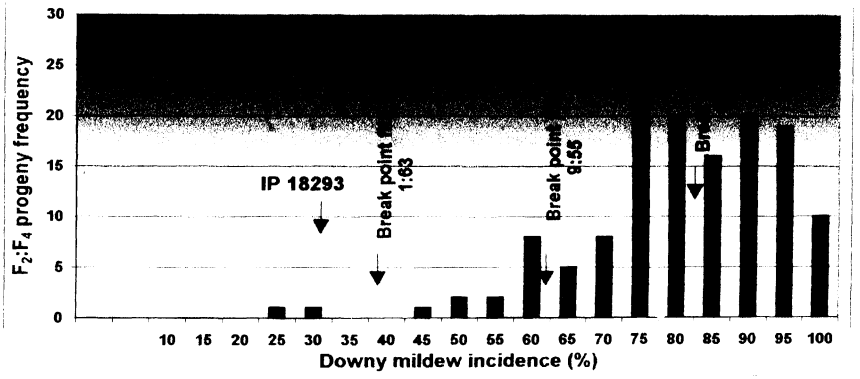


Figure 15. Frequency distribution of disease incidence (%) among F_2:F_4 progenies from the pearl millet cross (IP 18293 x Tift 238D1) when screened under greenhouse conditions in Bangor, UK against a *Sclerospora graminicola* population from Bamako, Mali



4.1.11. CLUSTERING ANALYSIS

The dendrogram was constructed based on the mean downy mildew disease percentages for the 142 F_2F_4 families against each of the eight Indian and African pathogen populations used in this study (Fig 16a) A separate dendrogram was also constructed using only data from screens against the six Indian pathogen populations (Fig 16b) These results revealed that there were two groups of pearl millet downy mildew pathogen populations used in this study One group consists of the more virulent Indian pathogen populations (New Delhi and Durgapura) and the two African pathogen populations (Niger and Mali) The second group consists of the four less virulent Indian downy mildew pathogen populations (Jodhpur Jamnagar Jalna and Patancheru)

4.2. PARENTAL POLYMORPHISM

The parental lines (IP 18293 and Tift 238D1) were screened against a total of 220 probe-enzyme combinations (55 probes · 4 restriction enzymes) to identify polymorphic combinations Forty-one single copy probes were used along with nine two-copy probes and five low-copy probes Autoradiograms showing results of parental line screening against some probe-enzyme combinations are given as Figures 17 and 18 Approximately 40% of the probes were polymorphic between the parental lines Among the four restriction endonuclease enzymes, *Hind*III gave more polymorphic combinations (50 9%) followed by *Dra*I (42 5%) *EcoR*V (36 6%) and *EcoR*I (29 1%) The list of 33 selected polymorphic probe-enzyme combinations is given in Table 5

4.3. MARKER SEGREGATION AND SEGREGATION DISTORTION

The segregation ratios of the marker loci were compared with the expected ratio and results are presented with the calculated χ^2 values in Table 6 Of the 33 probes used to construct a skeleton map in this mapping population, 19 probes were single-copy sequences and nine were two-copy sequences Among the five low-copy probes, *Xpsm*413 2 is a low copy sequence assigned to the linkage group 4

The segregating pattern of marker loci from 142 F_2 progenies of the (IP 18293 Tift 238D1)-based mapping population was compared with the expected ratios of 1 2 1 (1 homozygote of parent P_1 2 hetrozygote 1 homozygote of parent P_2) and the

Figure 16. Dendrogram showing relationships among eight *Sclerospora graminicola* populations from India and Africa based on % similarity in rank order of disease incidence for 142 F_2:F_4 progenies of pearl millet cross (IP 18293 x Tift 238D1), when screened against these under severe greenhouse conditions

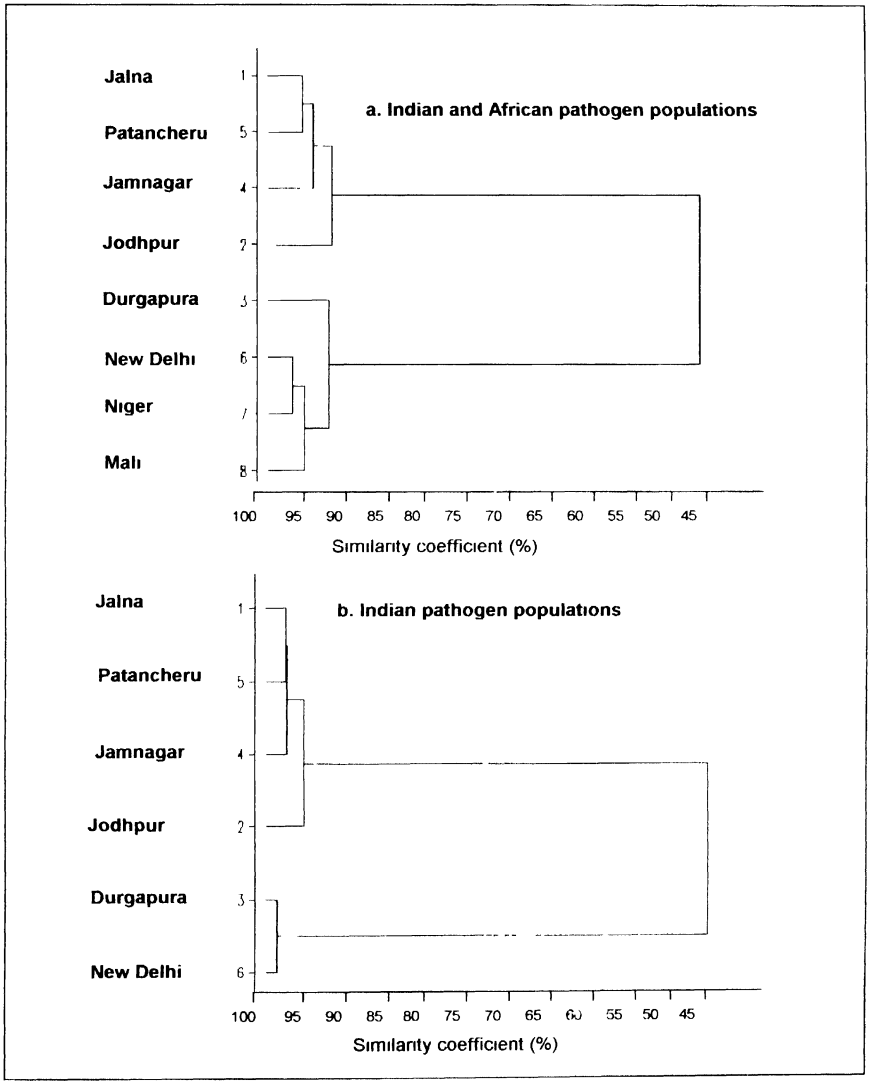
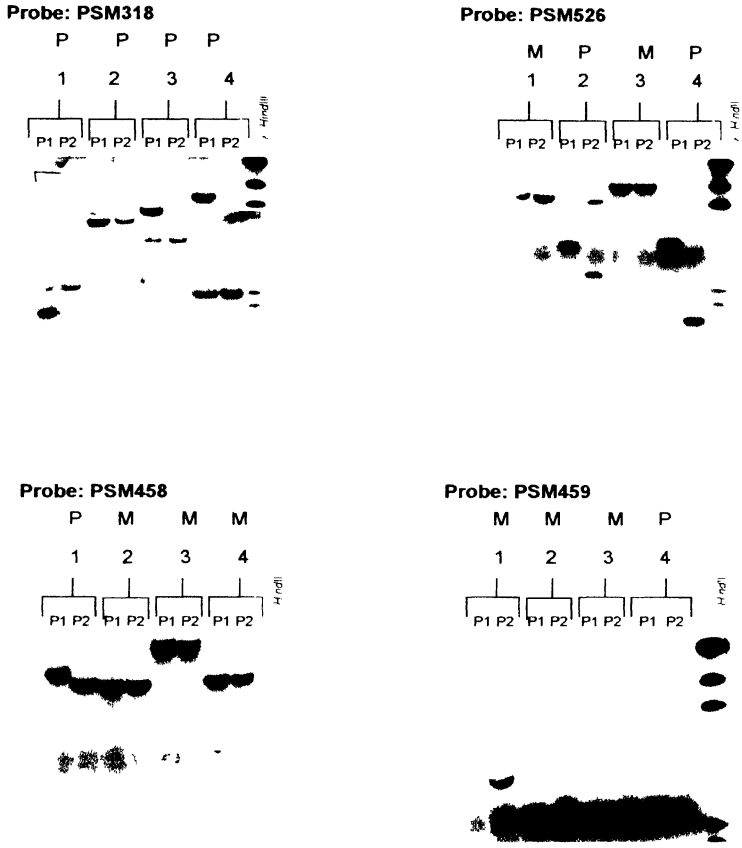
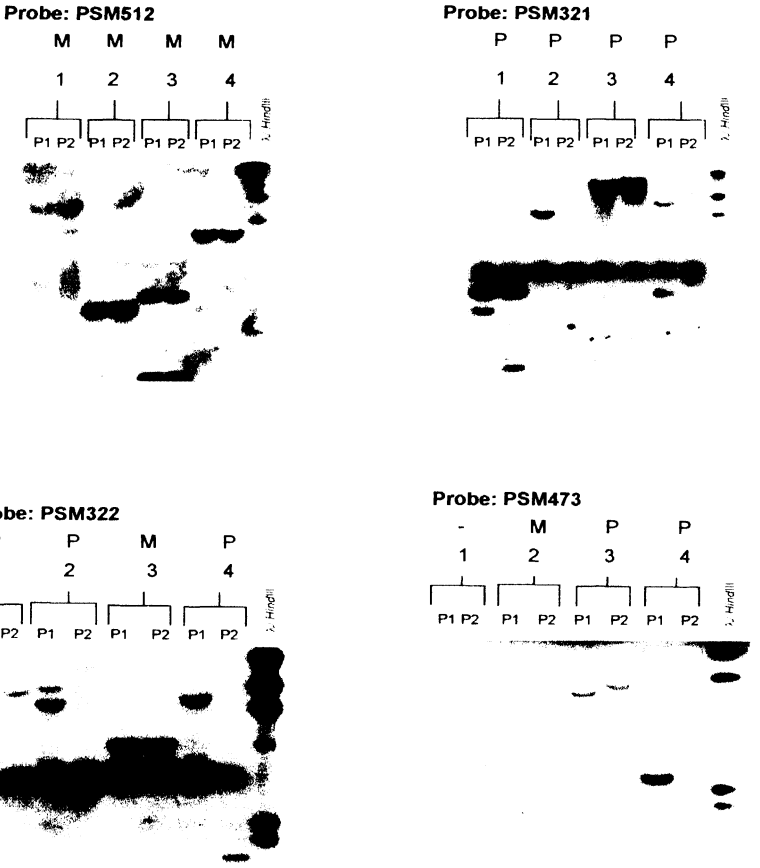


FIGURE 17. PARENTAL SCREENING AGAINST 16 DIFFERENT PROBE-ENZYME COMBINATIONS (PROBES PSM318, PSM526, PSM458 AND PSM459)



P1 = Tift 238D1 (Susceptible parent for pearl millet downy mildew)
P2 = IP 18293 (Resistant parent for pearl millet downy mildew)
1. *Hind*III, 2. *Eco*RI 3. *Eco*RV 4. *Dra*I = Endonuclease restriction enzymes
P = Polymorphic combination
M = Monomorphic combination

FIGURE 18. PARENTAL SCREENING AGAINST 16 DIFFERENT PROBE-ENZYME COMBINATIONS (PROBES PSM512, PSM321, PSM322 AND PSM473)



P1 = Tift 238D1 (Susceptible parent for pearl millet downy mildew)
P2 = IP 18293 (Resistant parent for pearl millet downy mildew)
1. *Hind*III, 2. *Eco*RI 3. *Eco*RV 4. *Dra*I = Endonuclease restriction enzymes
P = Polymorphic combination
M = Monomorphic combination

Table 5. The finalized list of 33 polymorphic probe-enzyme combinations selected for mapping following the screening of parental lines IP 18293 and Tift 238D1 against of 220 combinations.

Number	Probe	Restriction enzyme	Insert size	Copy number	Linkage group	Locus
1	PSM037	*HindIII*	0 7 kb	1 copy	3	*Xpsm037*
2	PSM084	*EcoRV*	1 3 kb	1 copy	4	*Xpsm084*
3	PSM087	*DraI*	0 7 kb	2 copies	1 and 6	*Xpsm087 2*
4	PSM108	*DraI*	0 5 kb	1 copy	3	*Xpsm108*
5	PSM196	*DraI*	2 2 kb	2 copies	1 and 4	*Unlinked*
6	PSM214	*EcoRI*	0 4 kb	1 copy	2	*Xpsm214*
7	PSM269	*HindIII*	0 6 kb	1 copy	7	*Xpsm269*
8	PSM280	*EcoRI*	0 8 kb	2 copies	1	*Xpsm280*
9	PSM318	*HindIII*	1 2 kb	1 copy	5	*Xpsm318*
10	PSM321	*DraI*	1 0 kb	1 copy	2	*Xpsm321*
11	PSM322	*HindIII*	0 7 kb	1 copy	2	*Xpsm322*
12	PSM341	*EcoRI*	0 8 kb	1 copy	1	*Xpsm341*
13	PSM413	*EcoRV*	2 0 kb	Low copy	4	*Xpsm413 2*
14	PSM425	*EcoRI*	2 0 kb	Low copy	1	*Xpsm425*
15	PSM443	*HindIII*	0 4 kb	2 copies	2	*Xpsm443*
16	PSM458	*HindIII*	0 7 kb	1 copy	2	*Xpsm458*
17	PSM459	*DraI*	0 9 kb	2 copies	6 and 1	*Xpsm459 1*
18	PSM464	*EcoRI*	0 4 kb	2 copies	4	*Xpsm464*
19	PSM466	*HindIII*	1 0 kb	Low copy	2	*Xpsm466*
20	PSM473	*DraI*	0 7 kb	1 copy	3	*Xpsm473*
21	PSM510	*HindIII*	1 6 kb	2 copies	3 and 7	*Xpsm510 2*
22	PSM515	*HindIII*	1 7 kb	Low copy	1	*Xpsm515*
23	PSM526	*DraI*	1 7 kb	Low copy	7	*Xpsm526*
24	PSM588	*HindIII*	1 8 kb	1 copy	6	*Xpsm588*
25	PSM592	*DraI*	1 1 kb	1 copy	2	*Xpsm592*
26	PSM618	*DraI*	1 2 kb	1 copy	7	*Xpsm618*
27	PSM648	*DraI*	0 6 kb	1 copy	4	*Xpsm648*
28	PSM686	*DraI*	0 7 kb	2 copies	3	*Xpsm686*
29	PSM713	*EcoRI*	1 5 kb	1 copy	6	*Xpsm713*
30	PSM716	*EcoRI*	1 2 kb	1 copy	4	*Xpsm716*
31	PSM718	*EcoRI*	0 7 kb	1 copy	7	*Xpsm718*
32	PSM735	*EcoRV*	1 6 kb	2 copies	5 and 7	*Xpsm735 1*
33	PSM815	*HindIII*	0 8 kb	1 copy	5	*Xpsm815*

Table 6. Segregation patterns observed among 152 F_2 individuals across 33 polymorphic marker loci compared with the expected 1:2:1 ratios in the mapping population based on the cross IP 18293 x Tift 238D1.

Linkage Group	Marker Loci	Data segregation						Total	Expected segregation ratio			Chi-square calculated values	Table value (at d f = 2) 9 21 (1%) ** 5 99 (5%) *	Genotypes in	
		A	H	B	-	C	D		1A	2H	1B			Surplus	Deficit
LG1	*Xpsm*280	33	76	30	13	0	0	152	34 8	69 5	34 8	1 35	NS		
	*Xpsm*515	34	76	27	11	2	2	152	34 3	68 5	34 3	2 36	NS		
	*Xpsm*425	31	73	30	16	2	0	152	33 5	67 0	33 5	1 09	NS		
	*Xpsm*087 2	20	38	16	69	3	6	152	18 5	37 0	18 5	0 49	NS		
	*Xpsm*341	29	83	28	11	0	1	152	35 0	70 0	35 0	4 84	NS		
LG2	*Xpsm*322	50	59	21	17	1	4	152	32 5	65 0	32 5	14 05	**	A	H and B
	*Xpsm*466	26	37	21	66	0	2	152	21 0	42 0	21 0	1 79	NS		
	*Xpsm*458	49	59	32	11	0	1	152	35 0	70 0	35 0	7 59	*	A	H
	*Xpsm*214	34	49	14	48	0	7	152	24 3	48 5	24 3	8 26	*	A	B
	*Xpsm*321	37	64	30	8	0	13	152	32 8	65 5	32 8	0 82	NS		
	*Xpsm*592	47	54	24	27	0	0	152	31 3	62 5	31 3	10 78	**	A	H and B
	*Xpsm*443	34	77	24	14	0	3	152	33 8	67 5	33 8	4 16	NS		
LG3	*Xpsm*037	41	55	34	19	0	3	152	32 5	65 0	32 5	3 83	NS		
	*Xpsm*108	32	57	24	36	1	2	152	28 3	56 5	28 3	1 14	NS		
	*Xpsm*510 2	42	70	25	13	0	2	152	34 3	68 5	34 3	4 28	NS		
	*Xpsm*473	29	70	22	29	0	2	152	30 3	60 5	30 3	3 79	NS		
	*Xpsm*686	25	62	17	41	1	6	152	26 0	52 0	26 0	5 08	NS		
LG4	*Xpsm*464	17	32	10	84	0	9	152	14 8	29 5	14 8	2 11	NS		
	*Xpsm*716	36	64	25	26	0	1	152	31 3	62 5	31 3	2 01	NS		
	*Xpsm*648	21	34	12	48	21	16	152	16 8	33 5	16 8	2 43	NS		
	*Xpsm*084	19	31	14	73	11	4	152	16 0	32 0	16 0	0 84	NS		
	*Xpsm*413 2	26	62	19	45	0	0	152	26 8	53 5	26 8	3 62	NS		

Linkage Group	Marker Loci	Data segregation						Total	Expected segregation ratio			Chi-square	Table value	Genotypes in	
		A	H	B	-	C	D		1A :	2B :	1B	calculated values	(at d.f = 2) 9.21 (1%) ** 5.99 (5%) *	Surplus	Deficit
LG5	*Xpsm*815	38	46	14	50	4	0	152	24.5	49.0	24.5	12.12	**	A	B
	*Xpsm*318	43	73	19	14	1	2	152	33.8	67.5	33.8	9.43	**	A	H and B
	*Xpsm*735.1	49	68	15	19	1	0	152	33.0	66.0	33.0	17.64	**	A	B
LG6	*Xpsm*459.1	24	59	43	26	0	0	152	31.5	63.0	31.5	6.24	*	B	A
	*Xpsm*588	21	67	31	31	0	2	152	29.8	59.5	29.8	3.57	NS		
	*Xpsm*713	20	62	23	46	1	0	152	26.3	52.5	26.3	3.61	NS		
LG7	*Xpsm*713	45	53	18	35	0	1	152	29.0	58.0	29.0	13.43	**	A	H and B
	*Xpsm*269	33	72	30	9	0	8	152	33.8	67.6	33.8	0.73	NS		
	*Xpsm*618	30	74	20	23	1	4	152	31.0	62.0	31.0	6.26	*	H	B
	*Xpsm*526	31	96	18	5	2	0	152	36.3	72.6	36.3	17.54	**	H	A and B
Unlinked	*Xpsm*196	30	61	23	38	0	0	152	28.5	57.0	28.5	1.42	NS		

calculated χ^2 values for each and every individual marker locus are presented in the Table 6. The χ^2 table values were calculated for 5% and 1% probability level at 2 degrees of freedom. Twenty-two out of 33 co-dominant marker loci recorded non-significant χ^2 values when compared with table values of 5.99 at the 5% and 9.21 at 1% probability levels. The remaining eleven markers deviated significantly from the expected ratios at both levels except the marker loci *Xpsm*458, *Xpsm*214, *Xpsm*459.1 and *Xpsm*618, which were found to be non-significant at the 1% probability level with calculated χ^2 values ranging from 6.24 to 8.26. The highest degrees of distortion, with significant χ^2 values ranging from 9.43 to 17.64 at 5% level were observed for seven loci on LG2, LG5 and LG7. The orders of these most significantly deviating markers (from most distorted to least distorted) *Xpsm*735.1, *Xpsm*526, *Xpsm*322, *Xpsm*718, *Xpsm*815, *Xpsm*592 and *Xpsm*318.

Autoradiograms with the scoring details of some of the probe-enzyme combinations are given in Figures 19-27.

4.4. Linkage map from IP 18293 × Tift 238D1

Loci detected by a total of 33 RFLP probe-enzyme combinations and three morphological markers were used to construct a skeleton linkage map for the new pearl millet mapping population based on cross (IP 18293 × Tift 238D1). A linkage map of 561.8 cM was constructed with seven linkage groups (Fig. 28). Linkage group numbers were assigned as per Liu *et al.* (1994) using loci identified by common single-copy probes. Mapmaker/Exp multipoint analysis was used for construction of the linkage map, using a threshold value of 2.0 and a recombination fraction of 0.5. After making seven linkage groups, markers were placed based on "group", "sequence" and "map" commands. Markers with satisfactory orders were then anchored in each linkage group and the "framework" command was used. Markers orders with fewer candidate errors and high log-likelihood were preferred for anchoring each linkage group. Unlinked marker loci were placed in appropriate linkage groups using the "build" command. Care was taken particularly to properly place loci detected by low copy and multiple copy probes before assigning them to appropriate linkage groups. Locus ordering and distance were compared with the published base map (Fig. 29) of pearl millet from cross LGD-B-10 × ICMP 85410 (Liu *et al.*, 1994).

FIGURE 19. AUTORADIOGRAMS OBTAINED FROM SEGREGATING F_2 PROGENIES OF THE (IP 18293 X TIFT 238D1)-BASED MAPPING POPULATION FOR PROBES PSM280 AND PSM515

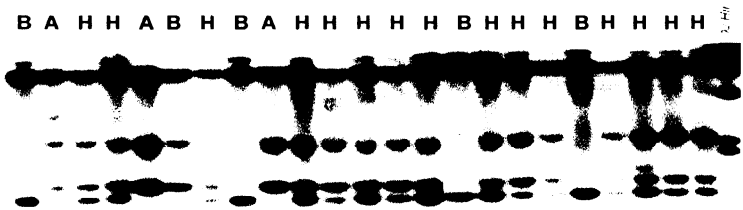
3D1 Segregating F_2 progenies from (IP 18293 × Tift 238D1)

B A H H A A B B H H H H H H H H H A B A H A H

Probe: PSM280 **Enzyme: *EcoR*I**

3D1 Segregating F_2 progenies from (IP 18293 × Tift 238D1)

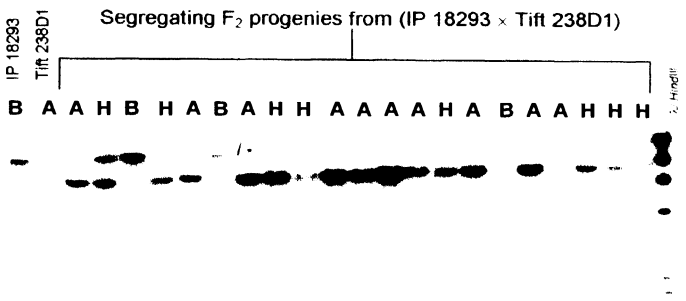
B A H H A B H B A H H H H H B H H H B H H H H



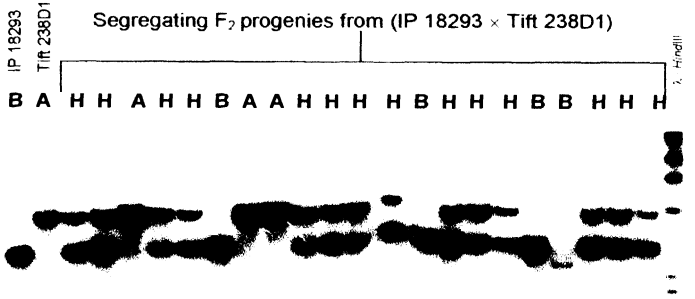
Probe: PSM515 **Enzyme: *Hind*III**

A = homozygous for parent Tift 238D1 allele
B = homozygous for parent IP 18293 allele
H = heterozygote
λ/*Hind*III = Molecular size markers with sizes of 23.1 kb, 9.4 kb, 6.6 kb, 4.4 kb, 2.3 kb and 2.0 kb.

FIGURE 20. AUTORADIOGRAMS OBTAINED FROM SEGREGATING F_2 PROGENIES OF THE (IP 18293 X TIFT 238D1)-BASED MAPPING POPULATION FOR PROBES PSM322 AND PSM341



Probe: PSM322 **Enzyme: *Hind*III**

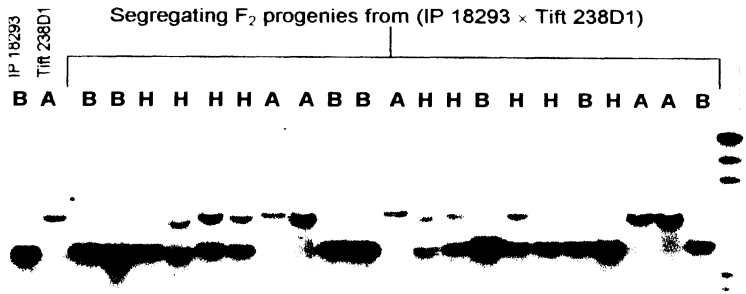


Probe: PSM341 **Enzyme: *Eco*RI**

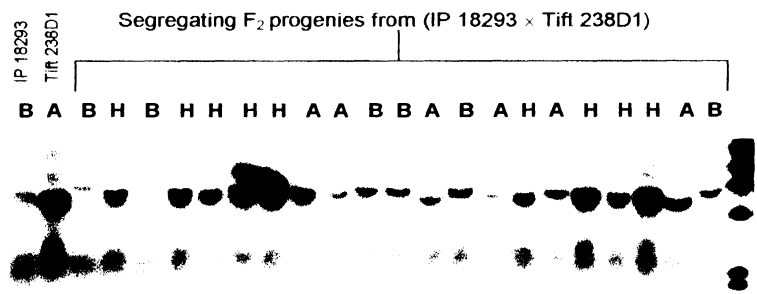
A = homozygous for parent Tift 238D1 allele
B = homozygous for parent IP 18293 allele
H = heterozygote

λ/*Hind*III = Molecular size markers with sizes of 23.1 kb, 9.4 kb, 6.6 kb, 4.4 kb, 2.3 kb and 2.0 kb.

FIGURE 21. AUTORADIOGRAMS OBTAINED FROM SEGREGATING F_2 PROGENIES OF THE (IP 18293 X TIFT 238D1)-BASED MAPPING POPULATION FOR PROBES PSM718 AND PSM37



Probe: PSM718 **Enzyme: *EcoR*I**

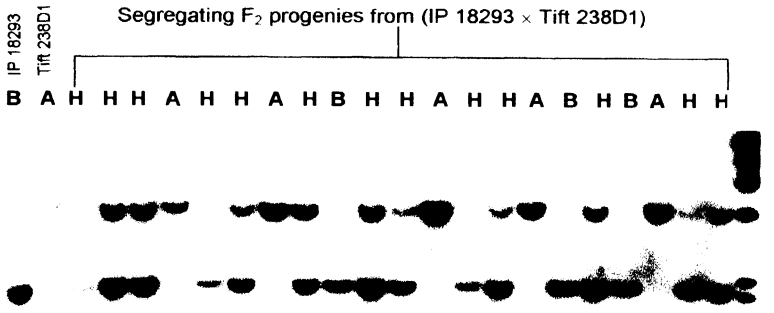


Probe: PSM37 **Enzyme: *Hind*III**

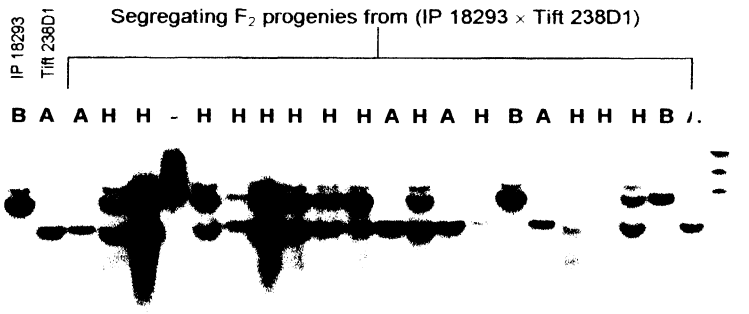
A = homozygous for parent Tift 238D1 allele
B = homozygous for parent IP 18293 allele
H = heterozygote

λ/*Hind*III = Molecular size markers with sizes of 23.1 kb, 9.4 kb, 6.6 kb, 4.4 kb, 2.3 kb and 2.0 kb.

FIGURE 22. AUTORADIOGRAMS OBTAINED FROM SEGREGATING F_2 PROGENIES OF THE (IP 18293 X TIFT 238D1)-BASED MAPPING POPULATION FOR PROBES PSM473 AND PSM510



Probe: PSM473 **Enzyme: *Dra*I**

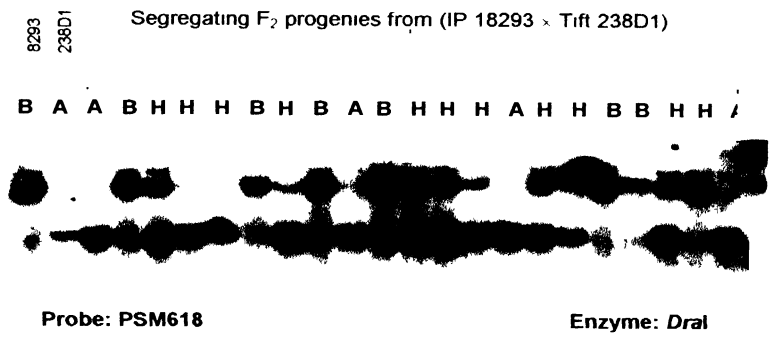


Probe: PSM510 **Enzyme: *Hind*III**

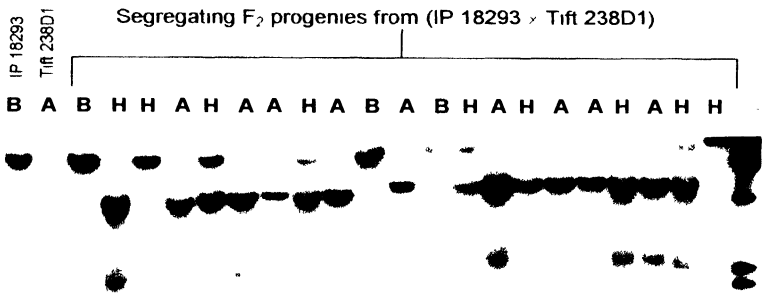
A = homozygous for parent Tift 238D1 allele
B = homozygous for parent IP 18293 allele
H = heterozygote

λ/*Hind*III = Molecular size markers with sizes of 23.1 kb, 9.4 kb, 6.6 kb, 4.4 kb, 2.3 kb and 2.0 kb.

FIGURE 23. AUTORADIOGRAMS OBTAINED FROM SEGREGATING F PROGENIES OF THE (IP 18293 X TIFT 238D1)-BASED MAPPING POPULATION FOR PROBES PSM618 AND PSM716



Probe: PSM618 **Enzyme: *Dra*I**

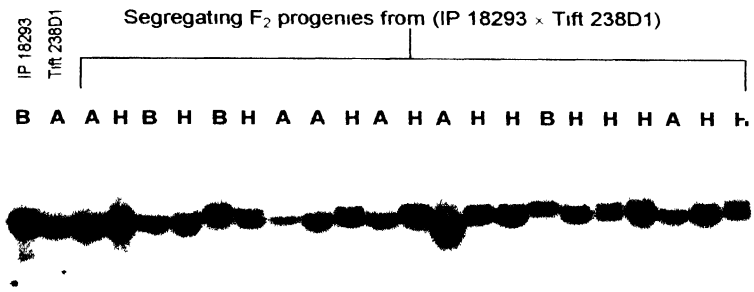


Probe: PSM716 **Enzyme: *Eco*RI**

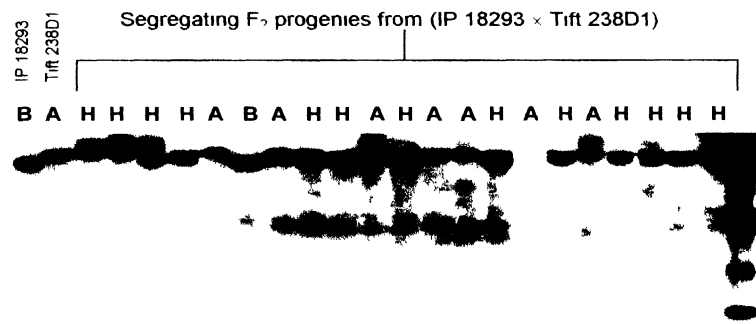
A = homozygous for parent Tift 238D1 allele
B = homozygous for parent IP 18293 allele
H = heterozygote

λ/*Hind*III = Molecular size markers with sizes of 23.1 kb, 9.4 kb, 6.6 kb, 4.4 kb, 2.3 kb and 2.0 kb.

FIGURE 24. AUTORADIOGRAMS OBTAINED FROM SEGREGATING F_2 PROGENIES OF THE (IP 18293 X TIFT 238D1)-BASED MAPPING POPULATION FOR PROBES PSM321 AND PSM458



Probe: PSM321 **Enzyme: *Dra*I**

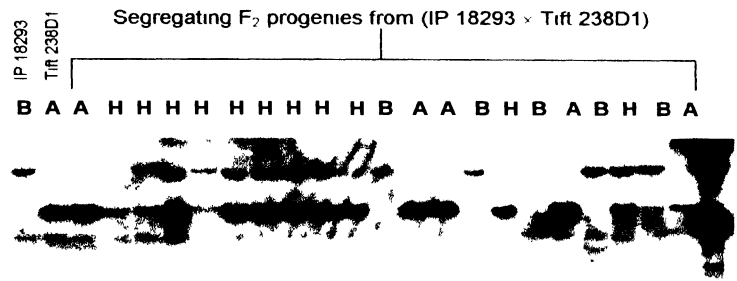


Probe: PSM458 **Enzyme: *Hind*III**

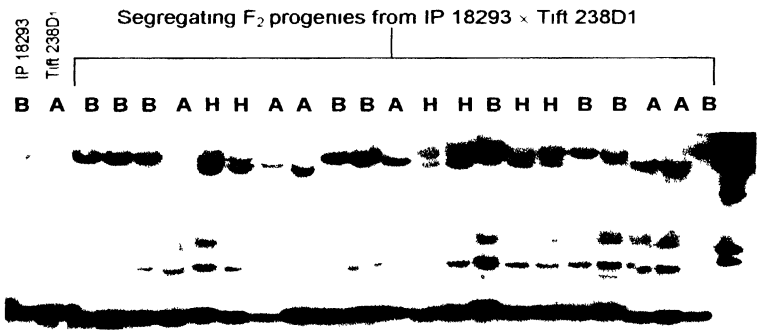
A = homozygous for parent Tift 238D1 allele
B = homozygous for parent IP 18293 allele
H = heterozygote

λ/*Hind*III = Molecular size markers with sizes of 23.1 kb, 9.4 kb, 6.6 kb, 4.4 kb, 2.3 kb and 2.0 kb.

FIGURE 25. AUTORADIOGRAMS OBTAINED FROM SEGREGATING F_2 PROGENIES OF THE (IP 18293 X TIFT 238D1)-BASED MAPPING POPULATION FOR PROBES PSM648 AND PSM425



Probe: PSM648 **Enzyme: *Dra*I**

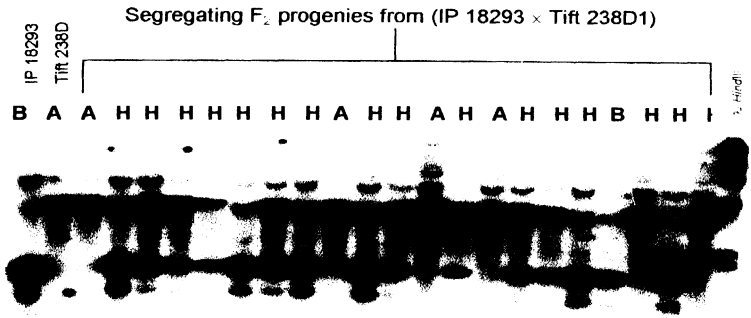


Probe: PSM425 **Enzyme: *EcoR*I**

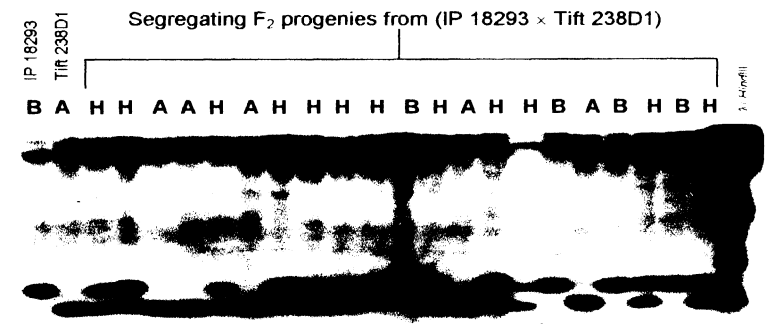
A = homozygous for parent Tift 238D1 allele
B = homozygous for parent IP 18293 allele
H = heterozygote

λ/*Hind*III = Molecular size markers with sizes of 23.1 kb, 9.4 kb, 6.6 kb, 4.4 kb, 2.3 kb and 2.0 kb.

FIGURE 26. AUTORADIOGRAMS OBTAINED FROM SEGREGATING F_2 PROGENIES OF THE (IP 18293 X TIFT 238D1)-BASED MAPPING POPULATION FOR PROBES PSM526 AND PSM84



Probe: PSM526 **Enzyme: *DraI***

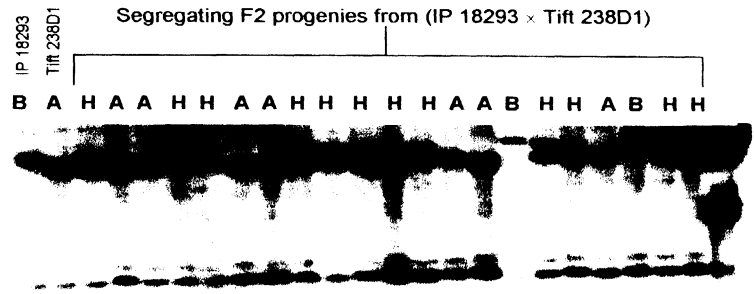


Probe: PSM84 **Enzyme: *EcoRV***

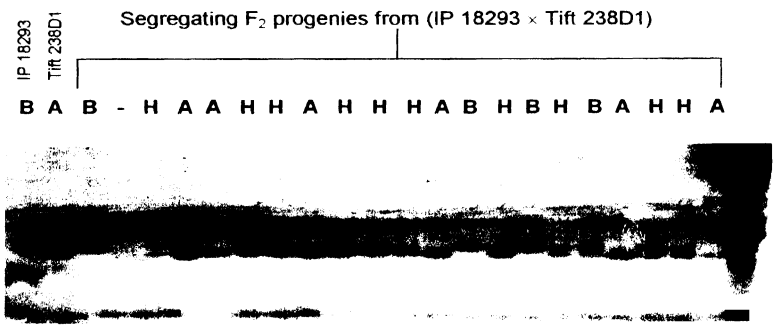
A = homozygous for parent Tift 238D1 allele
B = homozygous for parent IP 18293 allele
H = heterozygote

λ/*Hind*III = Molecular size markers with sizes of 23.1 kb, 9.4 kb, 6.6 kb, 4.4 kb, 2.3 kb and 2.0 kb.

FIGURE 27. AUTORADIOGRAMS OBTAINED FROM SEGREGATING F_2 PROGENIES OF THE (IP 18293 X TIFT 238D1)-BASED MAPPING POPULATION FOR PROBES PSM735 AND PSM592



Probe: PSM735 **Enzyme: *EcoRV***



Probe: PSM592 **Enzyme: *DraI***

A = homozygous for parent Tift 238D1 allele
B = homozygous for parent IP 18293 allele
H = heterozygote
λ/*HindIII* = Molecular size markers with sizes of 23.1 kb, 9.4 kb, 6.6 kb, 4.4 kb, 2.3 kb and 2.0 kb.

Figure 28. RFLP-based skeleton linkage map for cross IP 18293 x Tift 238D1. The seven major linkage groups were ordered from 1 to 7 per Liu *et al*. (1994), using loci identified by common single copy probes. Numbers on the left hand bar are map distances in Haldane centiMorgans, and the abbrevated locus names are on the right hand side of bar

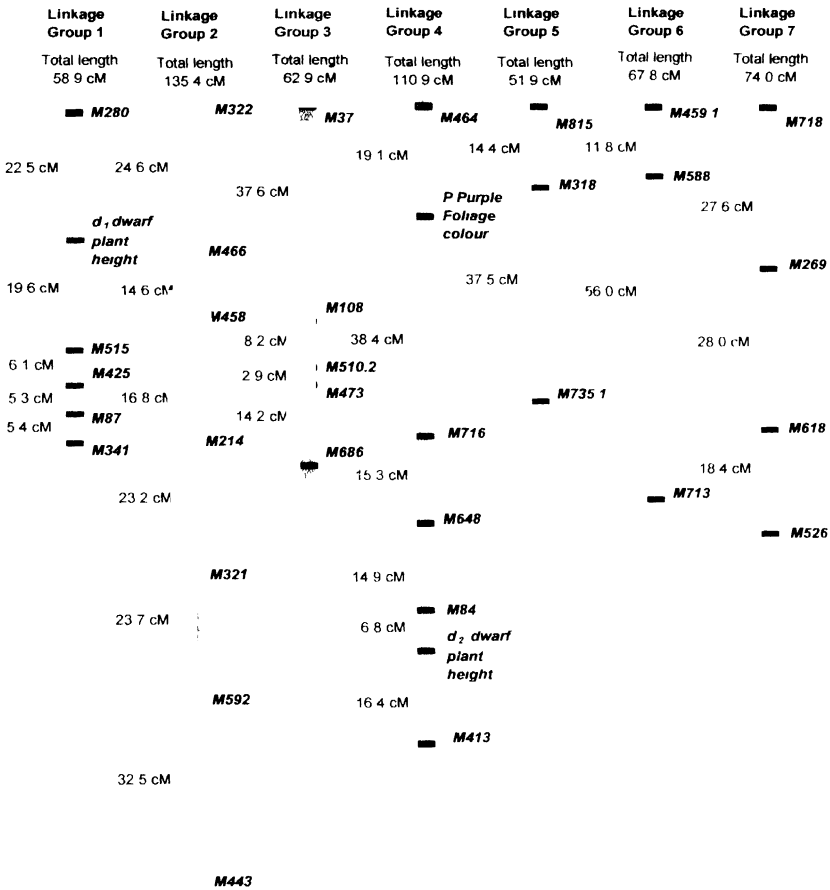
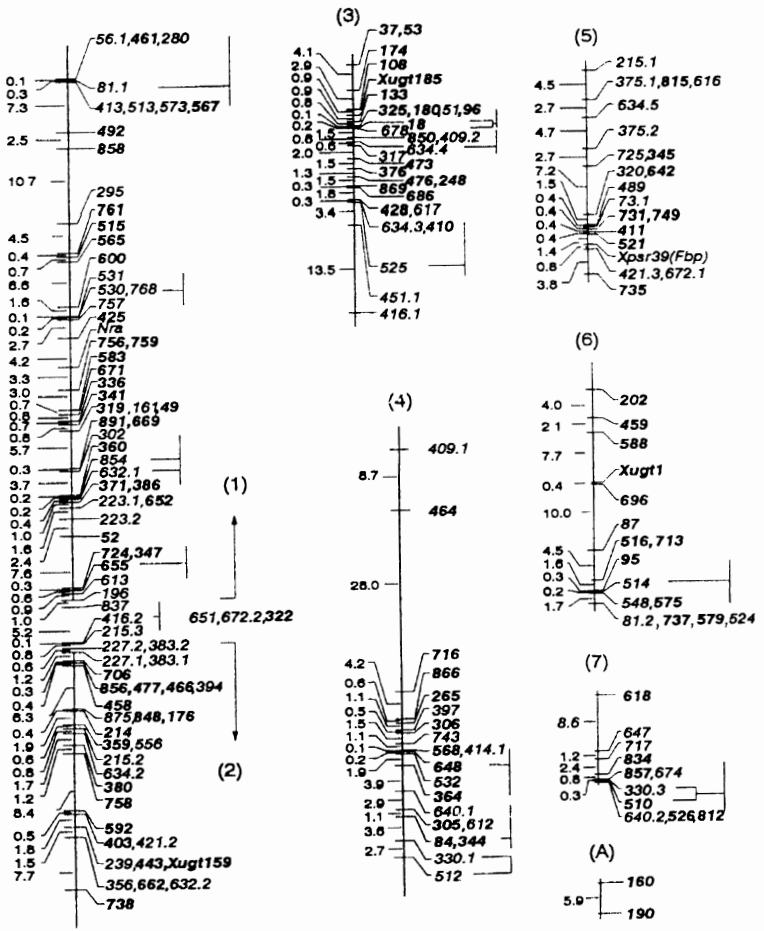


Figure 29. Pearl millet RFLP-based linkage map for a cross LGD-B-10 × ICMP 85410 (Liu *et al.*, 1994). Numbers on the left hand bar are map distances in Kosambi centiMorgans and on the right hand side are abbreviated locus names



4.4.1. Linkage Group 1

Total map length of linkage group 1 is 58 9 cM It consists of five loci detected by PSM RFLP probes (PSM280, PSM515, PSM87 and PSM341) and one morphological marker (d_1 dwarf plant height) This d_1 plant height marker data was obtained from the testcross progenies of Tift 238D1 · purple F_2 plants of (IP 18293 · Tift 238D1) The d_1 plant height locus was placed between marker loci *Xpsm*280 and *Xpsm*515 (Fig 28) This was the best order among all the other combinations considered with high a negative LOD value of –204 66 and a small number of candidate errors (putative double cross-overs)

4.4.2. Linkage Group 2

Seven markers were placed in linkage group 2 which had a longest total length of 136 4 cM among linkage groups for this mapping population The order of the loci is *Xpsm*322, *Xpsm*466, *Xpsm*458, *Xpsm*214, *Xpsm*321, *Xpsm*592 and *Xpsm*443, and a LOD value of –320 57 was observed (Fig 28)

4.4.3. Linkage Group 3

This linkage group is the third smallest in this newly constructed skeleton map, with a total length of 62 9 cM Five markers were placed with intramarker distances of 37 8 8 2, 2 9 and 14 2 cM for marker loci *Xpsm*37, *Xpsm*108 *Xpsm*510 2 *Xpsm*473 and *Xpsm*686 For this marker order, a highly negative log likelihood value of –181 89 was obtained with only a few candidate errors

4.4.4. Linkage Group 4

Linkage group 4 is the second longest one in this skeleton map, with a Haldane map length of 110 9 cM Along with two morphological markers, five RFLP loci were placed with an average distance of 18 6 cM The purple foliage colour marker locus *P* is placed in between RFLP marker loci *Xpsm*464 and *Xpsm*716 The d_2 dwarf plant height locus is placed near to RFLP marker locus *Xpsm*84 with a distance of 6 8 cM (Fig 28) A log likelihood score of –252 7 was obtained by keeping these seven marker loci in this order of *Xpsm*464, *P*, *Xpsm*716, *Xpsm*648, *Xpsm*84, d_2 and *Xpsm*413

4.4.5. Linkage Group 5

Three RFLP markers were placed in linkage group 5, with a distance of 14 4 cM between *Xpsm*815 and *Xpsm*318 and 37 5 cM between *Xpsm*315 and *Xpsm*735 1 This is the shortest linkage group for this mapping population with a total length of 51 9 cM

4.4.6. Linkage Group 6

The total length of this linkage group is 67 8 cM with only three marker loci of *Xpsm*459 2, *Xpsm*588 and *Xpsm*713 (Fig 28) With this marker locus order no candidate error was noticed and a highly negative log likelihood value of −129 4 was obtained

4.4.7. Linkage Group 7

Four marker loci were placed in linkage group 7 with an average distance of 24 7 cM The total linkage group length is 74 0 cM A highly negative log likelihood value of −193 63 was recorded for the best marker locus order, *Xpsm*718, *Xpsm*269, *Xpsm*618 and *Xpsm*526 (Fig 28)

4.5. QTL mapping

For QTL mapping, the linkage map constructed from all 142 individuals from the F_2 population of cross (IP 18293 x Tift 238 D1) was used Two software packages, Mapmaker/QTL (Lincoln *et al* , 1992b) and QTL Cartographer (Basten *et al* , 1997), were used The skeleton linkage map of 32 RFLP loci and three morphological marker loci, generated from this cross was used as such without eliminating any of the markers for Mapmaker/QTL interval mapping analysis But for QTL Cartographer analysis, a separate linkage map output data file from Mapmaker/Exp was used after eliminating the three morphological markers d_1 plant height from linkage group 1, and d_2 plant height and *P* purple foliage colour from linkage group 4 This gave a reduced map length of 502 1 cM for 32 loci as compared to the 561 8 cM map originally obtained for the 35 loci by decreases of 17 2 cM in linkage group 1 and 42 5 cM in linkage group 4

4.5.1. Mapmaker/QTL

The interval mapping method as implemented in Mapmaker/QTL was used with a LOD of 2 0 as threshold value for QTL significance This software calculates weight (additive) and dominance effects and estimates the portion phenotypic variation

explained by individual QTLs, using the free (unconstrained) genetic model QTL Combined effects of multiple QTLs were calculated by multiple QTL models for two QTLs, three QTLs, and four QTLs etc The qualifying criteria for accepting a multiple QTL model was a LOD score of two more than the highest LOD score of the models having one less QTL

$$LOD_n = LOD_{(n-1)} + 2$$

where

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

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

4.5.2. QTL CARTOGRAPHER

Composite interval mapping as implemented in QTL Cartographer (Basten *et al*, 1997) was used with a threshold likelihood ratio of 11 50 This software package also calculates weight (additive) and dominance effects at each position under four different null hypotheses It gave results in 21 columns including the chromosome test position likelihood ratio test statistics, dominance effect, additive effect, and r^2 values for all four hypotheses The results can be viewed in a graphical mode, which shows the position of the QTLs as likelihood peaks (Fig 30 to 38)

In this (IP 18293 · Tift 238D1)-based experimental population, genotyping was assessed in the F_2 and phenotypic trait data were taken from the F_2-derived F_4 self bulk families produced by selfing F_2 plants for two successive generations So the dominance effect observed in the F_4 families was reduced to 25% of that expected in the F_2 generation So for 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 4D/W ratio, the inheritance patterns were assessed as

0 21-0 80 = Partially dominant inheritance
0 81-1 20 = Dominant inheritance
> 1 20 = Over-dominant inheritance

In this mapping population the male parent (marker allele homozygotes scored as A) was Tift 238D1 (downy mildew susceptible with d_1 dwarf gene) and the female parent (marker allele homozygotes scored as B) was IP 18293 (downy mildew resistant, with d_2 dwarf gene) If weight is positive the alleles from IP 18293 increase susceptibility (ie resistance alleles are from the susceptible parent) Similarly if weight is negative, the allele from IP 18293 reduces disease incidence, (ie the allele from Tift 238D1 decreases resistance or increases susceptibility) Again this fully depends on the marker genotype scoring codes and the software used In this study, the parental line IP 18293 was scored as "B" and the susceptible male parental line Tift 238D1 was scored as "A" so the above explanation holds good for Mapmaker/QTL

Results are described below from both analyses (interval mapping and composite interval mapping) for identification of QTLs with significant effects that were detected from screening of the mapping population F_4 families against Indian and African pearl millet downy mildew pathogen populations In addition QTLs for plant height were also mapped

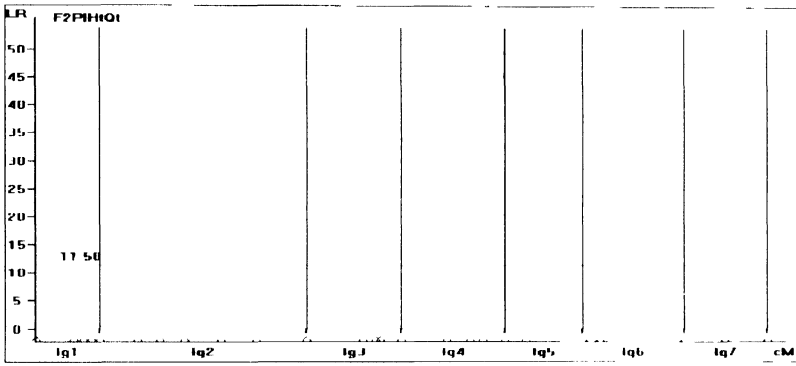
4.5.3. QTLs FOR PLANT HEIGHT

Mapmaker/QTL identified two single QTLs for plant height, one each on LG1 and LG4 This was confirmed by a two-QTL model with an acceptable LOD score greater than 11 31 (9 31+2 00) Two different recessive dwarfing loci were identified, one on linkage group 1 with reduced height associated with homozygosity for the alleles of Tift 238D1 at the d_1 dwarf locus and the other on linkage group 4 for which reduced height was associated with homozygosity for the alleles from IP 18293 at the d_2 dwarf locus (Fig 30 and Table 7) This was confirmed by the composite interval mapping analysis, which indicated a small shift in position of the QTL on linkage group 4 from the middle to the end of the linkage group An acceptable three QTL model from Mapmaker/QTL suggests that there is a possibility of one more plant height QTL present at the top of linkage group 1, with the alleles from Tift 238D1 conferring a partially dominant reduction in plant height

Table 7. QTL results for plant height using QTL Cartographer composite interval mapping and Mapmaker/QTL interval mapping methods for single-QTL and multiple-QTL models

Using QTL Cartographer					
Linkage group/ Marker	**Position**	**Additive effect**	**Dominance effect**	**Likelihood ratio**	**Inheritance**
LG1/M87 2-M341	8 0 cM	-8 9	24 3	36 3	d_1 dwarf locus recessive dwarf allele from Tift 238D1
LG4/M84-M413 2	6 0 cM	25 4	13 5	49 2	d_2 dwarf locus, over-dominant tall allele from Tift 238D1
Using Mapmaker/QTL					
Linkage group/Marker	**Position**	**Additive effect**	**Dominance effect**	**LOD**	**Variance explained**	**Inheritance**
LG1/ M087 2-M341	4 0 cM	16 2	15 0	5 4	17 0%	d_1 dwarf locus recessive dwarf allele from Tift 238D1
LG4/M648-M084	12 0 cM	-25 6	10 4	9 1	31 3%	d_2 dwarf locus, recessive dwarf alleles from IP 18293
Two QTL model [Acceptable LOD score is 9.13 + 2.00 = 11.13]						
LG1/M87 2-M341	0 0 cM	19 5	25 2			Recessive d_1 dwarf from Tift 238D1
LG4/M648-M084	9 4 cM	-30 9	15 9	23 8	64 0%	d_2 dwarf locus, recessive dwarf from IP 18293
Three QTL model [Acceptable LOD score is 23.8 + 2.0 = 25.8]						
LG1/M280-tctd1ht	19 3 cM	13 8	-2 0			Partially dominant dwarf alleles from Tift 238D1
LG1/M87 2-M341	5 4 cM	11 8	26 8			d_1 dwarf locus, recessive dwarf alleles from Tift 238D1
LG4/M648-M84	9 7 cM	31 0	16 5	28 0	71 1%	d_2 dwarf locus, recessive dwarf alleles from IP 18293

Figure 30. QTL likelihood map showing likelihood ratio (LR) for plant height using the composite interval mapping method implemented in QTL Cartographer



4.6. QTLs for downy mildew Resistance

4.6.1. Downy mildew resistance QTLs for the ICRISAT, Patancheru pathogen population

For this pathogen population, two downy mildew resistance QTLs have been identified from both analyses Single-QTL models from Mapmaker/QTL showed two peaks associated with large phenotypic variation in LG2 and LG6 (Table 8) The QTL identified on LG6 (M588-M718) seems to be an artifact, but QTL Cartographer suggested that it was a true QTL with over-dominant resistance contributed by alleles from resistant parent IP 18293 (Fig 31) Variance explained for downy mildew reaction against the Patancheru pathogen population by the LG2 QTL was 22 6 percent Mapmaker/QTL detected no multiple QTL models that agreed with the minimum acceptable limit of LOD score 6 4 (4 4+2 0 = 6 4)

4.6.2. Downy mildew resistance QTLs for the MAHYCO, Jalna pathogen population

A single downy mildew resistance QTL for the Jalna pathogen population was identified on LG2 by both the composite interval mapping and interval mapping analyses (Fig 32) The portion of observed phenotypic variation explained by this QTL was 23 6%, with dominant inheritance of resistance from resistant parental line IP 18293 and recessive inheritance of susceptibility alleles from susceptible parent Tift 238D1 A slight difference of 2 0 cM in the position of the QTL on LG3 (M592+2 0 cM) in composite interval mapping (M592+0 0 cM) as compared to interval mapping by Mapmaker/QTL was noticed (Table 9), but this is not significant

4.6.3. Downy mildew resistance QTLs for GAU, MRS Jamnagar pathogen population

A single downy mildew resistance QTL explained a high proportion of phenotypic variation (52 3%) for reaction to this pathogen population, and was observed for both analyses (Fig 33) This single QTL was located on LG2 (M592+10 0 cM) Over-dominant resistance from IP 18293 and recessive susceptibility from Tift 238D1 was the observed mode of inheritance (Table 10)

Table 8. QTL results for downy mildew resistance against a pathogen population from ICRISAT, Patancheru using QTL Cartographer composite interval mapping and Mapmaker/QTL interval mapping methods for single-QTL and multiple-QTL models

Using QTL Cartographer

Linkage group/ Marker	Position	Additive effect	Dominance effect	Likelihood ratio	Inheritance
LG2/M592-M443	4 0 cM	10 6	-5 2	21 7	Over-dominant resistance from IP 18293
LG6/M588-M713	36 0 cM	25 4	-25 6	39 2	Over-dominant resistance from IP 18293

Using Mapmaker/QTL

Linkage group/ Marker	Position	Additive effect	Dominance effect	LOD	Variance explained	Inheritance
LG2/M592-M443	12 0 cM	-11 1	-8 8	4 4	22 6%	Over-dominance resistance from IP 18293
LG6/M588-M713	34 0 cM	-27 9	-29 4	9 9	63 4%	Artifact over-dominant resistance from IP 18293

Table 9. QTL results for downy mildew resistance against a pathogen population from MAHYCO, Jalna using QTL Cartographer composite interval mapping and Mapmaker/QTL interval mapping methods for single-QTL and multiple-QTL models

Using QTL Cartographer

Linkage group / Marker	Position	Additive effect	Dominance effect	Likelihood ratio	Inheritance
LG2/M592-M443	2 0 cM	16 5	-4 1	40 7	Recessive susceptibility from Tift 238D1

Using Mapmaker/QTL

Linkage group / Marker	Position	Additive effect	Dominance effect	LOD	Variance explained	Inheritance
LG2/M592-M443	0 0 cM	-11 9	-3 7	7 4	23 6%	Dominant resistance from IP 18293

Figure 31. QTL likelihood map showing likelihood ratio (LR) for resistance to ICRISAT, Patancheru downy mildew pathogen population using the composite interval mapping method implemented in QTL Cartographer

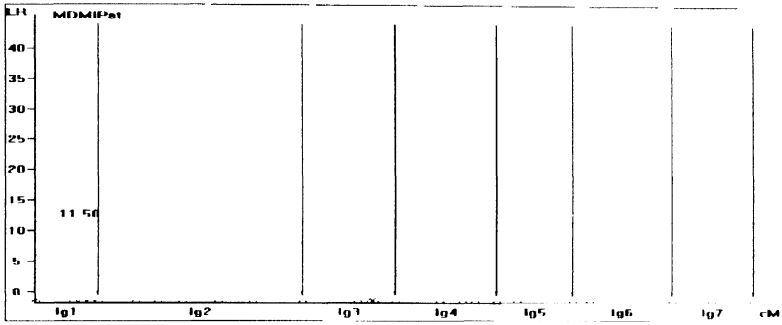


Figure 32. QTL likelihood map showing likelihood ratio (LR) for resistance to MAHYCO, Jalna pathogen population using the composite interval mapping method implemented in QTL Cartographer

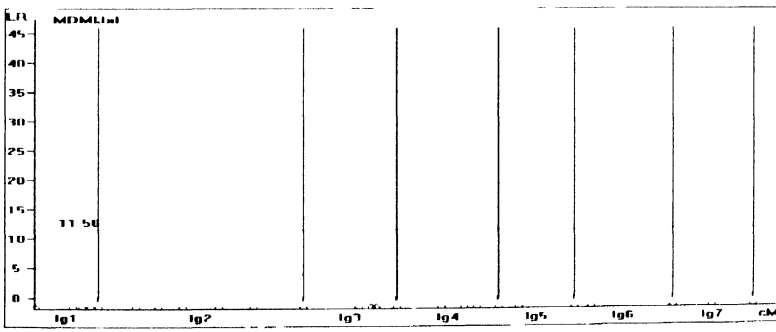


Table 10. QTL results for downy mildew resistance against a pathogen population from GAU, MRS Jamnagar using QTL Cartographer composite interval mapping and Mapmaker/QTL interval mapping methods for single-QTL and multiple-QTL models

Using QTL Cartographer

Linkage group/ Marker	Position	Additive effect	Dominance effect	Likelihood ratio	Inheritance
LG2/M592-M443	10 0 cM	25 4	-9 5	45 1	Recessive susceptibility from Tift 238D1

Using Mapmaker/QTL

Linkage group/ Marker	Position	Additive effect	Dominance effect	LOD	Variance explained	Inheritance
LG2/M592-M443	10 0 cM	-21 0	-12 6	11 4	52 3%	Over-dominant resistance from IP18293

4.6.4. Downy mildew resistance QTLs for the CAZRI, Jodhpur pathogen population

QTL Cartographer identified three QTLs for resistance to this pathogen population on linkage groups 2 3 and 6 (Fig 34) But Mapmaker/QTL has revealed only two QTLs one on LG2 and the other on LG3 Single-QTL models from interval mapping indicated the QTL on LG3 (M473) to be a major one with a large phenotypic effect accounting for 26 9% of observed variation and a high LOD value of 6 35 (Table 11) The significantly superior two-QTL model explained of about 39 9% of variation in disease reaction among mapping population progenies The inheritance of the identified QTLs showed over-dominant resistance from the resistant parent IP 18293 In addition to these two identified QTLs QTL Cartographer results showed that one more QTL on the LG6 was also responsible for resistance against this pathogen population with over-dominant mode of inheritance from IP 18293

4.6.5. Downy mildew resistance QTLs for the RAU, ARS Durgapura pathogen population

There was no significant QTL identified by the composite interval mapping method for this Duragapura pathogen population (Fig 35) This pathogen population was one of the most highly virulent ones tested and produced very heavy disease incidence on essentially all of the F_4 families on this mapping population Mapmaker/QTL interval mapping identified two single-QTLs individually explaining small percentages of the observed phenotypic variation (12 8 and 9 5%) in LG3 and LG7 respectively (Table 12) Not a single significant multiple-QTL model was found as all had LOD values of less than 5 04 (3 04+2 00) two units above of the best single-QTL model LOD score that required support

4.5.6. Downy mildew resistance QTLs for the IARI, New Delhi pathogen population

The QTL Cartographer composite interval mapping method was not able to identify any downy mildew resistance QTL from this mapping population effective against the New Delhi pathogen population (Fig 36) Phenotypic values for mapping progenies were skewed strongly towards the susceptibility The Mapmaker/QTL interval

Figure 33. QTL likelihood map showing likelihood ratio (LR) for resistance to GAU, Jamnagar pathogen population using the composite interval mapping method implemented in QTL Cartographer

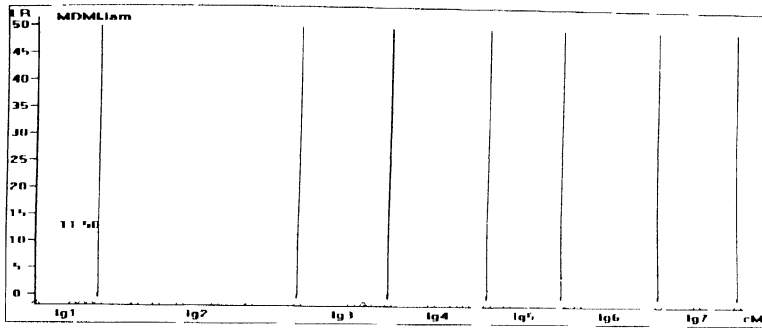


Figure 34. QTL likelihood map showing likelihood ratio (LR) for resistance to CAZRI, Jodhpur downy mildew pathogen population using the composite interval mapping method implemented in QTL Cartographer

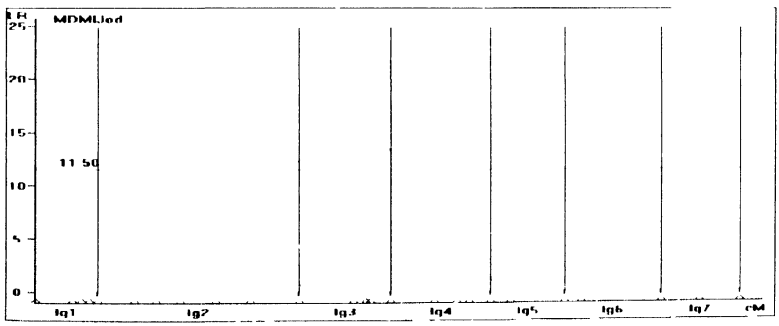


Table 11. QTL results for downy mildew resistance against a pathogen population from CAZRI, Jodhpur using QTL Cartographer composite interval mapping and Mapmaker/QTL interval mapping methods for single-QTL and multiple-QTL models

Using QTL Cartographer

Linkage group/ Marker	Position	Additive effect	Dominance effect	Likelihood ratio	Inheritance
LG2/M592 -M443	32 0 cM	4 1	-6 3	12 3	Over-dominant resistance from IP 18293
LG3/M473 -M686	14 0 cM	6 0	-6 4	13 7	Over-dominant resistance from IP 18293
LG6/M588 -M713	10 0 cM	14 2	-13 5	11 6	Over-dominant resistance from IP 18293

Using Mapmaker/QTL

Linkage group/ Marker	Position	Additive effect	Dominance effect	LOD	Variance explained	Inheritance
LG2/M592 -M443	32 5 cM	-6 2	-7 3	4 7	15 8%	Over-dominant resistance from IP 18293
LG3/M473 -M686	12 0 cM	-9 9	-8 6	6 3	26 9%	Over-dominant resistance from IP 18293
Two QTL model (Acceptable LOD score is 6.35 + 2.00 = 8.35)						
LG2/M592-M443	32 2 cM	-4 7	-6 8			Over-dominant resistance from IP 18293
LG3/M473-M686	10 6 cM	-9 7	-8 6	10 5	39 9%	Over-dominant resistance from IP 18293

Table 12. QTL results for downy mildew resistance against a pathogen population from RAU, ARS Durgapura, using QTL Cartographer, composite interval mapping and Mapmaker/QTL interval mapping methods for single-QTL and multiple-QTL models

Using QTL Cartographer

Linkage group/ Marker	Position	Additive effect	Dominance effect	Inheritance
-	-	-	-	-

Using Mapmaker/QTL

Linkage group/ Marker	Position	Additive effect	Dominance effect	LOD	Variance explained	Inheritance
LG3/M473-M686	8 0 cM	-6 9	-9 8	3 0	12 8%	Over- dominant resistance from IP 18293
LG7/M618-M526	14 0 cM	-8 6	-5 2	2 4	9 6%	Over-dominant resistance from IP 18293

Table 13. QTL results for downy mildew resistance against a pathogen population from IARI, New Delhi, using QTL Cartographer, composite interval mapping and Mapmaker/QTL interval mapping methods for single-QTL and multiple-QTL models

Using QTL Cartographer

Linkage group/ Marker	Position	Additive effect	Dominance effect	Inheritance
-	-	-	-	-

Using Mapmaker/QTL

Linkage group/ Marker	Position	Additive effect	Dominance effect	LOD	Variance explained	Inheritance
LG7/M618-M526	2.1 cM	-5.7	-2.5	2.3	8.9%	Over- dominant resistance from IP 18293

Figure 35. QTL likelihood map showing likelihood ratio (LR) for resistance to RAU, Duragapura pathogen population using the composite interval mapping method implemented in QTL Cartographer

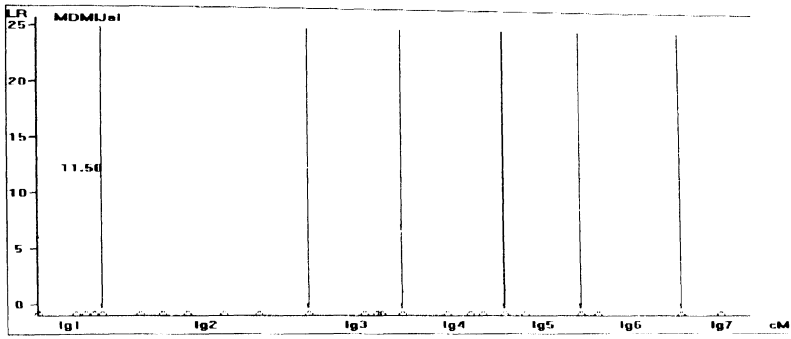
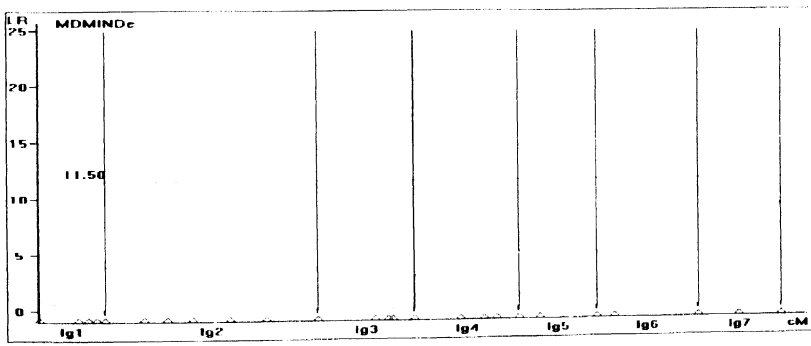


Figure 36. QTL likelihood map showing likelihood ratio (LR) for resistance to IARI, New Delhi downy mildew pathogen population from India using the composite interval mapping method implemented in QTL Cartographer



mapping method identified a single QTL in LG7 that could explain a low percentage (8.9%) of the observed phenotypic variation. The mode of inheritance of this downy mildew resistance QTL was over-dominant resistance from IP 18293 (Table 13).

4.5.7. Downy mildew resistance QTLs for the Sadore, Niger (African) pathogen population

For this pathogen population three downy mildew resistance QTLs were found using single-QTL model interval mapping as implemented in Mapmaker/QTL. These were located in linkage groups LG1, LG4 and LG6. Out of these three individually significant QTLs, the best one was observed on LG4. It had a high LOD value of 5.89 and individually explained 40.2% of observed phenotypic variation. The mode of inheritance for this QTL was over-dominant resistance from the resistant parent IP 18293. For the QTL on LG1, recessive resistance from IP 18293 was noted.

The critical LOD score for the two-QTL model was 7.89 (5.89+2.00). Among all two-QTL models, a high LOD value of 8.00 was observed for one with a QTL on LG1 (M515+3.0 cM) and the other on LG4 (*P*+7.0 cM). Compared to the single-QTL models, the position of the LG1 QTL has changed from M425 to M515. Similarly for the second QTL on LG4, a small shift was observed from *P*+12.0 cM in the single-QTL model to *P*+7.0 cM in the two-QTL model (Table 14). Neither of these change is significant and they are of no practical importance, as they would not affect marker choice for MAS.

In contrast, QTL Cartographer composite interval mapping showed three QTL peaks in linkage groups 2, 4 and 6 (Fig. 37). The QTLs on linkage groups 4 and 6 were the same ones that which Mapmaker/QTL identified. A new QTL detected on linkage group 2 might be an artifact or an expression of heterosis for downy mildew resistance. The remaining two QTLs on linkage groups 4 and 6 showed over-dominant susceptibility from the downy mildew susceptible parent Tift 238D1, which agrees with the recessive inheritance of resistance from IP 18293 in the single-QTL model interval mapping results from Mapmaker/QTL.

Table 14. QTL results for downy mildew resistance against a pathogen population from Sadore, Niger using QTL Cartographer composite interval mapping and Mapmaker/QTL interval mapping methods for single-QTL and multiple-QTL models

Using QTL Cartographer

Linkage group / Marker	Position	Additive effect	Dominance effect	Likelihood ratio	Inheritance
LG2/M592-M443	4.0 cM	0.5	-7.0	14.60	Artifact ? heterosis for resistance
LG4/M464-M716	0.0 cM	9.0	8.0	19.9	Over-dominant susceptibility from Tift 238D1
LG6/M588-M713	28.0 cM	8.1	9.7	16.1	Over-dominant susceptibility from Tift 238D1

Using Mapmaker/QTL

Linkage group / Marker	Position	Additive effect	Dominance effect	LOD	Variance explained	Inheritance
LG1/M425-M87.2	0.0 cM	-4.3	-2.8	2.3	7.8%	Over-dominant resistance from IP 18293
LG4/ *P*-M716	12.0 cM	-10.6	11.2	5.9	40.2%	Recessive resistance from IP 18293
LG6/M588-M713	28.0 cM	-9.4	10.9	2.6	47.1%	Recessive resistance from IP 18293
Two QTL model [5.89 + 2.00 = 7.89]						
LG1/M515-M425	3.0 cM	-4.22	-1.04			Over-dominant resistance from IP 18293
LG4/ *P* -M716	7.0 cM	-8.02	9.74	8.00	36.2%	Recessive resistance from IP 18293

4.5.8. Downy mildew resistance QTLs for the Bamako, Mali (Africa) pathogen population

Mapmaker/QTL detected two single downy mildew resistance QTLs for this pathogen population, one on LG1 and the other on LG4. But the composite interval mapping method from QTL Cartographer revealed only one QTL on the LG1, 4.0 cM away from marker locus *Xpsm*87.2 (Fig. 38).

From the interval mapping method, two QTLs were identified and the maximum LOD of these identified QTLs was 3.79 in the single-QTL model. Consequently, the critical LOD score for two-QTL models was 5.78 (3.78+2.00) (Table 15). A two-QTL model was accepted in which one QTL was on LG1 with the over-dominant resistance from IP 18293 and the second QTL was on LG4 with a recessive mode of inheritance from IP 18293. Together these two QTLs explained 37.5% of observed phenotypic variation in reaction of the 142 mapping progenies to this pathogen isolate.

Figure 37. QTL likelihood map showing likelihood ratio (LR) for resistance to Sadore, Niger downy mildew pathogen population from Africa using the composite interval mapping method implemented in QTL Cartographer

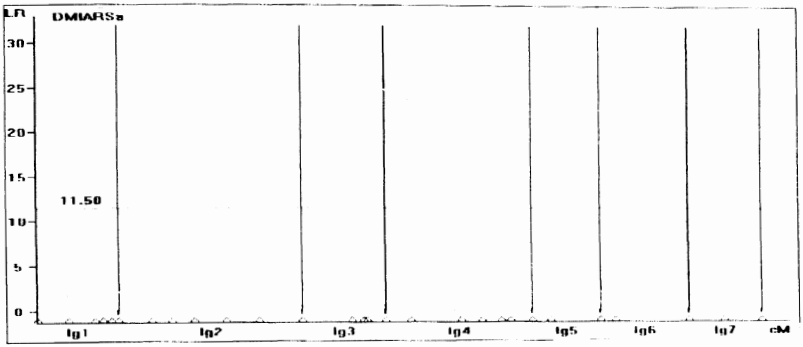


Figure 38. QTL likelihood map showing likelihood ratio (LR) for resistance to Bamako, Mali downy mildew pathogen population from Africa using the composite interval mapping method implemented in QTL Cartographer

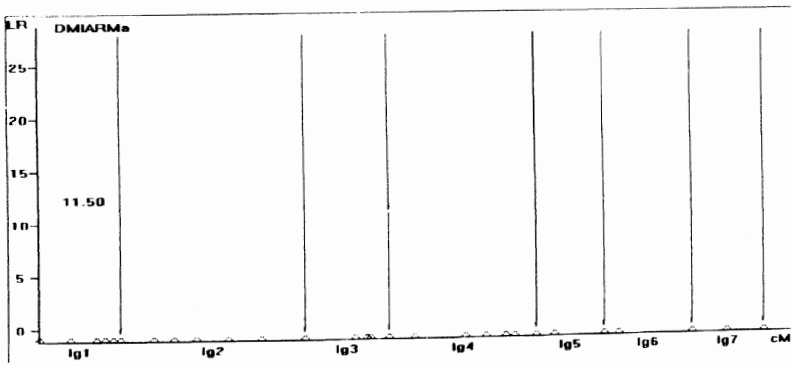


Table 15. QTL results for downy mildew resistance against a pathogen population from Bamako, Mali using QTL Cartographer composite interval mapping and Mapmaker/QTL interval mapping methods for single-QTL and multiple-QTL models

Using QTL Cartographer

Linkage group/ Marker	Position	Additive effect	Dominance effect	Likelihood ratio	Inheritance
LG1/M87.2-M341	4.0 cM	7.6	-2.2	19.4	Recessive susceptibility from Tift 238D1

Using Mapmaker/QTL

Linkage group/ Marker	Position	Additive effect	Dominance effect	LOD	Variance explained	Inheritance
LG1/M87.2-M341	4.0 cM	-7.2	-3.0	3.8	12.8%	Over- dominant resistance from IP 18293
LG4/ *P*-M716	16.0 cM	-9.9	8.6	3.8	26.9%	Recessive resistance from IP 18293
Two QTL model (Acceptable LOD score is 3.78+ 2.00 = 5.78)						
LG1/M087.2-M341	4.1 cM	-7.0	-1.0			Over- dominant resistance from IP 18293
LG4/ *P*-M716	13.4 cM	-9.8	7.4	7.8	37.5%	Recessive resistance from IP 18293

Discussion

5. DISCUSSION

5.1. DOWNY MILDEW INHERITANCE STUDIES

Segregation among the 142 F_2F_4 families for disease reaction against the Patancheru pathogen population gave best fits to the digenic ratios of 9:7 (for a break point of 20% DMI) and 15:1 (for a break point of 60% DMI), indicating the presence of two independently segregating dominant resistance genes (Table 2, Table 3 and Fig. 8) both of which must have at least one dominant resistance allele for family mean DMI <20%, and both of which must be homozygous recessive for susceptibility alleles for family mean DMI >60%. However the best fit for levels of susceptibility comparable to susceptible parent Tift 238D1 was the trigenic ratio of 63 resistant:1 susceptible, using a break point of 75% DMI.

Against Jalna pathogen population, it was clear that there are three genes controlling resistance with observed segregation fitting a series of trigenic ratios (37:27, 55:9 and 63:1), depending on the natural breakpoints used to separate resistant and susceptible progeny classes (Fig. 9).

Segregation patterns observed when the F_2F_4 families were screened against the Jamnagar pathogen population indicated three to four homozygous recessive genes were controlling susceptibility with ratios of 9:55, 9:7, and 13:3 (one is in homozygous recessive form) providing best fits to the observed distributions when considering the break points of 35, 55 and 90% DMI respectively (Table 2 and 3). When a break point at 15% DMI was used to separate resistant and susceptible progenies, a good fit to a 1:3 ratio suggested that one homozygous resistance gene was required for family mean DMI <15% (Fig. 10). Deswal and Govila (1994) reported that host plant resistance to downy mildew was controlled by duplicate gene action for the pathogen from one region of India (New Delhi) and by complementary interaction for the pathogen from another region (Tamil Nadu).

F_2F_4 families from the cross of resistant and susceptible pearl millet inbreds IP 18293 and Tift 238D1 segregated in a 3:1 ratio (resistant:susceptible) when screened against the pathogen population from Jodhpur and the natural break point of 20%

disease incidence was used to separate the resistant and susceptible classes (Fig 11) For this downy mildew pathogen population a single dominant gene appeared to condition resistance However it was also clear that as many as three homozygous recessive genes for susceptibility might be required for downy mildew incidence of >65% comparable to that of susceptible parent Tift 238D1

Resistance to the RAU-Durgapura and IARI-New Delhi pathogen populations also segregated among the 142 F_2F_4 families but the mean disease reactions of the families to these two pathogen strains were skewed strongly towards susceptibility (Fig 12-13) For these two pathogen populations the observed segregation patterns among the F_2F_4 families were observed to best fit ratios of 9 55 and/or 1 15 (moderately resistant susceptible) suggesting the role of epistatic interactions of 2 or 3 major genes in the control of disease reaction in this cross However it was not clear from this analysis whether the segregating resistance genes conferring disease reaction to these two pathogen populations were necessarily the same

A 9 55 ratio would indicate the presence of either a dominant inhibitor of resistance conferred by two duplicate dominant genes or the presence of a recessively inherited resistance that is required for effective expression of resistance conditioned by two additional duplicate dominant genes An alternative 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 that two dominant resistance genes of similar effect are only expressed when the recessive susceptibility allele is homozygous at the failed resistance gene 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 a pearl millet leaf spot disease caused by *Bipolaris setariae*

Disease reaction was also skewed towards susceptibility in screens against both of the African pathogen populations (Figs 14 and 15) Against the pathogen population from Bamako Mali it was observed that two homozygous/heterozygous dominant resistant gene and one homozygous recessive resistance gene were required to get a mean progeny downy mildew incidence of less than 65% and homozygous resistance

alleles" were required at all three loci for F_2F_4 family mean DMI <40%. But in the case of the pathogen population from Sadore, Niger, digenic interaction was noticed and homozygous resistance alleles were required at two independently segregating loci for family mean DMI <60%.

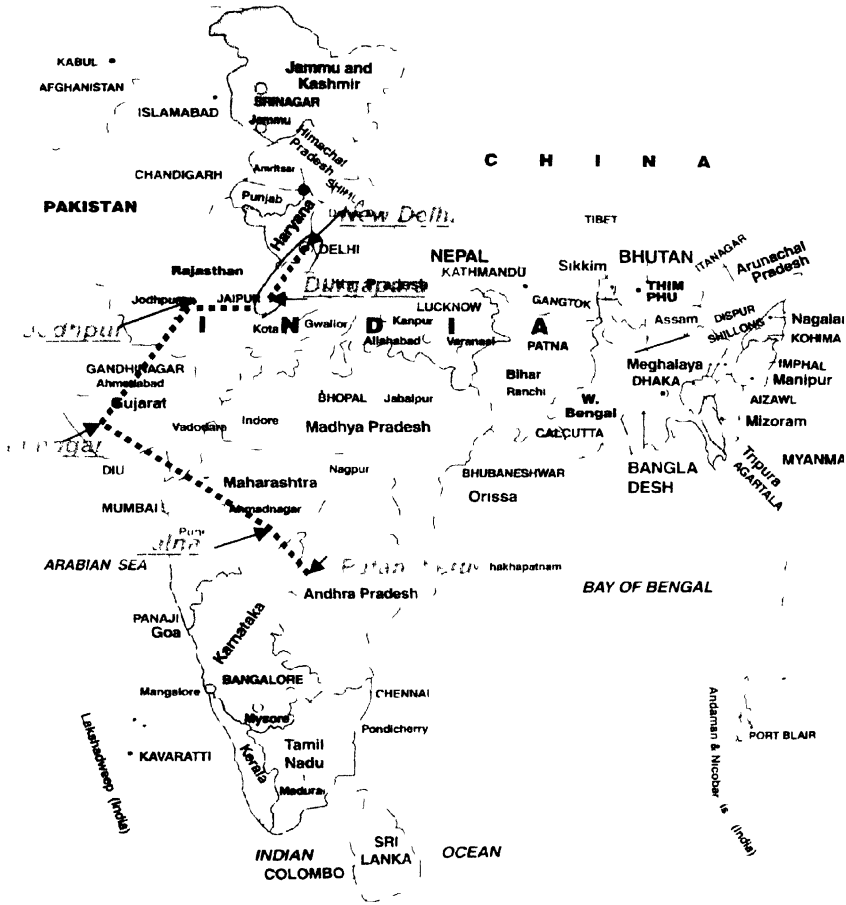
5.1.1. PEARSON RANK CORRELATION COEFFICIENT

Pearson rank correlation coefficient values based on disease reactions of the 142 F_2F_4 progenies used in this study (Table 4) showed that there are strong relationships between the pathogen populations from Jalna and Jamnagar; Patancheru and Jamnagar; and Patancheru and Jalna; as well as between those from Durgapura and New Delhi. These could be related to the distribution of geographical origins of these pathogen populations as Durgapura (in Jaipur, Rajasthan) and New Delhi are the two northern most locations from which pathogen populations were obtained in India for this study, while the southern-most were those from Patancheru and Jalna and the Jamnagar and Jodhpur pathogen populations originated from locations between these two extremes. Different host genotypes probably having been involved in shaping the virulence gene architectures of pathogen populations from these three regions of India (Fig. 39) Likewise strong relationships were observed between the two African pathogen populations based on mapping population F_2F_4 progeny rank in seedling disease screens.

5.1.2. CLUSTERING ANALYSIS

The dendrogram based on similarity in reaction of F_2F_4 families to India and African pathogen populations clearly showed two different groups among the pathogen isolates used in this study. One group is highly virulent (New Delhi, Durgapura, Niger and Mali) and another is less virulent (Patancheru, Jodhpur, Jalna and Jamnagar) (Fig. 16a). The dentrogram constructed using only Indian pathogen populations shows three groups: highly virulent (New Delhi and Duraganura), moderately virulent (Jodhpur and Jamnagar) and less virulent (Patancheru and Jalna) (Fig. 16b). So the northern Indian pathogen populations are shown to be more virulent than those from south and central India, at least against the array of host-plant resistances segregating in this particular pearl millet mapping population. This is supported by the results of the Pearson rank correlation analysis discussed above. Pathogen variability of pearl millet downy

Figure 39. Map showing the distribution of Indian pearl millet downy mildew pathogen populations used to screen in this (IP 18293 x Tift 238D1)-based pearl millet mapping population



mildew population and their host-cultivar reactions to different pathogen populations have previously been studied by Ball (1983), Thakur *et al.* (1997). In contrast to those earlier studies, this clustering analysis has provided useful groupings of pearl millet downy mildew pathogen populations that can be used by breeders and pathologists in planning future downy mildew resistance gene deployment strategies

5.2. PARENTAL POLYMORPHISM

As expected, the amount of marker polymorphism (40%) exhibited in this pearl millet mapping population (IP 18293 × Tift 238D1) is higher than that normally found in predominantly self-pollinating cereal crop species like rice (McCouch *et al.*, 1988) and wheat (Chao *et al.*, 1989; Devos *et al.*, 1992). In barley the amount of polymorphism observed was less than 28% (Graner *et al.*, 1991; Heun *et al*, 1991). Normally in inbreeding species, the level of polymorphism is expected to be generally lower than in the out-crossing species such as pearl millet (Miller and Tanksley, 1990). Naturally out-crossing crop species tend to have high levels of DNA polymorphism and virtually any cross that does not involve related individuals will provide sufficient marker polymorphism (Helentjaris, 1987). In pearl millet Liu *et al.* (1994) reported 56% average pair-wise polymorphism for probe-enzyme combinations among elite hybrid parental lines. In this study, the percentage of polymorphism varied with the restriction enzymes. The restriction enzyme *Hind*III recorded the highest level of polymorphism followed by *Dra*I, *Eco*RV and *Eco*RI. Base substitutions in the restriction sites are responsible for the difference in reaction of restriction probe-enzyme combinations. Out of 220 probe-enzyme combinations, only two probes revealed polymorphism against all four restriction enzymes considered (*Hind*III, *Dra*I, *Eco*RV and *Eco*RI). Likewise only six probes showed monomorphism across all four of these restriction enzymes.

5.3. MARKER SEGREGATION AND SEGREGATION DISTORTION

In this (IP18293 × Tift 238D1)-based mapping population, 67% of the marker loci segregated per the expected ratio of 1:2:1 Segregation distortions can be the result of genetic elements that exhibit the phenomenon of meiotic drive, that is, the mechanics of the meiotic divisions cause one member of a pair of heterozygous alleles or heteromorphic chromosomes to be transmitted to progeny in excess of the expected Mendelian proportion of 50% (Sandler and Novitski, 1957; Sandler and Goli, 1985, Lyttle, 1991). They can also occur due to gametic selection (especially among male

gametes of the selfed F_1 plants that contribute to F_2 seed production or through selective influences of the gynoecium including genetic including genetic incompatibility), environmental effects, and differential competitive ability of genetically variable pollen (Lyttle, 1991; Xu, 1997). Segregation distortion has been reported in a wide range of organisms, including plants in which species or strain 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) As an extreme example, a rice intraspecific recombinant inbred population (CO39/Moroberekan) was reported to have 98.8% of marker loci showing skewing towards the indica parent (Wang *et al.*, 1994). Segregation distortion is most commonly observed in interspecific crosses; however, previous studies showed distortion phenomena also occur in intraspecific pearl millet crosses (Liu *et al.*, 1994; Busso *et al*, 1995).

In this (IP 18293 × Tift 238D1)-based mapping population about 33% of the marker loci showed distorted segregation. Interestingly, in the segregation distorted marker loci, the proportion of male parent Tift 238D1 alleles was commonly found in excess to that for female parental alleles (except in one marker locus *Xpsm*459.1), when compared to the expected female and male parent ratio of 1:1. This kind of segregation distortion with biased skewed marker towards the alleles from a male parent has previously been reported in pearl millet cross LGD × ICMP 85410 (Liu *et al*, 1994). In both of these cases, the female parent of the cross producing the pearl millet mapping population had at least one major negative fitness attribute that could be expected to distort segregation in linked genomic regions. The reasons for this kind of distortion could be due to gametic or hybrid selection for genes affecting fitness at different developmental stages (O'Donoughue *et al.*, 1992) so that the sample of F_2F_3 plants used to provide tissue 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 this (IP 18293 × Tift 238D1)-based mapping population of 142 F_2F_4 progenies, the marker loci with highest degree of segregation distortion were found in LG5 (*Xpsm*815, *Xpsm*318 and *Xpsm*735.1) with surplus alleles from pearl millet downy

mildew susceptible parental line Tift 238D1 In LG2 distortions with increased frequency of heterozygotes were noticed for loci *Xpsm458*, *Xpsm214* and *Xpsm592* Marker loci in the top and the bottom of the LG7 also showed distortion with higher than expected frequencies of heterozygotes From these results we can conclude that at least three distortion-causing loci are present in this segregating population one each on LG2, LG5 and LG7 It has previously been reported that such segregation distortion is likely to be a general property of pearl millet (Liu *et al* , 1994)

5.4. LINKAGE MAPPING

The base map for pearl millet has a length of 287 7 cM and was produced for an F_2 population from the cross LGD-1-B-10 × ICMP 85410 (Liu *et al* , 1994) This base map was used for comparison with the present skeleton map and this is presented in the Figure 29 In the present study a linkage map of 561 8 cM length was constructed for an F_2 population based on the cross IP 18293 × Tift 238D1 Despite the substantial increase over the base map, still this is the shortest in terms of genome map length among all major cereals For an example RFLP marker-based genetic linkage map of rice constructed with approximately 800 probes had a length of 1491 cM (Causse *et al* , 1994) and with the addition of new markers has later reached a length of 1680 cM (Price *et al* , 2000) In barley a 1453 cM length linkage map has been reported (Graner *et al* , 1991, Heun *et al* 1991) For sorghum an RFLP-based linkage map of 1530 cM length has been constructed using maize and sorghum genomic probes (Pereira *et al* , 1994, Subudhi and Nguyen, 2000) 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 genetic linkage map of rye (*Secale cereale* L), a cross-pollinated diploid like pearl millet, has recently been extended to 1140 cM by addition of RAPD and isozyme markers (Masojc *et al* , 2001) In pearl millet, to locate the QTLs of our interest in a new cross, a skeleton map of with less than 40 probe-enzyme combinations has been constructed with an average map distance of <20 cM between markers (Liu *et al* , 1994)

Liu *et al* (1994) expected an increase in the length of the pearl millet linkage map This expectation has been borne out by subsequent mapping studies in pearl millet using different parental combinations The increase in map length has been achieved by adding new RFLP markers from pearl millet and other cereal crops (Devos *et al* , 2000) along with AFLP and SSR markers John Innes Centre, UK is the institute responsible

for the pearl millet consensus map and it has most recently updated this in the year 1996 The most current version is readily accessible through the web (http //jiio5 jic bbsrc ac uk 8000/cgi-bin/webace?db=millet)

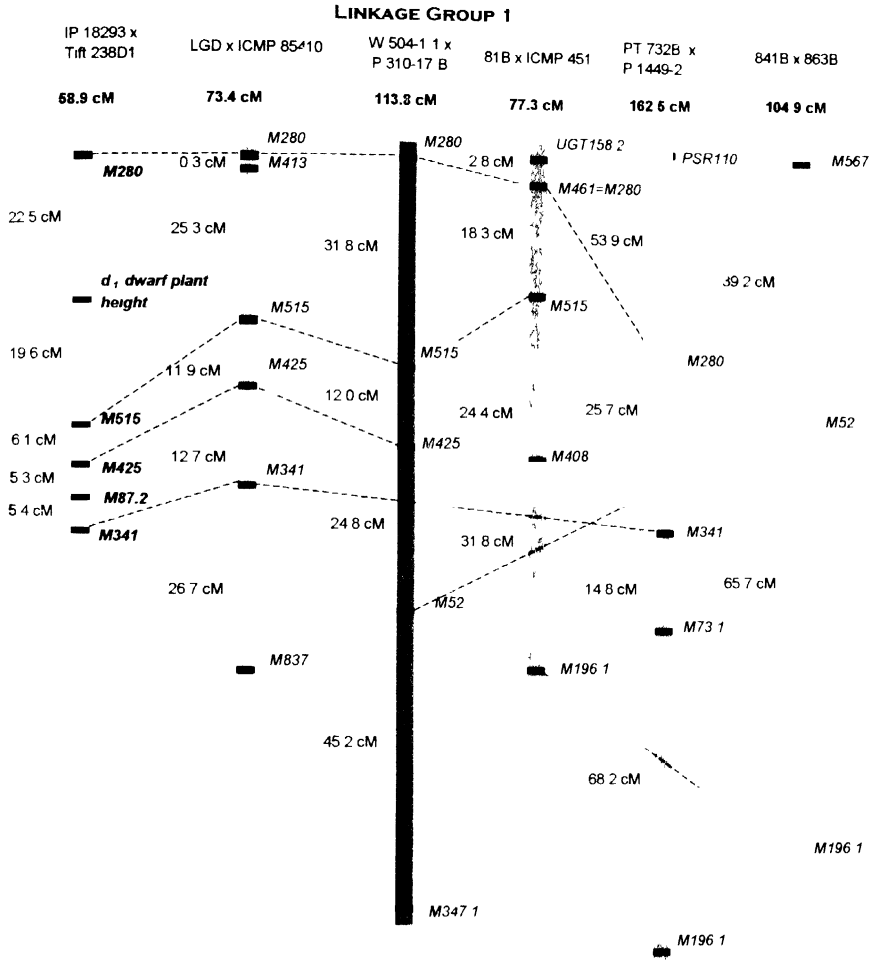
Marker order and the length of the map obtained in the present study are compared below with the published base pearl millet map of Liu *et al* (1994) Along with this previously published map, other recently constructed maps based on crosses 841B × 863B, 81B-P6 × ICMP 451-P6 and PT 732B × P 1449-2 (John Innes Centre Devos *et al*, unpublished) and W 504-1-1 × P 310-17-B (ICRISAT Maria Kolesnikova-Allen personal communication) The total length of these previously developed maps ranged from 287 7 cM (LGD × ICMP 85410) to 695 2 cM (81B-P6 × ICMP 451-P6) with an average map length of 500 75 cM which is nearly equal to the newly constructed map reported here

For an easy comparison the available pearl millet linkage maps including the present one have been numbered from one to six Map number one is the newly developed map from based on cross IP 18293 × Tift 238D1 and map number two is the base map reported by Liu *et al* (1994) based on cross LGD-1-B-10 × ICMP 85410 Likewise, map numbers 3 4, 5 and 6 represent W 504-1-1 P 310-17-B 81B-P6 ICMP 451-P6 PT 732B × P 1449-2 and 841B × 863B respectively Comparison has made on an individual linkage group basis (Figs 32-39) Total length of each linkage group is given on the top and common markers across the maps and are joined together with dotted lines for easy comparison

5.4.1. LINKAGE GROUP 1

Marker order of LG1 is same across all six mapping populations However the present population has comparatively short map length of only 58 9 cM (Fig 40) The reason could be because only pearl millet RFLP makers have been used in this study whereas for the maps 5 and 6 additional markers from rice RFLP probes and other markers from SSRs have been used An alternative explanation is that no polymorphic probe-enzyme combinations between parents IP 18293 and Tift 238D1 were detected for map positions below *Xpsm*341 on this linkage group In the present study a clear separation of LG1 and LG2 was noted, but in the original base map a pseudo-linkage of

Figure 40. A comparison of the genetic linkage maps for linkage group 1 in five previously available pearl millet mapping populations with the new map based on cross IP 18293 x Tift 238D1



LG1 and LG2 was observed. The maximum length of LG1 among all the existing maps is from map 5 (162.5 cM) followed by map 3 (113.8 cM). The average distance of LG1 in this newly developed map is 9.81 cM and markers are fairly well dispersed across the whole linkage group except in the bottom portion. The d_1 dwarfing gene locus is placed near the top of this linkage group.

5.4.2. LINKAGE GROUP 2

There is an increase in map length for this linkage group in the first mapping population compared with mapping populations 2 and 3. A small crossover in the order of markers was noticed between the locus *Xpsm*322 and *Xpsm*592 in map 5, but basically the marker orders are the same in all maps (Fig. 41). Markers were very well distributed in map 1 and the recombination level is particularly high in this linkage group in this mapping population. Without addition of new markers, the length of map 1, LG2 has been almost doubled compared that in map 2 (the original base map). The lengthiest map for this linkage group was observed in map 6 with 179.0 cM followed by 175.8 cM in map 4, both of which included two additional loci detected with RFLP probe PSM708 (at the top and bottom of the linkage group).

5.4.3. LINKAGE GROUP 3

Basically there were no differences in the order of the marker loci detected in this linkage group across the six maps (Fig. 42). The first map from the present study has given the second longest linkage group 3 with a length of 62.9 cM. Even though the map order and the common markers are same as that of the base map, the new linkage map has almost doubled the map length for LG3. In particular, the gap between the marker loci *Xpsm*37 and *Xpsm*108 has been increased by nearly five times compared to the base map reported (Liu *et al.*, 1994). Part of this may be due to the use of the Kosambi mapping function in that earlier study, and use of the Haldane function in the subsequent ones, but this alone is not enough to explain the increased length observed for the (IP 18293 × Tift 238D1)-based LG 3 map.

5.4.4. LINKAGE GROUP 4

LG4 in map 1 is quite lengthy at 110.9 cM. The reason might be the two additional morphological marker loci of for *P* purple foliage colour and d_2 dwarf plant height (Fig. 43). Newly placed AFLP markers and SSR markers in map 4 increased the

Figure 41. A comparison of the genetic linkage maps for linkage group 2 in five previously available pearl millet mapping populations with the new map based on cross IP 18293 x Tift 238D1

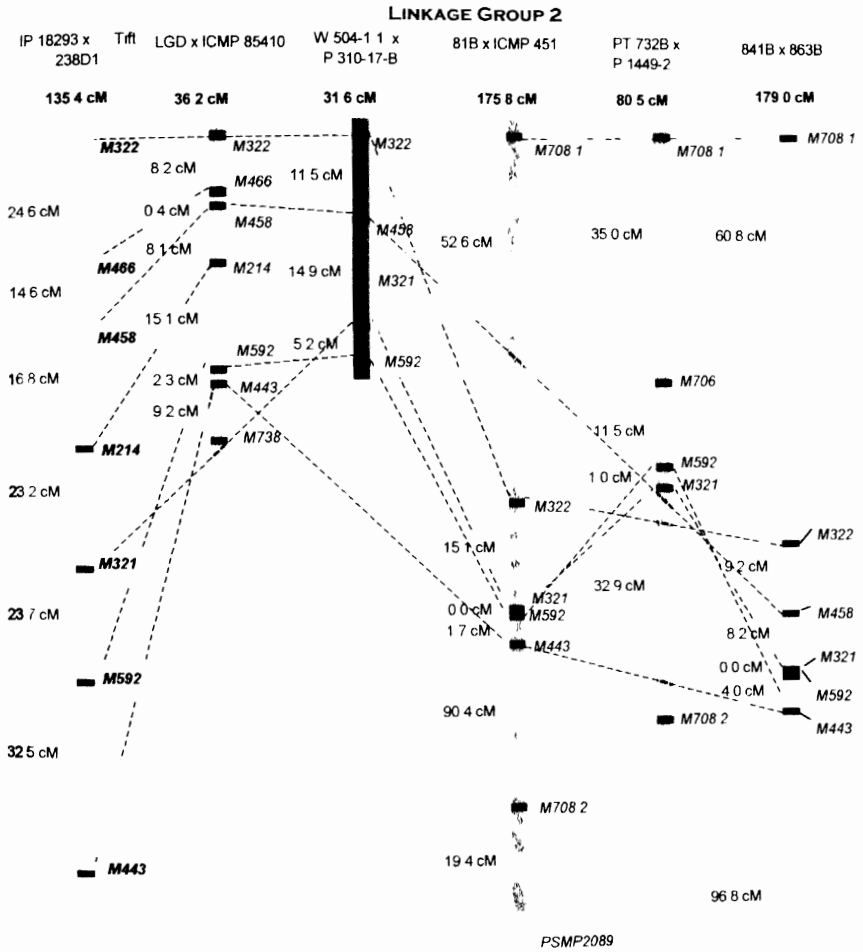


Figure 42. A comparison of the genetic linkage maps for linkage group 3 in five previously available pearl millet mapping populations with the new map based oncross IP 18293 x Tift 238D1

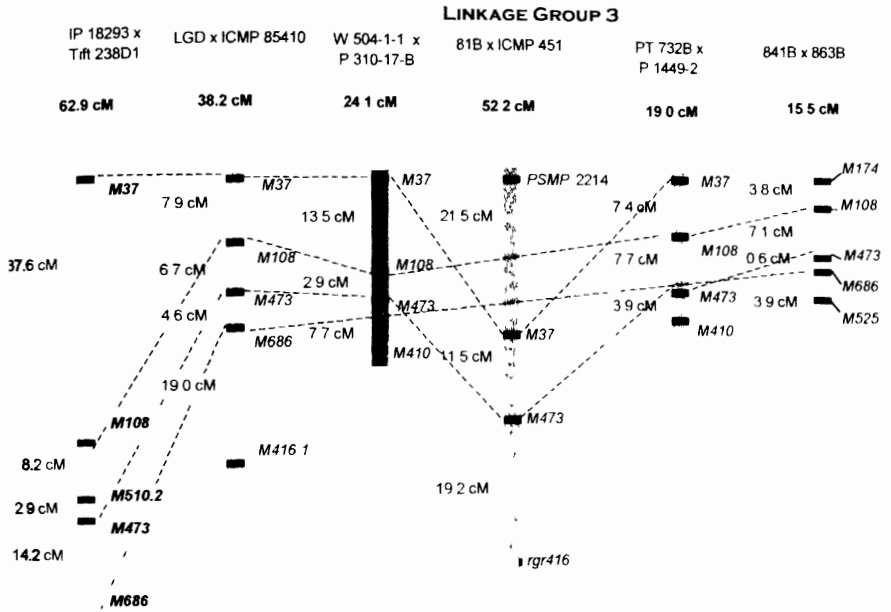
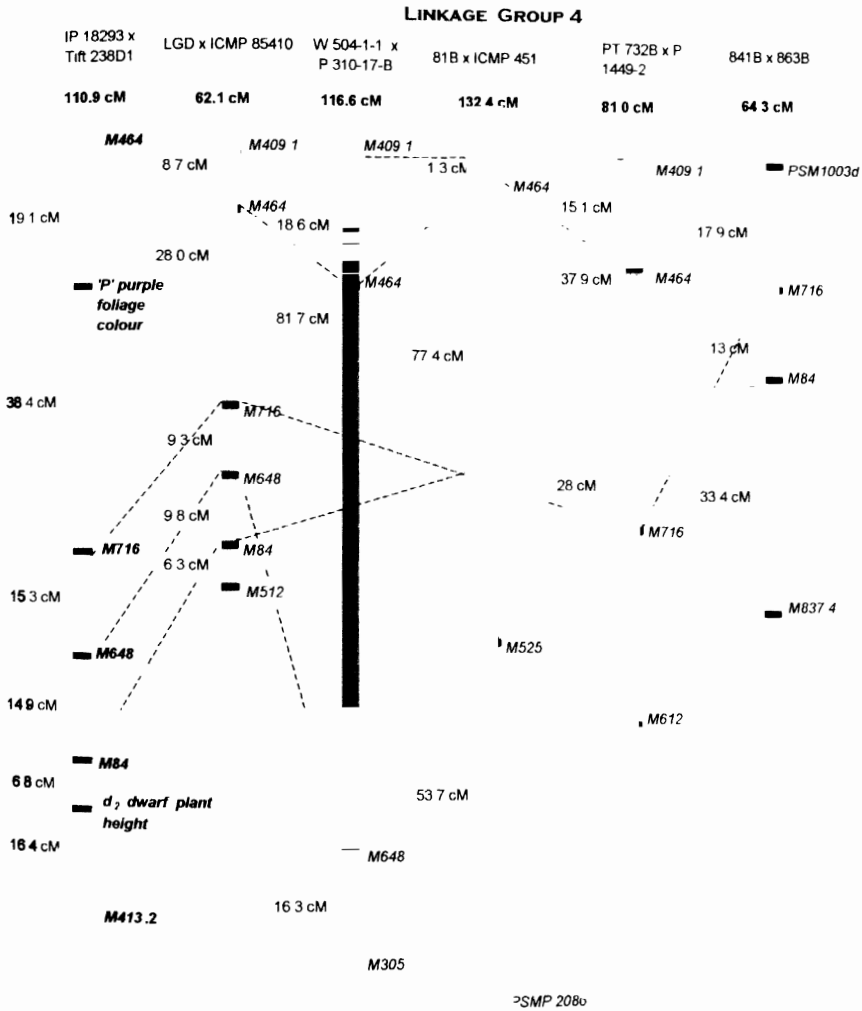


Figure 43. A comparison of the genetic linkage maps for linkage group 4 in five previously available pearl millet mapping populations with the new map based on cross IP 18293 x Tift 238D1



map lengths for this linkage group to 132 4 cM Marker order is the same as that of the base map LGD × ICMP 85410 In the top of the LG4 of map 1 (newly developed map) the distance between marker loci *Xpsm*464 and *Xpsm*716 has doubled in comparison with the base map (map 2) with the inclusion of one morphological marker for purple foliage colour Basically all the markers were dispersed evenly through out the linkage group 4 in map 1 with an average distance of 18 5 cM A marker detected by PSM413 a low copy probe has been placed at the bottom of this linkage group whereas in the base map the locus detected by this probe was placed on the top of LG1 (Fig 40) so here the locus is *Xpsm*413 2

5.4.5. Linkage group 5

LG5 is the shortest one (51 9 cM) in the newly developed map (map 1) with only three marker loci but these are very well distributed throughout its length (Fig 44) Also this 51 9 cM is the second longest for this linkage group among all six maps Map 4 is the longest for this linkage group of 102 9 cM due solely to the presence of newly added pearl millet SSR series markers at both ends

5.4.6. Linkage group 6

A nearly two-fold increase in the length of LG6 of the newly developed map 1 has been observed as compared with the base map Mainly this was because of the increase in the distance between the marker loci *Xpsm*588 and *Xpsm*713 (Fig 45) which is an artifact of the different mapping functions used in calculating map 1 (Haldane) and map 2 (Kosambi) Even though only three markers were used in map 1 for this linkage group these are dispersed through its whole length Marker order remains the same as in the remaining five maps for this linkage group The map from 841B x 863B is the longest for this linkage group because of the newly linked *Xpsm*870 marker for which linkage to *Xpsm*713 was only detectable following the addition of the intervening SSR marker *Xpsmp*2002

5.4.7. Linkage group 7

There is a clear indication of increase in the length of LG7 in map 1 compared with maps from the other five pearl millet mapping populations (Fig 46) As for other linkage groups maps 4 and 6 reported greater map length because of added SSR marker loci (in this case *Xpsmp*2013 and *Xpsmp*2033)

Figure 44. A comparison of the genetic linkage maps for linkage group 5 in five previously available pearl millet mapping populations with the new map based on cross IP 18293 x Tift 238D1

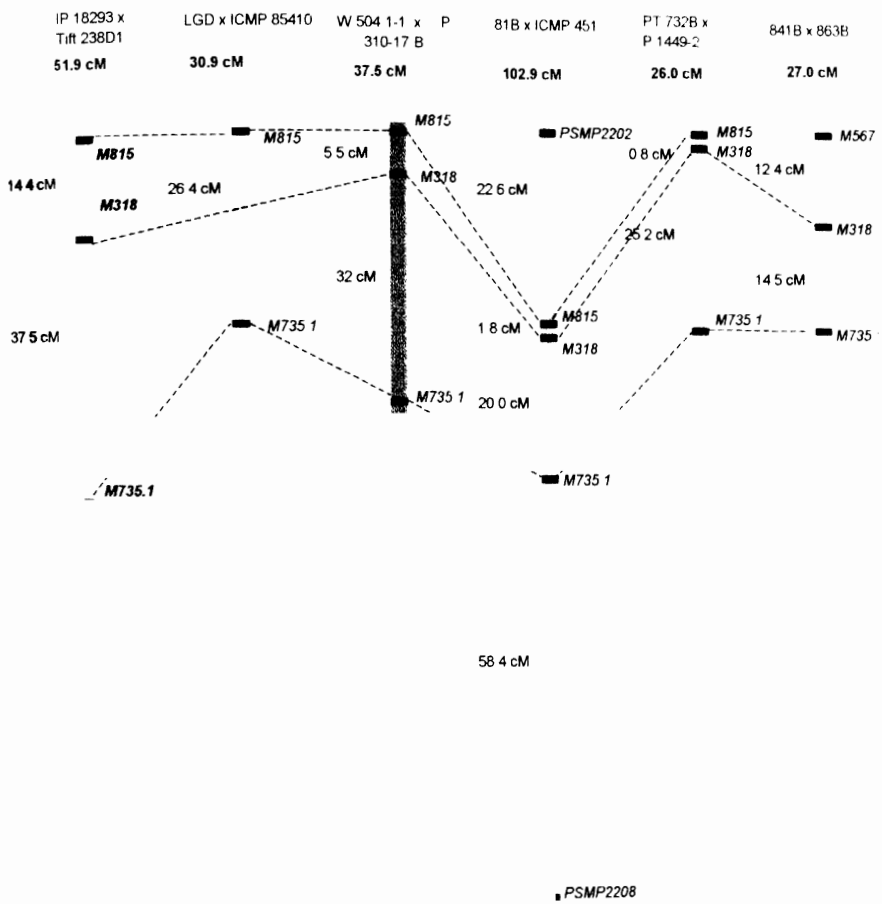


Figure 45. A comparison of the genetic linkage maps for linkage group 6 in five previously available pearl millet mapping populations with the new map based on cross IP 18293 x Tift 238D1

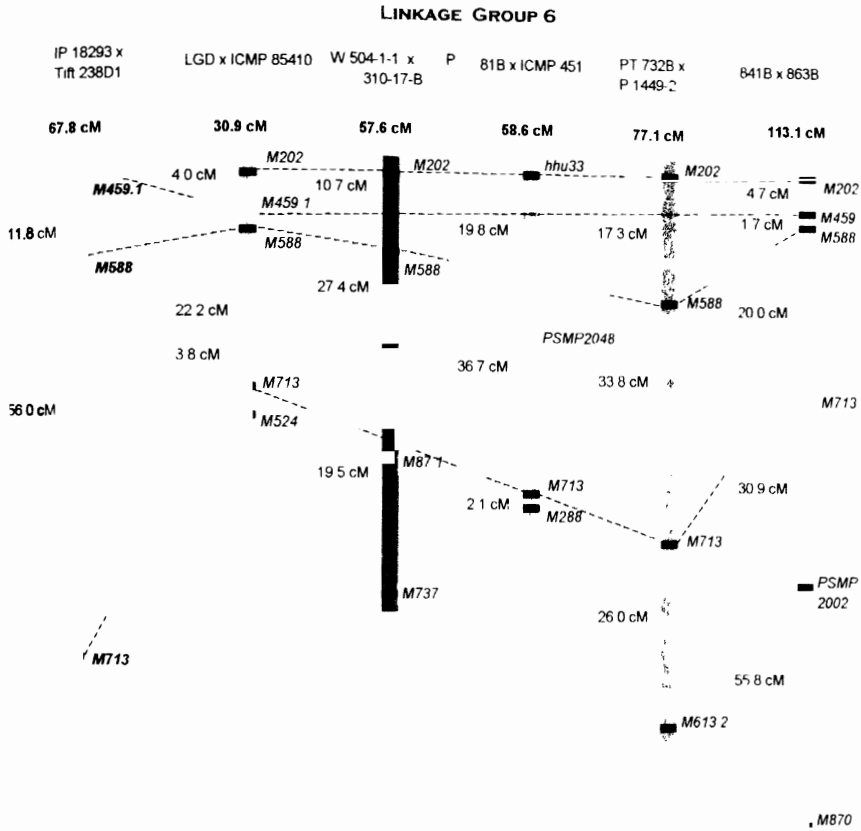
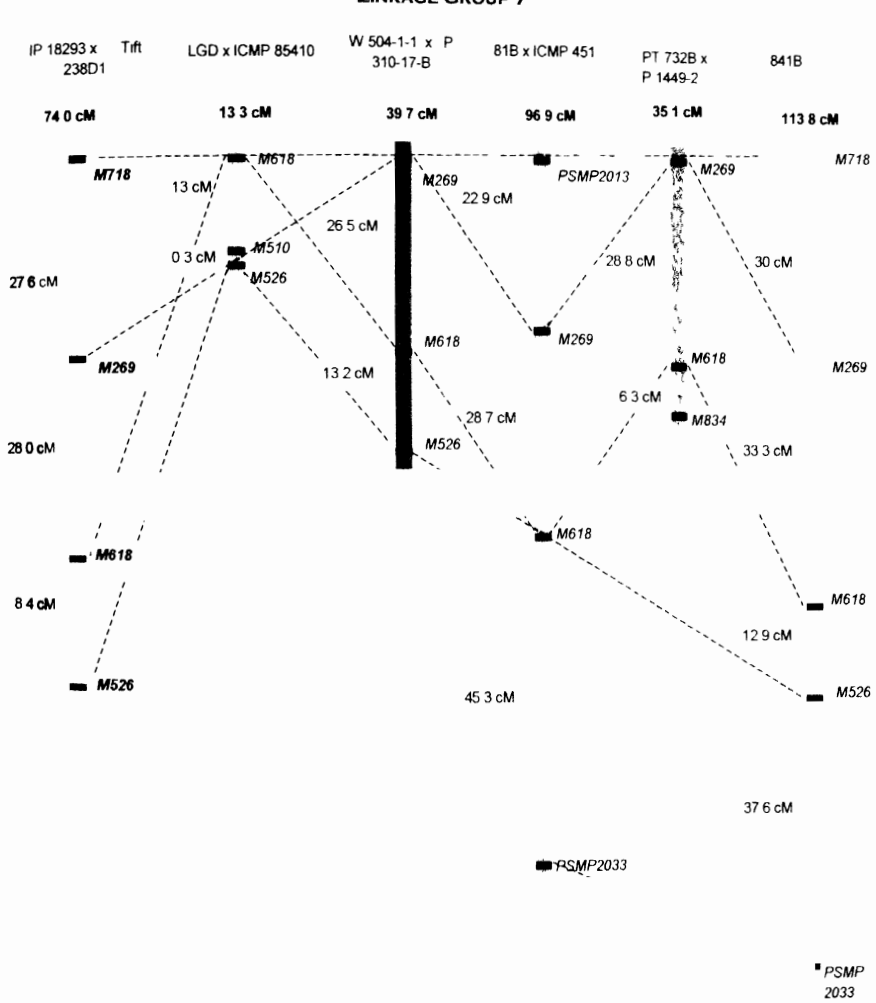


Figure 46. A comparison of the genetic linkage maps for linkage group 7 in five previously available pearl millet mapping populations with the new map based on cross IP 18293 x Tift 238D1



5.5. PLANT HEIGHT

Plant height is generally considered to be a relatively simply inherited trait with a few loci largely affecting its expression In this experimental population an attempt was made to place the d_1 and d_2 dwarfing genes on an RFLP-based genetic linkage map The results of interval mapping as implemented in Mapmaker/QTL suggested that there were two QTLs responsible for the d_1 dwarf plant height, one QTL on the top of LG1 and the other at the bottom of this same linkage group However QTL Cartographer results indicated that there was only one QTL for d_1 dwarf plant height on LG1 which was controlled by recessive dwarf alleles from male parent Tift 238D1 The d_2 dwarfing gene from parent IP 18293 was located on LG4 by both software packages The segregation of two independently inherited dwarfing genes was confirmed in a significant two-QTL model for quantitatively assessed plant height by Mapmaker/QTL The purple foliage colour locus *P* was also placed in this newly constructed linkage map It fits well near the top of LG4 between RFLP marker loci *Xpsm*464 and *Xpsm*716 These placements of morphological marker loci were done hypothetically and may be prone to large error Once the integration of RFLP and the morphological markers has been accomplished, probability of statements can then be made concerning the association between a QTL and the qualitative genetic loci However, tests for allelism using molecular techniques will be necessary to confirm any putative association

5.6. QTL MAPPING FOR DOWNY MILDEW RESISTANCE

QTL analysis detects chromosomal regions that contain genes for quantitative traits This approach can be used by breeders to predict quantitative effects for specific genotypes by the analysis of their DNA marker profiles Pearl millet downy mildew resistance has historically been considered to be quantitative trait, significantly affected by the environment Host plant resistance against downy mildew was continuously distributed in the F_2F_4 progenies used in this study as has been found in most previous studies on the genetics of pearl millet downy mildew resistance (Singh *et al*, 1980, Basavaraju *et al*, 1981, Dass *et al*, 1984, Shinde *et al*, 1984) However this does not necessarily imply that its inheritance is complex and that many resistance genes are segregating It is usually difficult to obtain accurate and unbiased data for this type of quantitative trait But getting accurate phenotypic data without the influence of environment is an always indispensable prerequisite for precise QTL mapping (Paterson *et al*, 1991, Liu *et al*, 1994) The larger the environmental effect on the character (i e

Table 16. Significant QTLs for downy mildew resistance against Indian and African pathogen populations from the results of Mapmaker/QTL

Single-QTL model							Qualified multiple-QTL model				
Pathogen[a]	LG[b]	Position[c]	LOD[d]	Variation[e]	Add[f]	Domi[g]	Position	Variation	Add	Domi	Inheritance[h]
Indian											
Patancheru	2	M592+12.0 cM	4.41	22.6%	-11.07	-8.84					OD-IP
	6	M588+34.0 cM	7.94	63.4%	-27.92	-29.44					Artifact ?
Jodhpur	2	M592+32.0 cM	4.71	15.8%	-6.24	-7.28	M592+32.2 cM		-4.73	-6.86	OD -IP
	3	M473+12.0 cM	6.85	26.9%	-9.92	-8.56	M473+10.6 cM	39.9%	-9.69	-8.61	OD-IP
Jalna	2	M592+0.0 cM	7.41	23.6%	-11.97	-3.71					D-IP
Jamnagar	2	M592+10.0 cM	11.4	52.3%	-21.00	-12.68					OD-IP
New Delhi	7	M618+4.0 CM	2.3	9.3%	-6.01	-2.24					OD-IP
Durgapura	3	M473+8.0 cM	8.0	12.8%	-6.82	-9.76					OD-IP
African											
Niger	1	M425+0.0 cM	2.32	7.8%	4.25	-2.97					OD-IP
	4	*P*+12.0 cM	5.89	40.2%	-10.56	11.29	M515+3.0 cM		-4.22	-1.04	R-IP
	6	M588+28.0	2.55	47.1%	-9.35	10.94	*P*+7.0 cM	36.21%	-8.02	9.74	R-IP
Mali	1	M087+4.0 cM	3.75	12.8	-7.22	-3.02	M87.2+4.1 cM		-7.06	-1.03	OD-IP
	4	*P*+16.0 cM	3.78	26.9	-9.92	8.61	*P*+13.4 cM	37.5%	-9.80	7.35	R-IP

[a] Downy mildew pathogen population
[b] Linkage groups
[c] Position of the QTL with the marker loci
[d] Log Likelihood score
[e] Percentage of variation explained for downy mildew resistance
[f] Additive effect
[g] Dominance effect
[h] Estimated mode of inheritance for resistance.
(OD = Over-dominance, D = Dominance, R = Recessive, IP = Resistant parent IP 18239)

the lower its heritability), the less likely that statistically significant QTL(s) will be detected Estimates of heritabilities can be improved by controlling environmental errors and increasing replication So in order to reduce environmental effects in our present study an improved inoculation method was used with replication of progenies screened in time (at Patancheru) or in space (at Bangor)

Fairly detailed linkage maps developed so far in many crop species have determined that for many traits traditionally thought of as being polygenically inherited a small number of QTLs can control a large proportion of the observed variation (Masur *et al*, 1993, Kjear *et al*, 1991, Paterson *et al*, 1991) Even with fairly high heritabilities segregation of just two genes can result in a continuous distribution This is particularly so in the present study as the progeny screened were replicated F_4 families in which segregation of heterozygotes would result in less distinct phenotypic classes than if the F_2 population itself had been screened under conditions of perfect heritability (but no replication) (Jones *et al* 1995)

At least seven different downy mildew resistance QTLs effective against pathogen populations of downy mildew from India and Africa were detected segregating in the (IP18293 · Tift 238D1)-based mapping population and are tabulated in Table 16 Although independent inheritance of resistance to different populations of *Sclerospora graminicola* has been observed in previously published studies a common resistance QTL was found against four Indian pathogen populations (Patancheru Jalna Jamnagar and Jodphur) in this present study These pathogen populations appear to be less virulent than the rest of the pathogen populations used in this study This particular QTL was located on LG2 near to the marker locus *Xpsm*592 with its position varying within a distance of 0 0 to 32 00 cM across these four pathogen populations This resistance QTL appeared to be most effective against the pathogen population from Jamnagar against which it accounted for 52% of observed phenotypic variation among the mapping population of F_4 progeny bulks

A resistance QTL for the pathogen population from Jodhpur was identified on LG3 in addition to the common QTL on LG2 (Mb92) This QTL on LG3 was found for both the Jodhpur and Duragapura pathogen populations Likewise for the Patancheru pathogen population, a possible resistance QTL on LG6 (perhaps an artifact) of IP

18293 was observed in addition to the common one on LG2 These additional QTLs were specific to individual pathogen populations or groups of pathogen populations from geographically adjacent locations Many previous reports have suggested that differences in host reaction to pearl millet downy mildew at different locations were because of pathogen variation (Bhat 1973 Girard 1975 Ahmad *et al* 1978 Thakur *et al* 1978 Shetty and Ahmed 1981 Thakur *et al* 1992)

Results from the screening of (IP18293 Tift 238D1)-based mapping population $F_2 F_4$ families showed that African pathogen populations from Niger and Mali were more virulent than most of the Indian pathogen populations Among the six Indian pathogen populations the two from New Delhi and Durgapura were found to be most pathogenic on these F_4 families

In this study it was clear that there were different resistance QTLs effective against the Indian and African pathogen populations But across the two African populations a common resistance QTL was identified on LG4 From the QTL mapping results for Niger other than this common QTL two more resistance QTLs were identified on LG1 and LG6 These seemed to be specific to this pathogen population (the latter may in fact have been an artifact as the significant LOD peak did not extent to either flanking marker) Likewise for the Mali pathogen population a QTL on LG1 was identified This QTL was probably different from that effective against the pathogen population from Niger 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 of *Sclerospora graminicola* populations In contrast to the results the earlier investigation of Jones *et al* (1995) reported several downy mildew resistance QTLs segregating in the (LGD x ICMP 84510)-based mapping population that were against individual pathogen populations but found only one possible block of resistance QTLs effective against more than one African downy mildew pathogen population

For the pathogen populations from Jalna Jamnagar and New Delhi only a single QTL was detected despite of a continuous segregation for resistance It is therefore likely that other resistance QTLs were also segregating but were not detected This could have been because of these QTLs explained a very low proportion of variation in

disease reaction, or that the effect of QTLs were masked by epistatic interactions of the genes, the number of marker loci were not sufficient to locate the resistance QTLs, or the power and precision of the analysis was insufficient to detect more number of QTLs because of the relatively small mapping population size and short map length. However, distribution of the F_4 families was strongly skewed towards susceptibility in screens against both the New Delhi and Durgapura pathogen populations. This may be yet another reason for the failure to locate more QTLs for resistance to these two pathogen populations. If QTLs remained undetected in these two screens, then it is likely that there were also more QTLs contributing towards resistance that have genes undetected in other screens. It was also noted that for all the screens in the current study, the portion of variation in disease reactions among the F_4 self bulk progenies that was explained by detected QTLs varied from 7.8 to 53.2%. So obviously, more resistance QTLs exist that still remain undetected.

For all the resistance QTLs detected in the current study, an increase in downy mildew resistance was inherited from the resistant female parent of the mapping population, IP 18293. For many QTLs, the mode of inheritance of resistance was estimated to be over-dominance with one or two recessive resistances in case of African pathogen populations and one dominant resistance for the Jalna pathogen population. 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.*, 1978; Pethani *et al.*, 1980; Basavaraju *et al.*, 1981; Shinde 1984; Mehta and Dang, 1987). In the present study, except for a few QTLs, all resistance loci detected were characterized by over-dominance. Several earlier studies (Jones *et al.*, 1995; Singh *et al.*, 1978; Dass *et al.*, 1984) have also revealed over-dominance as a component the downy mildew resistance inheritance in pearl millet.

Two resistance QTLs of small effect, effective against the Sadore, Niger pathogen population (one of which was also effective against the Bamako, Mali pathogen population) showed recessive inheritance of resistance from resistant parent IP 18293. Previous studies (Singh *et al.*, 1978) on the downy mildew inheritance in pearl millet reported recessive resistance genes and this type of resistance inheritance has also been observed in other plant-pathogen systems (Day, 1974; De Wit, 1992).

Earlier studies (Paterson *et al.*, 1988; Stuber *et al.*, 1992; Bubek *et al.*, 1993; Pé *et al.*, 1993; Van Ooijen, 1994) compared different methods for identification of QTLs results and showed that there were no major differences in the QTL maps produced. In the present study along with the interval mapping procedure implemented in Mapmaker/QTL, composite interval mapping as implemented in QTL Cartographer was used. Here too, there were no major differences recorded in the QTL maps obtained by these two procedures.

5.7. General Discussion

In this QTL mapping study, some of the QTLs for downy mildew resistance were found to be non-pathogen population specific. Such disease resistances are expected to be durable for a number of years. These resistance QTLs are of considerable interest because the durability of resistance is major concern for plant breeders. Several pathogen populations specific QTLs were also identified. Such disease resistances tend to have reduced durability as all that is required for them to be overcome by the pathogen is for them to be deployed in or near regions where the counterpart virulence is already present in the pathogen population. It should be stressed that there is little knowledge regarding the stability of disease resistance QTL alleles when transferred to different genetic backgrounds and/or when evaluated in different environments. Tanksley and Hewitt, (1988) and Witcombe and Hash (2000) discussed breeding programs based on MAS and the implication of this for QTL allele introgression.

Mapping of additional downy mildew resistance QTLs that individually explain a high percentage of observed variation in disease reaction and subsequent marker-assisted transfer of such identified QTLs into agronomically elite hybrid is needed if breeding for pearl millet downy mildew resistance is to be effective in the long term. Characterization of virulence gene variation within and between populations of *Sclerospora graminicola* also has to be done, so that plant breeders and pathologist can more efficiently identify host-plant resistance appropriate for deployment in a given target region. A resistance gene deployment strategy of pyramiding genes in cereal hybrids using MAS has been discussed as a possible solution by Witcombe and Hash (2000) and Hash and Witcombe (in press).

Alternative procedures of marker-assisted transfer of QTLs for downy mildew as well as drought tolerance in pearl millet were given by Hash *et al.* (2000). The first successful application of MAS for pearl millet improvement has been enhancement of downy mildew of resistance of inbred pollinator line H 77/833-2 (parent of popular pearl millet hybrid HHB 67). Several improved versions of this pollinator have been developed at ICRISAT (Sharma, 2001). This has demonstrated a time and cost-effective route for the application of molecular marker-based downy mildew resistance breeding in this crop.

After identifying flanking markers for the resistance QTLs segregating in the pearl millet mapping population based on cross IP 18293 × Tift 238D1, it doesn't necessarily hold that these marker loci will serve as indicators of disease resistance for all cross combinations of pearl millet. For each and every new cross, one has to construct a separate skeleton linkage map to study the segregation pattern of resistance. But advances in biotechnology will undoubtedly provide less time-consuming and less costly alternative solutions in future. High-density maps of molecular markers permit new gene-cloning approaches, such as map-based cloning or positional cloning, which make it possible to actually clone the gene(s) responsible for the QTL. To achieve this there should be comprehensive genomic libraries of relatively large DNA fragments, typically in BAC (Bacterial Artificial Chromosome) vectors, with closely linked DNA markers, ideally less than few hundred-kilo bases apart (Xu, 1997). Martin *et al.* (1993) isolated the tomato gene *Pto*, conferring resistance to bacterial pathogen *Pseudomonas syringe*, by this approach.

Quantitative traits are usually controlled by many genes, each having a different effect and location in the genome. Separation of multiple QTLs (linked and unlinked) is the first step in genetic manipulation. Molecular marker-based mapping provides possibilities not only for the resolution of multiple QTLs but also for the identification of individual plants with favourable combinations of QTL alleles. Closely linked multiple QTLs can be separated into single clonable genetic factors by mapping and selection approaches, including fine mapping to improve resolution power, minor QTL mapping to eliminate the overshadowing effect of major QTLs, and regional mapping to saturate the chromosomal region of interest.

Even though marker-based QTL mapping and marker-assisted selection now play a prominent role in the field of plant breeding, examples of successful, practical outcomes are rare (Young, 1999). An increasingly extensive body of literature on DNA marker mapping and quantitative trait loci has quickly grown to provide researchers with the theoretical basis for using marker technology in their research. This in turn has lead to more than 400 research articles in this field. Is all this fuss really warranted? For some applications, rigid assessments are probably premature. If we take an example of computers in the year 1940, the prototype mainframe computer, 'Eniac' with 18,000 vacuum tubes was envisioned to have a very limited market size (<5) by IBM, yet subsequent innovations have made computers as a common indispensable item of our day-to-day life. So molecular breeders must reassess their research programs with rigorous experimental guidelines and ambitious goals with integration of genomics and bioinformatics, to achieve the goal of improved selection efficiency in a directed, time and cost-effective manner.

Keeping all this in mind, there are tremendous opportunities to improve and increase downy mildew resistance in pearl millet. In near future it is possible to transfer resistance QTLs to elite hybrid parental lines in a well-directed manner and with cost-effective breeding strategies. It will be an achievable task to release resistant pearl millet hybrids that can in turn help to feed the poorest among the poor risk-averse farmers of semi-arid regions, who have limited purchasing power, and whose subsistence mostly depends on their farm earnings.

Summary

6. SUMMARY

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an important staple food crop for millions of rural people living in semi-arid regions of tropical and sub-tropical Asia and Africa. In parts of the USA, South America and Southern Africa it is cultivated for feed and forage purposes. Pearl millet is a crop that can be grown in adverse agro-climatic conditions like drought, heat, and infertile sandy soils. It is the only crop that gives a sure source of grain yield to the farmers whose subsistence is totally dependant on farming in hot, dry marginal agricultural environments. Among the diseases affecting pearl millet, downy mildew is the most devastating. This is caused by a pseudo-fungal pathogen [*Sclerospora graminicola* (Sacc.) J. Schröt.]. Severely affected tillers produce leafy structures instead of grains on the panicle.

The most efficient, effective, environmentally safe and economical means to control downy mildew of pearl millet is the use of resistant cultivars. Earlier studies on this host-pathogen interaction have shown that resistance is polygenically controlled. The parental lines IP 18293 (downy mildew resistant, d_2 dwarf, purple foliaged) and Tift 238D1 (downy mildew susceptible, green foliaged, d_1 dwarf) were used for this new mapping population. Replicated screening of F_2F_4 segregating progenies from the (IP 18293 x Tift 238D1)-based mapping population, against downy mildew pathogen populations from India (Patancheru, Jalna, Jodhpur, Jamnagar, Durgapura and New Delhi) were done at ICRISAT and against African pathogen populations (Niger and Mali) at Bangor, UK. The segregating F_2F_4 progenies showed continuous disease reaction inheritance patterns that varied across the pathogen populations. Depending on the pathogen population, resistance appeared to be controlled by monogenic, digenic or trigenic Mendelian ratios. From the observed segregation patterns, it was shown that at least three genes were controlling resistance to this range of pathogen populations.

Cluster analysis and Pearson rank correlation values for F_2F_4 progeny disease incidence revealed relationships between the diverse Indian and African downy mildew pathogen populations. The northern Indian pathogen populations (Duragapura and New Delhi) and the African pathogen populations (Niger and Mali) were shown to be more virulent than those from southern and central India (Patancheru, Jalna, Jamnagar and

Jodhpur), at least with regard to the host-plant resistance segregating in this mapping population

Detailed genetic linkage maps in plants are very useful tools for studying genome structure and evolution, identifying introgression between genomes and localizing genes of interest RFLP markers have simple genetic segregation patterns and are potentially unlimited in number Detailed RFLP linkage maps have been constructed for several important crops such as maize, tomato lettuce potato and rice The first RFLP-based genetic linkage map of pearl millet was constructed by Liu *et al* (1994) In the present study an (IP 18293 × Tift 238D1)-based F_2F_3 population was used for map construction and its F_2F_4 segregating progenies were phenotyped for their resistance behaviour against eight downy mildew pathogen populations The amount of marker polymorphism exhibited in this pearl millet mapping population was 40% as high as that of maize and other outbreeding crop species From the 220 probe-enzyme combinations assessed in parental screening, 33 RFLP probe-enzyme combinations were selected for linkage map construction Using these 33 selected RFLP loci a skeleton map with a length of 561 8 cM was produced for the (IP 18293 × Tift 238D1)-based mapping population using Mapmaker/Exp Most of the selected markers segregated as expected with the Mendelian segregation ratio of 1 2 1 However 33% of marker the loci exhibited distorted segregation This was mostly because of the excess of heterozygotes and alleles from male parent Tift 238D1 Marker orders in all linkage groups were the same as base map of Liu *et al* (1994) Along with this base map the linkage map for this population was compared with all the previously constructed pearl millet maps

Although the use of most conventional morphological markers is not usually practicable in breeding programs those that are available should not be ignored In this present study marker loci controlling dwarfness (d_1 and d_2) and purple foliage colour (P) were used along with the RFLP markers The d_1 dwarfing gene has been placed on the top of the linkage group 1 and the d_2 dwarfing gene on linkage group 4 The purple colour locus P was also placed on linkage group 4 using this newly constructed (IP 18293 × Tift 238D1)-based pearl millet mapping population

Molecular markers are rapidly being adopted by crop improvement researchers globally as an effective and appropriate tool for basic and applied studies of biological components in agricultural systems. Use of markers in applied breeding programs can range from facilitating appropriate choice of parents for crosses, to mapping/tagging of gene blocks associated with economically important traits. Reports on linkage between quantitative trait loci effects and marker genotypes have been available in the literature quite a long time. Molecular markers tightly linked to different disease resistance genes have potential importance in facilitating selection procedures particularly for pyramiding two or more different resistance genes with the intension of producing a more durable and broad-spectrum resistance. In this study host-plant resistance QTLs were identified from parental line IP 18239 for six Indian and two African downy mildew pathogen populations. For QTL mapping, the interval-mapping method implemented in Mapmaker/QTL and the composite interval mapping method from QTL Cartographer were used. A total of seven different resistance QTLs were identified from screens of the mapping population progenies against these eight different pathogen populations. Among these, a common resistance QTL was identified on linkage group 2 which was effective against four Indian pathogen populations (Patancheru, Jodhpur, Jalna and Jamnagar). Such disease resistances are expected to be durable for a number of years. Likewise a common resistance QTL was identified on linkage group 4 for the African pathogen populations from Sadore, Niger and Bamako, Mali. These resistance QTLs are of considerable interest because the durability of resistance is of major concern for plant breeders. Several pathogen populations specific resistant to downy mildew QTLs were also identified, but these are not expected to contribute to durable resistance unless deployed as components of uniform or segregating resistance gene pyramids.

QTL mapping and DNA markers also provide insights into facets of quantitative inheritance patterns. In this present mapping population all identified resistance QTLs were from the resistant parent IP 18293, and for these an over-dominant inheritance pattern was most commonly observed. These identified resistance QTLs can now be transferred to genetic backgrounds of elite hybrid parental lines through marker-assisted selection breeding programs. Flanking markers of the identified QTLs can facilitate selection of resistant progenies during this backcrossing process whereas other marker loci can be used in reducing the length of the donor segments carried along with the introgressed genes and/or selecting for recovery of recurrent parent alleles on non-

carrier chromosomes Marker-assisted selection can also be used to a pyramid several resistant genes into a single male-sterile line (and its maintainer) or pollinator line

DNA markers, of course, do have defects preventing their general use in breeding programs They are expensive, require comparatively more time in the initial stages to standardize, and require relatively sophisticated laboratory set up Each of these difficulties can be overcome by new methodologies like automated DNA extraction, high-throughput genotyping systems and PCR-based non-radioactive visualization techniques Undoubtedly these technological innovations in the field of molecular breeding, along with the advancement of bioinformatics will bring enormous benefits to plant breeding complementing classical plant breeding methods to achieve our goal in comparatively shorter time and a more directed manner

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7. REFERENCES

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Appendices

I. PREPARATION OF BUFFERS AND OTHER CHEMICALS

0.5 M EDTA (1000 mL)

Na_2 EDTA	186 1 g
Sterile dH_2O	800 mL

Adjust to pH 8 0 with NaOH pellets make the volume 1 liter with sterile dH_2O and autoclave

5 M NaCl (1000 mL)

NaCl	292 2 g
Sterile dH_2O	800 mL

Make the volume 1 liter with sterile dH_2O and Autoclave

1 M Tris-Cl

Trizma base	121 1 g
Sterile dH_2O	800 mL

Adjust to pH 8 5 with conc HCl, make the volume 1 liter with sterile dH_2O and autoclave

20% SDS (2000 mL)

SDS	400 g
Sterile dH_2O	1200 mL

Stir until dissolved and make the volume 2 liters with sterile dH_2O

Buffer S (2000 mL)

1 M Tris-Cl, pH 8 5	200 mL
5 M NaCl	40 mL
0 5 M EDTA, pH 8 0	200 mL
20% SDS	200 mL

Make the volume 2 liters, store warm

Proteinase K (10 mg/mL)

Proteinase K	100 mg
Sterile dH_2O	10 mL

Stir thoroughly, dispense in 1 mL aliquots, store at -20°C

0.5 M Tris-Cl

Trizma base	60 507 g
Sterile dH_2O	800 mL

Adjust pH to 8 0 with 6 N HCl, make the volume 1 liter with sterile dH_2O and autoclave

$T_{50}E_{10}$ buffer

0 5 M Tris-Cl, pH 8 0	100 mL
0 5 M EDTA, pH 8 0	20 mL
Sterile dH_2O	600 mL

Make the volume 1 liter with sterile dH_2O

$T_{10}E_1$ buffer

0 5 M Tris-Cl, pH 8 0	20 mL
0 5 M EDTA, pH 8 0	2 0 mL
Sterile dH_2O	600 mL

Make the volume 1 liter with sterile dH_2O

Chloroform (24:1)

Chloroform	240 mL
Isoamyl alcohol	10 mL

70% Ethanol

100% ethanol	700 mL
Sterile dH_2O	300 mL

RNase (10mg/mL)	
RNase	100 mg
Sterile dH_2O	10 mL
Dissolve by placing in boiling water for 20 min; cool slowly; dispense into 1 mL aliquots store at -20°C	
3 M Sodium acetate	
Sodium acetate	408.24 g
Sterile dH_2O	600 mL
Adjust to pH 5.2 with glacial acetic acid; make the volume 1 liter with dH_2O and autoclave	
10 X TBE	
Trizma base	108 g
Sterile dH_2O	500 mL
Boric acid	55 g
0.5 M EDTA	40 mL
Adjust to pH 8.4 with 6N HCl, make the volume 1 liter with dH_2O	
50 X TAE	
Trizma base	242 g
Sterile dH_2O	500 mL
0.5 M EDTA; pH 8.0	100 mL
Glacial acetic acid	57.1 mL
Make the volume 1 liter with dH_2O	
1X TAE	
50x TAE	20 mL
Sterile dH_2O	980 mL
0.25 M HCl	
Concentrated HCl	43 mL
Sterile dH_2O	1957 mL

Kesara's loading buffer	
Bromophenol blue	0.10 g
Xylene cyanol	0.10 g
Glycerol	10 mL
$Na_2EDTA.2H_2O$	0.372 g
Make the volume 20 mL with 1x TAE and dispense into 2 mL screw-cap tubes, store at 4°C	
4 M NaOH	
NaOH pellets	160 g
Sterile dH_2O	800 mL
Make the volume 1 liter with dH_2O	
0.4 M NaOH	
NaOH pellets	96 g
Sterile dH_2O	2000 mL
Stir and make the volume 6 liters with dH_2O	
20 X SSC	
NaCl	877 g
Sodium citrate	441 g
Sterile dH_2O	4000 mL
Dissolve and make the volume 5 liters with dH_2O	
2 X SSC	
20x SSC	200 mL
Sterile dH_2O	1800 mL
Loading buffer	
Sucrose	4 g
Bromophenol blue	25 mg
0.5 M EDTA pH 8	400 µL
Make the volume 10 mL with sterile dH_2O	

5 X HSB	
NaCl	175.3 g
PIPES	30.3 g
Na_2.EDTA.$2H_2O$	7.45 g
Sterile dH_2O	800 mL
Adjust to pH 6.8 with 4 M NaOH; make the volume 1 liter, autoclave	

Denhardt's III	
Gelatin	2 g
Ficoll-400	2 g
PVP-360	2 g
SDS	10 g
Sodium pyrophosphate	5 g
Dissolve and make the volume to 100 mL with dH_2O; store at 65°C	

Carrier DNA	
Salmon sperm DNA	5 g
Sterile dH_2O	1 liter
Dissolve and autoclave then dispense into 50 mL aliquots; store at -20°C	

Wash 1 for ^{32}P Blots	
20x SSC	200 mL
20% SDS	50 mL
dH_2O	1700 mL

Wash 2 for ^{32}P Blots	
20x SSC	20 mL
20% SDS	50 mL
dH_2O	1700 mL

Stripping solution	
20% SDS	50 mL
20x SSC	10 mL
Sterile dH_2O	1940 mL

Prehybridization solution (OLB labeling)	
Denhardt's III	10 mL
Carrier DNA	10 mL
5 X HSB	20 mL
Sterile dH_2O	60 mL
Warm it before use, take 15 mL/reaction	

Hybridization solution (OLB labeling)/ reaction	
OLB A	5 µL
BSA	2 µL
Klenow fragments	2 µL
Probe (denatured)	3 µL
	2 µL

Prehybridization solution (For New England bio-lab kit)	
Na_2HPO_4	35.5 g
BSA	5 g
SDS	35 g
dH_2O	300 mL
Adjust pH to 7.2 with phosphoric acid (H_3PO_4), make the volume 500 mL with dH_2O. Take 5 mL/reaction with 1 mL of carrier DNA	

Buffered phenol (100 mL)	
Phenol	100 mL
Trizma base	6.021 g
8-hydroxyquinaline	0.1 g
Sterile dH_2O	100 mL

Stir and remove the upper phase, again add 100 mL of dH_2O and repeat as above. pH should be 7-8. Store cool.

Hybridization solution *(For New England bio-lab kit)*	
10X Labelling buffer	5 µL
dNTP mixture	6 µL
α-^{32}P dCTP	5 µL
Klenow fragments	1 µL

7.5 M Ammonium acetate	
Ammonium acetate	57.75 g
Sterile dH_2O	75 mL
Make the volume 100 mL with dH_2O	

LB medium 500 mL	
NaCl	5 g
Trypton	5 g
Yeast extract	2.5 g
Make the volume to 500 mL, adjust pH to 7.2 with 1 N NaOH and autoclave	

LB + Agar medium 500 mL	
Nacl	5 g
Trypton	5 g
Yeast extract	2.5 g
Agar	7.5 g
Make the 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 the volume to 100 mL, filter it and store at 4°C	

dNTP mixture (For New England bio-lab kit)	
dATP	50 µL
dTTP	50 µL
dGTP	50 µL

Sol. A (for plasmid extraction)	
Lysozyme	4 mg
GTE solution	1 mL
Every time prepare freshly dissolve and take 200 µL/reaction	

Ampicillin 50 mg/mL (5 mL)	
Ampicillin	250 mg
Sterile dH_2O	5 mL

Sol. B (for plasmid extraction)	
10% SDS	1 mL
1 N NaOH	2 mL
Sterile dH2O	7 mL
Every time prepare freshly dissolve and take 300 µL/reaction	

Developer	
Developer A powder	172.5 g
Developer B powder	11.2 g
Warm sterile dH_2O up to 52°C	700 mL
Slowly add A and B make the volume to 1 litre 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 the volume 1 liter with sterile dH_2O	

1% Agarose	
Agarose	2.5 g
TAE/TBE	250 mL
Take TAE or TBE based on the tank buffer	

II. GENOTYPIC DATA POINTS SCORED FROM THE RFLP-BASED AUTORADS

No.	Linkage Group	Probe	Enzyme	F_2 Segregating population from IP 18293 x Tift 238D1																					
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	LG1	M280	*EcoRI*	A	A	A	H	H	H	A	H	H	B	B	A	H	H	H	H	H	H	H	B	H	B	H
2		M515	*HindIII*	A	-	A	A	H	H	A	D	H	B	-	A	H	H	-	A	B	D	-	B	B	H	A
3		M425	*EcoRI*	A	A	A	A	H	H	A	H	H	B	B	A	H	H	H	H	B	H	H	C	B	C	A
4		M087.2	*DraI*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	B	B	B	A
5		M341	*EcoRI*	A	A	A	A	H	H	A	H	H	B	B	A	H	H	H	H	B	H	H	B	B	H	H
6	LG2	M322	*HindIII*	A	H	A	A	-	B	H	A	H	A	H	A	B	H	A	H	H	A	H	H	H	A	H
7		M466	*HindIII*	-	-	-	-	-	B	H	A	B	H	H	A	B	-	H	H	H	A	B	B	A	H	H
8		M458	*HindIII*	A	H	-	-	-	B	B	A	H	H	A	A	B	B	H	H	H	A	A	B	H	A	H
9		M214	*EcoRI*	A	A	A	H	-	-	B	A	-	-	H	A	B	-	A	H	H	A	A	-	-	-	-
10		M321	*DraI*	D	D	D	D	D	B	B	D	B	D	D	-	B	B	D	D	B	D	D	A	H	A	H
11		M592	*DraI*	A	H	H	H	-	B	B	A	B	-	A	A	B	B	H	H	-	A	A	-	A	A	H
12		M443	*HindIII*	-	-	-	-	-	B	H	A	B	H	H	A	B	-	H	H	H	A	B	H	H	A	H
13	LG3	M037	*HindIII*	A	-	A	H	B	-	-	-	-	-	-	-	D	D	-	B	A	A	-	H	H	H	B
14		M108	*DraI*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	-	A	H
15		M510.2	*HindIII*	H	H	B	H	B	A	H	B	A	H	B	H	A	H	A	H	A	H	B	H	A	H	H
16		M473	*DraI*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	H	H	H
17		M686	*DraI*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	H	A	H
18	LG4	M464	*EcoRI*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	A	-	-
19		M716	*EcoRI*	H	H	H	A	H	D	H	H	H	H	H	H	H	B	H	H	A	B	H	B	H	H	A
20		M648	*DraI*	H	H	A	H	B	A	H	H	A	A	A	-	H	B	H	H	A	B	A	H	H	H	A
21		M084	*EcoRV*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	H	H	A
22		M413.2	*EcoRV*	A	A	-	H	-	A	H	H	H	H	H	H	H	A	H	H	A	B	A	H	A	H	A
23	LG5	M815	*HindIII*	H	H	-	-	-	A	A	A	C	-	-	-	A	-	B	H	H	H	A	H	-	H	B
24		M318	*HindIII*	H	H	H	H	A	A	A	H	A	H	H	H	A	A	B	H	H	H	A	H	A	H	H
25		M735.1	*EcoRV*	H	H	A	A	-	A	H	H	H	H	H	H	A	A	B	A	H	H	A	H	H	H	B
26	LG6	M459.1	*DraI*	A	A	B	A	-	B	A	H	H	B	B	-	H	-	H	A	B	H	B	B	B	H	H
27		M588	*HindIII*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	B	B	H	H
28		M713	*EcoRI*	H	H	H	H	H	H	H	B	H	H	A	H	A	A	H	H	B	A	H	H	B	H	B
29	LG7	M718	*EcoRI*	A	H	A	H	B	H	H	A	H	H	B	B	B	D	B	H	A	B	H	H	A	A	-
30		M269	*HindIII*	D	H	A	H	B	B	B	H	A	H	B	H	-	A	H	B	A	B	H	H	D	H	B
31		M618	*DraI*	H	C	-	D	-	-	B	A	D	A	B	-	B	A	-	H	A	-	-	H	H	A	H
32		M526	*DraI*	H	H	H	H	B	B	B	H	A	A	B	B	B	A	H	H	H	B	H	H	H	H	H
33	Unlinked	M196	*DraI*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	A	H	B

No.	Linkage Group	Probe	Enzyme	F_2 Segregating population from IP 18293 x Tift 238D1																							
				24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
1	LG1	M280	*EcoRI*	H	H	A	A	H	A	A	H	H	H	H	H	B	A	H	H	B	-	A	A	B	H	B	H
2		M515	*HindIII*	H	H	A	A	B	H	H	H	H	H	H	B	B	A	H	B	H	A	H	H	B	H	B	A
3		M425	*EcoRI*	H	H	A	A	B	B	H	H	B	H	H	B	B	A	A	B	-	-	H	-	B	H	B	A
4		M087.2	*DraI*	H	H	A	A	B	B	H	H	B	H	H	B	H	A	A	B	H	H	H	A	H	-	B	A
5		M341	*EcoRI*	H	H	A	A	B	B	H	H	B	H	H	B	H	A	A	B	H	A	H	-	B	A	B	A
6	LG2	M322	*HindIII*	B	H	A	A	H	A	A	H	-	B	H	H	H	-	H	H	A	H	A	B	D	D	B	H
7		M466	*HindIII*	H	H	H	A	A	A	A	H	H	H	H	H	H	A	H	H	H	H	A	B	A	A	B	H
8		M458	*HindIII*	B	H	H	A	A	A	A	H	H	H	H	H	H	A	H	H	H	B	A	B	A	B	B	H
9		M214	*EcoRI*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	B	A	B	A	-	H	D
10		M321	*DraI*	B	H	A	A	H	A	A	H	H	B	H	H	H	A	H	H	H	B	H	B	H	B	H	H
11		M592	*DraI*	H	A	H	A	A	A	A	H	-	H	H	H	H	H	H	A	A	H	A	B	A	B	-	H
12		M443	*HindIII*	B	H	A	A	H	A	A	H	H	B	H	H	H	H	H	B	H	H	H	D	H	H	H	B
13	LG3	M037	*HindIII*	A	H	A	H	A	B	A	B	H	H	H	H	B	B	A	B	H	H	H	B	H	-	D	C
14		M108	*DraI*	A	A	B	B	H	B	A	B	A	H	-	A	B	B	H	B	H	H	B	H	B	-	H	B
15		M510.2	*HindIII*	A	H	B	H	H	H	A	B	A	H	H	A	H	B	A	H	H	H	B	H	B	H	H	B
16		M473	*DraI*	A	H	B	H	H	H	A	B	A	H	H	A	H	B	A	H	H	H	B	H	B	H	H	B
17		M686	*DraI*	D	H	B	H	H	H	D	B	-	H	H	A	H	B	A	-	H	H	B	H	B	C	H	B
18	LG4	M464	*EcoRI*	B	A	A	D	D	H	B	H	A	A	D	D	D	D	D	-	-	-	-	-	-	-	-	-
19		M716	*EcoRI*	H	A	A	H	A	B	B	H	A	H	A	A	H	A	H	B	H	H	H	A	A	H	H	A
20		M648	*DraI*	H	A	A	H	A	B	B	B	A	H	A	A	H	A	H	B	-	H	H	A	-	-	B	A
21		M084	*EcoRV*	H	A	A	H	A	B	B	H	A	H	A	A	H	A	H	B	H	H	H	A	A	B	B	A
22		M413.2	*EcoRV*	H	A	A	H	H	B	B	H	-	H	A	H	H	A	-	B	H	H	H	A	H	-	B	A
23	LG5	M815	*HindIII*	A	C	-	H	H	B	H	H	-	H	-	A	A	-	H	-	-	H	H	-	H	-	A	-
24		M318	*HindIII*	A	H	H	H	H	H	H	H	-	H	-	H	A	A	H	A	H	H	H	H	H	H	A	D
25		M735.1	*EcoRV*	H	A	H	H	H	H	H	B	B	A	A	H	H	A	H	H	H	A	A	H	A	A	A	H
26	LG6	M459.1	*DraI*	B	A	-	H	B	A	H	H	-	B	-	B	H	-	-	-	B	H	H	B	H	H	H	B
27		M588	*HindIII*	B	A	B	H	H	H	H	H	H	-	H	B	H	H	H	B	B	H	H	H	A	H	H	B
28		M713	*EcoRI*	B	B	-	H	H	H	C	B	B	H	H	H	B	-	H	-	-	H	A	H	-	A	H	B
29	LG7	M718	*EcoRI*	A	B	-	A	A	H	-	A	H	A	A	A	A	-	-	A	-	H	H	H	H	H	A	A
30		M269	*HindIII*	H	B	H	A	H	H	H	A	H	H	A	-	H	H	B	H	B	D	H	H	H	A	A	A
31		M618	*DraI*	H	B	H	A	H	H	H	H	A	H	H	H	A	A	H	H	H	H	A	H	-	-	H	A
32		M526	*DraI*	H	H	H	A	H	A	H	H	H	H	H	H	H	H	A	H	H	H	A	H	H	H	H	A
33	Unlinked	M196	*DraI*	B	H	A	A	A	B	H	A	H	B	H	H	A	H	A	A	A	H	H	B	A	A	H	B

No.	Linkage Group	Probe	Enzyme	F2 Segregating population from IP 18293 x Tift 238D1																							
				48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
1	LG1	M280	*EcoRI*	H	H	H	B	B	H	B	H	H	A	A	H	H	B	H	H	H	A	H	H	B	B	B	B	A
2		M515	*HindIII*	A	B	H	B	H	H	B	A	H	A	A	H	H	B	A	A	H	A	H	B	B	C	C	B	A
3		M425	*EcoRI*	-	B	H	B	H	H	B	A	-	-	A	-	H	B	H	A	H	A	H	B	H	B	B	B	A
4		M087.2	*DraI*	A	-	C	-	H	H	-	-	-	-	A	-	A	-	-	-	-	-	-	-	-	-	-	-	-
5		M341	*EcoRI*	A	-	B	-	H	H	B	H	H	H	A	H	A	B	H	H	A	A	H	B	H	B	H	B	D
6	LG2	M322	*HindIII*	-	H	A	H	H	B	A	B	A	A	H	A	H	H	H	A	H	A	H	H	H	A	A	A	A
7		M466	*HindIII*	B	A	A	-	H	B	A	B	H	A	A	H	H	-	-	-	-	-	-	-	-	-	-	-	-
8		M458	*HindIII*	H	H	H	B	A	B	A	B	H	A	A	H	H	B	A	H	A	A	D	H	A	H	H	H	H
9		M214	*EcoRI*	D	D	D	D	H	B	H	B	D	A	A	-	-	B	A	-	A	A	A	H	A	H	H	H	H
10		M321	*DraI*	-	H	A	H	H	B	A	B	H	A	A	H	H	B	A	A	H	H	A	H	H	B	H	H	H
11		M592	*DraI*	B	H	A	H	H	B	A	B	-	A	H	H	H	B	-	H	A	A	H	H	A	H	H	H	A
12		M443	*HindIII*	B	H	H	H	H	B	D	B	H	H	A	H	A	-	D	D	A	H	H	H	H	H	H	H	H
13	LG3	M037	*HindIII*	H	A	H	A	A	H	A	H	B	A	H	H	B	B	H	B	H	H	H	H	H	A	B	B	A
14		M108	*DraI*	D	A	H	A	A	H	H	-	-	A	A	-	H	-	B	B	H	H	A	H	A	H	H	B	H
15		M510.2	*HindIII*	H	A	H	A	A	H	B	B	B	A	A	H	H	A	B	-	D	-	A	H	D	-	-	-	-
16		M473	*DraI*	H	H	H	A	A	H	B	B	B	A	A	H	H	A	B	H	H	H	A	H	A	H	H	B	H
17		M686	*DraI*	H	H	H	A	H	H	B	B	-	A	A	H	H	A	H	H	H	H	H	H	H	H	H	B	A
18	LG4	M464	*EcoRI*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19		M716	*EcoRI*	H	A	A	-	B	H	H	H	H	A	H	A	H	-	-	-	-	-	-	-	-	-	-	-	-
20		M648	*DraI*	-	A	H	H	B	H	-	-	-	-	-	-	-	-	C	-	C	-	D	-	-	H	-	-	H
21		M084	*EcoRV*	A	H	A	H	B	H	B	H	B	H	H	H	H	-	-	-	-	-	-	-	-	-	-	-	-
22		M413.2	*EcoRV*	H	A	H	-	B	A	B	H	B	-	H	-	H	H	H	A	H	H	H	H	-	H	-	-	H
23	LG5	M815	*HindIII*	-	H	H	-	B	B	-	H	A	-	B	-	A	A	-	H	H	A	H	H	H	A	A	-	A
24		M318	*HindIII*	H	A	B	B	B	H	H	H	A	A	H	B	H	-	H	A	H	H	H	A	A	A	A	C	A
25		M735.1	*EcoRV*	A	A	B	H	H	B	A	B	A	A	B	H	A	B	A	C	H	H	H	H	A	A	H	A	A
26	LG6	M459.1	*DraI*	A	B	H	B	H	A	A	H	-	B	H	-	-	A	H	B	B	A	H	H	A	H	H	-	H
27		M588	*HindIII*	A	B	H	H	B	A	A	A	H	B	H	D	H	H	H	B	H	H	B	H	H	B	H	H	H
28		M713	*EcoRI*	-	B	A	H	B	A	A	A	B	H	H	-	H	-	H	-	-	-	-	H	H	-	-	A	A
29	LG7	M716	*EcoRI*	-	-	H	-	H	H	H	-	A	H	H	H	H	H	B	A	H	-	A	-	A	-	A	-	A
30		M269	*HindIII*	H	H	H	H	A	B	H	B	H	H	B	H	H	A	B	A	H	H	H	H	A	B	H	H	H
31		M618	*DraI*	H	H	H	A	H	B	H	H	-	B	H	A	H	A	B	H	H	H	A	H	A	H	H	B	H
32		M526	*DraI*	H	H	H	H	H	B	H	H	H	B	H	A	H	A	H	H	H	H	H	H	H	H	H	B	A
33	Unlinked	M196	*DraI*	B	H	H	B	H	H	H	A	H	A	A	H	A	-	A	-	H	-	B	H	-	-	-	-	B

No.	Linkage Group	Probe	Enzyme	F_2 Segregating population from IP 18293 x Tift 238D1																							
				73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
1	LG1	M280	EcoRI	B	B	H	-	H	A	H	H	H	-	A	H	H	B	H	H	B	H	H	H	A	B	A	H
2		M515	HindIII	H	H	H	H	A	A	H	B	A	-	A	H	-	H	H	H	B	H	H	H	H	A	A	A
3		M425	EcoRI	H	-	H	-	H	A	H	B	H	H	H	H	H	H	H	H	B	H	H	H	A	A	A	A
4		M087.2	DraI	-	-	-	-	-	-	-	-	-	-	A	H	-	H	H	H	C	H	A	D	D	A	A	D
5		M341	EcoRI	B	H	H	-	H	A	H	B	H	A	H	H	H	H	H	H	B	H	H	H	B	-	A	H
6	LG2	M322	HindIII	H	A	A	A	A	H	H	A	-	-	B	-	-	A	H	H	H	B	D	H	H	A	A	A
7		M466	HindIII	-	-	-	-	-	-	-	-	-	A	B	H	-	H	H	A	B	B	H	B	A	H	H	H
8		M458	HindIII	H	H	B	H	H	A	H	H	-	H	B	A	-	A	A	A	B	A	B	B	B	A	H	B
9		M214	EcoRI	H	H	B	H	-	A	H	H	-	A	B	H	A	A	A	H	B	A	A	B	-	D	H	-
10		M321	DraI	B	A	B	A	H	H	H	B	A	H	H	A	H	D	H	H	H	H	A	B	H	A	H	H
11		M592	DraI	B	H	B	H	H	A	H	H	A	-	H	A	-	A	H	-	B	B	H	H	B	A	H	H
12		M443	HindIII	B		H	A	H	H	H	B	A	A	H	H	-	H	H	H	H	H	A	B	H	A	H	H
13	LG3	M037	HindIII	B	A	H	B	H	H	H	A	B	H	A	B	-	B	B	B	H	H	A	H	B	A	B	A
14		M108	DraI	H	B	A	H	B	B	H	B	H	H	H	H	H	H	H	H	H	H	B	H	A	H	B	A
15		M510.2	HindIII	H	H	A	-	B	B	H	B	H	A	H	H	-	H	H	H	H	H	H	H	A	H	B	A
16		M473	DraI	H	B	A	H	B	B	H	B	H	-	H	H	-	H	H	H	H	H	H	H	A	-	B	A
17		M686	DraI	A	B	H	B	A	B	H	H	A	-	A	H	-	H	H	H	H	H	A	H	H	-	H	H
18	LG4	M464	EcoRI	-	-	-	-	-	-	-	-	-	-	H	A	H	A	H	B	A	B	A	H	A	-	H	A
19		M716	EcoRI	-	-	B	-	H	B	H	H	H	H	H	A	H	A	B	H	A	B	A	A	A	A	H	A
20		M648	DraI	-	D	-	H	H	-	C	D	C	-	H	H	-	-	C	H	A	C	-	D	-	-	H	-
21		M084	EcoRV	-	-	-	-	-	-	-	-	-	-	C	C	C	D	C	C	-	C	D	-	D	D	-	-
22		M413.2	EcoRV	H	H	H	B	H	B	H	H	-	-	H	H	H	A	B	H	H	B	-	-	-	A	-	-
23	LG5	M815	HindIII	B	A	A	A	A	H	H	A	-	-	B	A	-	A	H	H	A	B	-	H	-	-	-	-
24		M318	HindIII	B	-	A	H	H	A	H	-	B	H	B	A	-	A	B	H	A	B	A	H	B	-	H	B
25		M735.1	EcoRV	B	H	A	A	-	A	B	A	-	-	H	H	A	H	H	A	A	-	A	H	H	-	H	H
26	LG6	M459.1	DraI	H	H	H	B	A	H	B	B	-	-	H	H	-	B	H	H	B	H	B	H	H	-	B	B
27		M588	HindIII	H	A	H	H	A	A	H	-	B	H	H	H	H	H	H	H	B	H	B	H	H	H	B	H
28		M713	EcoRI	-	-	H	-	H	H	H	B	H	A	A	H	H	B	H	B	H	H	B	H	H	A	H	A
29	LG7	M718	EcoRI	H	H	A	B	-	-	H	H	-	-	H	-	-	B	A	H	H	B	A	H	A	-	H	A
30		M269	HindIII	H	B	A	B	H	B	H	B	B	A	H	H	-	B	A	H	H	B	A	H	A	A	H	A
31		M618	DraI	H	B	A	H	B	B	H	H	H	-	H	H	-	H	H	H	H	H	H	H	A	-	-	A
32		M526	DraI	A	B	H	A	H	B	H	H	H	A	A	H	A	C	H	H	H	H	A	B	H	H	H	H
33	Unlinked	M196	DraI	H	H	A	H	A	B	H	H	H	H	B	B	H	A	H	H	A	H	H	H	H	H	H	B

N

No.	Linkage Group	Probe	Enzyme	F_2 Segregating population from IP 18293 x Tift 238D1																			
				97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118
1	LG1	M280	EcoRI	H	B	H	B	A	B	A	H	H	A	H	B	H	-	A	A	A	A	-	-	B	H
2		M515	HindIII	A	H	H	H	A	B	H	H	H	H	H	B	H	H	H	H	H	A	H	B	B	H
3		M425	EcoRI	A	H	H	H	-	B	H	H	H	H	H	A	H	A	A	H	H	H	H	B	B	H
4		M087.2	DraI	H	H	-	H	A	B	-	-	-	C	-	A	-	-	-	-	-	D	-	B	B	H
5		M341	EcoRI	H	H	H	H	A	B	H	H	H	H	H	A	H	H	H	H	H	H	H	B	H	H
6	LG2	M322	HindIII	-	C	-	H	A	A	B	B	A	H	H	H	H	A	H	H	B	B	A	A	H	H
7		M466	HindIII	A	A	B	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8		M458	HindIII	A	A	B	A	H	A	B	B	A	A	H	H	H	A	A	H	B	H	A	H	H	H
9		M214	EcoRI	A	A	H	A	-	H	H	H	H	H	-	H	-	-	H	H	-	-	H	H	H	H
10		M321	DraI	A	A	H	A	H	A	B	B	H	A	H	H	H	A	A	H	B	B	H	H	H	H
11		M592	DraI	H	A	B	A	-	A	-	-	A	-	-	-	-	A	H	A	-	B	A	A	A	H
12		M443	HindIII	A	A	H	A	-	A	B	B	H	A	A	A	A	A	A	H	B	H	H	H	H	H
13	LG3	M037	HindIII	B	H	B	A	A	A	H	A	A	H	A	A	A	A	B	H	H	-	H	H	H	H
14		M108	DraI	H	H	B	A	-	A	H	A	A	H	H	A	H	-	B	A	A	H	H	A	H	H
15		M510.2	HindIII	A	H	B	A	H	A	H	A	A	H	H	A	H	A	H	A	H	H	H	A	A	H
16		M473	DraI	H	H	B	A	H	A	H	H	A	H	H	D	H	A	H	A	H	H	H	H	A	H
17		M666	DraI	H	A	-	H	-	A	-	-	D	-	D	D	-	D	H	A	-	H	H	H	A	A
18	LG4	M464	EcoRI	A	H	-	H	A	H	B	H	H	B	H	H	-	-	A	H	H	H	B	H	H	H
19		M716	EcoRI	H	B	H	B	A	B	B	H	B	B	B	H	H	H	H	H	H	H	-	-	A	H
20		M648	DraI	-	H	-	B	D	C	-	-	-	C	H	-	-	-	-	-	-	A	C	D	H	C
21		M084	EcoRV	A	C	C	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22		M413.2	EcoRV	-	-	-	-	-	B	B	-	-	-	-	-	-	-	-	-	-	-	-	A	A	B
23	LG5	M815	HindIII	-	H	-	H	-	B	-	-	B	A	A	A	-	-	H	H	-	-	A	-	H	A
24		M318	HindIII	B	H	H	-	B	H	H	B	H	B	A	A	H	A	H	H	H	A	A	H	H	D
25		M735.1	EcoRV	H	H	-	H	-	H	H	A	H	-	-	H	-	-	H	A	H	A	A	A	B	H
26	LG6	M459.1	DraI	B	H	-	-	A	A	H	A	H	H	H	H	B	-	B	B	H	H	A	H	B	B
27		M588	HindIII	B	H	H	A	A	A	H	A	H	-	H	H	B	-	B	H	H	H	A	H	B	B
28		M713	EcoRI	H	H	H	H	B	B	H	A	H	H	H	A	B	B	H	H	B	H	-	-	H	H
29	LG7	M718	EcoRI	A	H	H	H	-	A	B	B	A	A	H	H	-	A	A	A	B	B	A	H	H	A
30		M269	HindIII	A	H	H	A	D	H	H	H	D	A	B	H	D	A	A	A	H	B	A	A	H	H
31		M618	DraI	A	H	-	A	B	H	H	H	H	H	B	H	D	A	A	A	H	H	H	H	H	H
32		M526	DraI	H	A	A	H	B	H	H	H	H	H	H	H	A	A	H	A	B	H	H	H	H	H
33	Unlinked	M196	DraI	H	H	A	H	B	H	H	A	B	A	B	H	A	H	H	H	A	H	A	H	H	H

No.	Linkage Group	Probe	Enzyme	F_2 Segregating population from IP 18293 x Tift 238D1																						
				119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	140	142
1	LG1	M280	EcoRI	B	H	H	A	B	B	H	H	H	H	H	H	H	H	H	A	B	-	H	A	H	H	A	H
2		M515	HindIII	B	H	H	H	H	B	A	H	H	H	H	H	B	H	H	H	B	H	H	H	H	H	H	A
3		M425	EcoRI	B	H	H	H	H	B	A	H	H	H	H	B	B	H	H	H	B	-	H	H	H	H	H	A
4		M087.2	DraI	B	H	H	H	H	B	A	H	H	H	H	H	B	H	-	H	B	H	H	H	H	-	-	A
5		M341	EcoRI	B	H	H	H	H	B	A	A	H	H	H	H	B	H	H	H	B	-	H	H	H	B	H	A
6	LG2	M322	HindIII	B	A	H	H	A	B	A	H	H	A	A	A	A	H	A	B	A	D	H	H	H	B	B	H
7		M466	HindIII	-	-	A	D	D	B	A	H	-	-	A	A	-	H	-	-	-	H	H	-	-	B	B	B
8		M458	HindIII	B	A	A	H	A	B	A	H	H	A	A	A	A	H	A	H	A	H	H	H	H	B	B	B
9		M214	EcoRI	H	A	A	H	H	H	H	-	A	H	H	A	A	H	-	H	H	H	H	H	H	B	B	-
10		M321	DraI	B	A	A	H	A	H	A	H	H	A	A	A	B	H	A	D	A	A	H	H	B	B	B	H
11		M592	DraI	B	A	H	H	A	B	A	H	H	A	A	A	A	H	H	-	A	H	H	H	H	B	B	H
12		M443	HindIII	B	A	A	H	A	H	A	H	H	H	H	A	H	H	A	H	A	A	H	H	B	H	B	H
13	LG3	M037	HindIII	B	B	H	A	-	H	A	H	B	H	H	A	A	A	B	B	H	B	A	H	H	-	A	B
14		M108	DraI	H	H	H	A	-	H	A	H	B	H	H	A	A	H	H	B	H	B	A	H	H	-	-	B
15		M510.2	HindIII	A	H	H	A	H	H	A	H	B	H	H	A	A	H	A	B	H	B	A	H	H	H	B	B
16		M473	DraI	A	H	H	A	H	H	A	H	B	H	H	A	H	H	A	B	H	B	A	H	H	-	-	B
17		M686	DraI	H	-	H	A	H	H	A	H	B	H	H	A	H	H	A	B	H	B	A	H	H	-	-	B
18	LG4	M464	EcoRI	A	-	H	-	-	D	H	H	-	-	D	B	A	H	-	H	H	-	H	B	H	-	-	A
19		M716	EcoRI	A	H	H	A	H	A	A	A	H	H	H	B	H	H	H	B	A	-	B	B	A	H	B	A
20		M648	DraI	-	C	D	D	C	D	A	D	D	D	D	C	D	B	C	C	D	C	C	C	-	H	B	H
21		M084	EcoRV	-	H	H	A	B	A	H	H	H	H	B	H	A	H	H	B	A	B	B	B	H	-	-	H
22		M413.2	EcoRV	-	H	H	A	H	A	H	H	A	H	B	H	H	H	A	B	H	H	H	B	H	H	B	H
23	LG5	M815	HindIII	-	H	A	H	H	A	A	H	C	A	A	C	A	H	-	H	H	H	H	H	A	A	H	A
24		M318	HindIII	H	H	A	H	H	A	A	H	H	A	A	H	A	H	H	H	H	H	H	H	A	A	H	A
25		M735.1	EcoRV	-	H	A	H	H	A	A	H	H	H	H	H	A	A	B	H	H	A	B	H	A	H	H	H
26	LG6	M459.1	DraI	A	H	B	A	B	H	A	A	H	B	B	B	H	B	A	A	H	B	H	H	H	A	B	H
27		M588	HindIII	A	H	B	A	B	H	A	A	H	B	B	B	H	B	A	A	H	B	H	H	H	-	-	H
28		M713	EcoRI	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	H	A
29	LG7	M718	EcoRI	B	A	-	H	A	H	A	H	H	H	A	A	A	H	-	H	A	-	H	H	-	H	-	H
30		M269	HindIII	B	A	H	H	H	B	H	H	A	H	H	H	H	H	H	H	D	D	A	H	H	B	B	H
31		M618	DraI	D	A	B	H	H	B	H	B	A	B	H	H	H	A	H	H	-	B	H	B	A	B	B	H
32		M526	DraI	A	A	H	H	H	H	H	H	A	H	H	A	H	A	H	H	H	B	A	H	A	B	B	H
33	Unlinked	M196	DraI	A	-	H	B	-	A	A	H	A	H	B	H	-	B	B	H	B	-	H	H	H	-	-	H

THE SOURCES OF APOMIXIS, THEIR MAINTENANCE AND UTILIZATION IN SORGHUM BREEDING AND THE LITERATURE ON SORGHUM APOMIXIS

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