Genetic diversity analysis, QTL mapping and marker-assisted selection for shoot fly resistance in sorghum [*Sorghum bicolor* (L.) Moench]

By

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M.Sc. (Agri.)

DISSERTATION

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2006

DEDICATED

TO

MY LATE BELOVED

PARENTS

CANDIDATE'S DECLARATION

I hereby declare that the dissertation or part thereof has not been previously submitted by me to any other University or Institution for a degree or diploma

Place: Parbhani **(S.P. Mehtre)**

Date: 27 January 2006

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CERTIFICATE - I

This is to certify that the dissertation entitled "**Genetic diversity analysis, QTL mapping and marker-assisted selection for shoot fly resistance in sorghum [*Sorghum bicolor* (L.) Moench]**" submitted to Marathwada Agricultural University, Parbhani in partial fulfillment of the requirement for the award of degree of **Doctor of Philosophy** in **Genetics and Plant Breeding** embodies the results of bonafide research carried out by **Shri. S.P. Mehtre** under my guidance and supervision and that no part of this dissertation has been submitted for any degree or diploma.

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

Place: Parbhani **(Dr. S.T. Borikar)**

Date: 27 January 2006 **Research Guide**

CERTIFICATE - II

This is to certify that the dissertation entitled "**Genetic diversity analysis, QTL mapping and marker-assisted selection for shoot fly resistance in sorghum [*Sorghum bicolor* (L.) Moench]**" submitted by **Shri. S.P. Mehtre** to the Marathwada Agricultural University, Parbhani in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in the subject of **Genetics and Plant Breeding** has been approved by the student's advisory committee after oral examination in collaboration with the external examiner.

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CHAPTER I
INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop globally after rice, maize, wheat and barley (FAO, 2004). It is grown in about 86 countries covering an area of about 47 million hectares (ha) with a grain production of 69 million tons (t) and average productivity of 1.45 t ha^{-1} (ICRISAT, 1996 and FAO, 2004). It is grown mostly in tropical and subtropical areas. Sorghum occupies 14.1 million ha in Asia. The major sorghum producing countries are Nigeria, Mali, Sudan, India, China, Pakistan, USA, Australia, Argentina, and Mexico. The grain is used as human food in various ways and both grain and stalk are used for animal feed. India is major producer of sorghum with the crop occupying an area of 9.9 million ha and yielding an annual production 8.00 million t during 2003/04 (FAS, 2005).

Productivity of sorghum is highly variable from county to country. Several constraints affect grain productivity. Among these, drought, pests (particularly sorghum shoot fly, spotted stem borer, midge, aphids and head bugs) and diseases (particularly anthracnose and grain molds) are the predominant ones. Grain yields in farmers' fields in Asia and Africa are generally low (500 to 800 kg ha^{-1}). One of the major factors causing these low sorghum grain yields is insect pest damage. Each year nearly 32% of the actual produce is lost due to insect pests in India (Borad and Mittal, 1983), 20% in Africa and Latin America, and 9% in the USA (Wiseman and Morrison, 1981). The annual loss of sorghum production due to shoot fly in India is estimated at nearly US$ 200 million (ICRISAT, 1992).

Sorghum shoot fly (*Atherigona soccata* Rond.) is a key pest of sorghum in many countries including India. Shoot fly female lays a cigar-shaped eggs on the lower leaf surface of young sorghum plants in the 1-7 leaf stage, i.e. 5-25 days after seedling emergence. Egg hatch in 1-2 days and first instar larvae move along the shoot to the growing point of the seedling. The larva cuts the growing point resulting in wilting and drying of the central leaf, causing the typical 'deadheart' symptom (Sharma *et al.*, 2003) that appear 1-4 weeks after seedling emergence. In order to compensate for the loss of central shoot, damaged plant produces side tillers that may subsequently be attacked by shoot fly. Larval development is completed in 8-10 days after which the insect pupates in the soil (plate1.1). Pupation lasts approximately eight

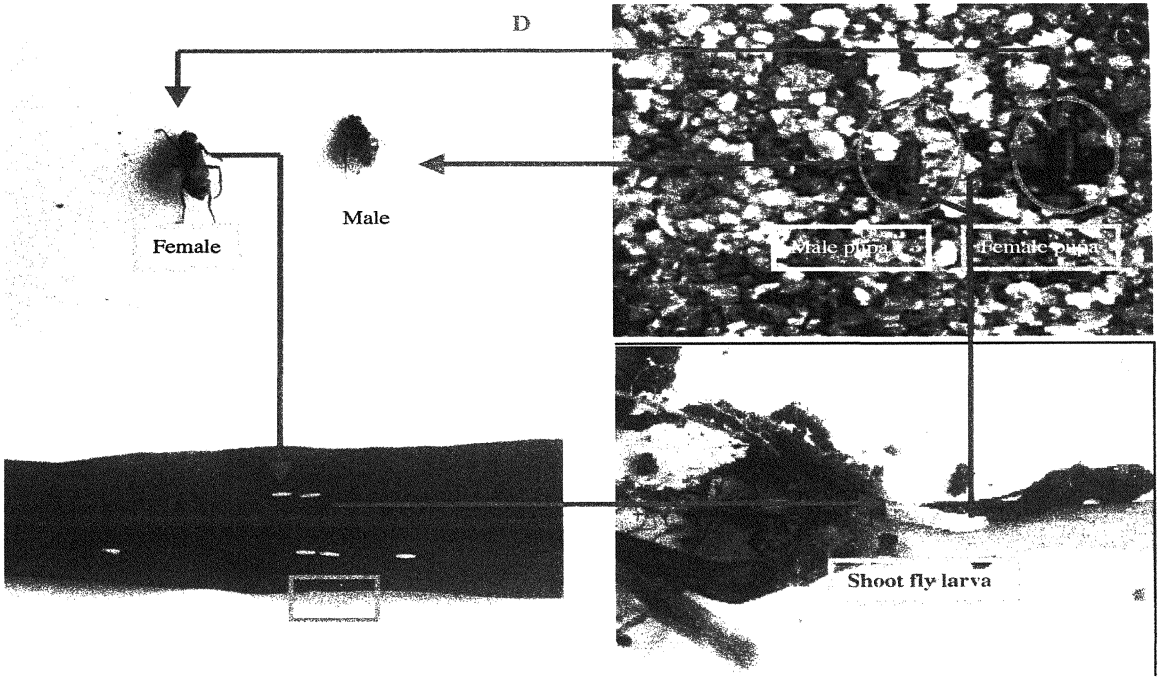


Plate 1.1 Various life stages of the sorghum shoot fly, *Atherigona soccata*. A = Eggs. B = Larva. C = Pupae. D = Adults.

days and the entire life cycle is completed in 17-21 days depending on prevailing weather conditions (Sharma *et al.*, 2003). Shoot fly incidence is high in late sown *kharif* (rainy season), early sown *rabi* (postrainy season) sorghum crops. The level of infestation even may go up to 90-100 % (Usman, 1972) and the losses due to this pest have been estimated to reach as high as 86% of grain and 45% of fodder yield (Sukhani and Jotwani, 1980). Adoption of chemical methods for insect control in staple food crops is not economically feasible for resource poor farmers of the semi-arid tropics (SAT) as the low crop value per acre precludes the use of insecticides for control of insects (Dhams, 1943). Therefore host plant resistance combined with timely sowing is the most realistic approach to minimize grain and stover yield losses due to insect pests such as sorghum shoot fly. Genetic variability for shoot fly resistance in plant exists in sorghum germplasm. Many of the germplasm sources for resistance to this pest have poor agronomic features and grain yield potential, and sources with high levels of resistance are not available in the cultivated species. Germplasm accessions with absolute resistance have been found in wild relatives of sorghum (*Sorghum purpurosericeum*, *S.nitidum*, *S. versicolor* and *S. australiense*) (Mote, 1984; ICRISAT, 1991); however, their utilization in sorghum breeding programs is hindered by crossing barriers.

Screening procedures have been standardized and low to moderate levels of resistance have been identified in several germplasm source materials (Sharma *et al.*, 1992). Several mechanisms of resistance have been identified in these resistant lines such as 'non-preference for oviposition' (components of which include trichomes, glossiness, and restricted leaf surface wetness), 'antibiosis', and 'tolerance' or 'recovery' (Sharma and Nwanze, 1997). Some of these resistance sources have been used in conventional breeding, but the levels of resistance available for selection among the segregating progenies were not high. The selection of sorghum genotypes for resistance to shoot fly by utilizing one or a few resistance parameters is inefficient because several components are involved in resistance and one or more genes govern each of these resistance components. Further, expression of many of these components is influenced by environmental variation; hence shoot fly resistance is a quantitative trait and shows a large amount of genotype × environment interaction. Marker-assisted selection has considerable potential to improve the efficiency of selection for quantitative traits (Hash and Bramel-Cox, 2000), such as shoot fly resistance, for which expression is sensitive to the testing environment. As resistance

to shoot fly are mostly quantitative in nature, it is important to identify quantitative trait loci (QTLs) from the viewpoint of genetics and breeding. The ultimate goal of such QTL analysis is to develop tools that are useful for marker-assisted selection in a practical breeding program aiming at increasing the level of resistance in agronomically elite backgrounds through gene pyramiding for shoot fly resistance.

Traditional quantitative genetic studies on shoot fly resistance with different sorghum genetic materials have been reported by many workers. Also recently QTL analysis for shoot fly resistance component trait has been carried out using a set of sorghum recombinant inbred lines (RIL population) derived from cross BTx623 × IS 18551 (Sajjanar, 2002; Folkerstama *et al.* 2005, unpublished). These studies revealed the complex nature of shoot fly resistance and quantitative inheritance of resistance for some of the component traits with possible genotype (G) × environment (E) interaction. Quantitative genetic analysis of shoot fly resistance requires replicated, multi-environment testing under a wide spectrum of shoot fly pressure because of the unpredictability of field screening environments. This can be accomplished by utilization of a RIL population. This allows measuring of the environmental (E) contribution and G × E contribution to total phenotypic variance allowing less biased estimates of genotypic (G) variance. In sorghum linkage maps have been developed using a number of Restricted Fragment Length Polymorphism (RFLP) (Subudhi and Nguyen, 2000), Amplified Fragment Length Polymorphism (AFLP) (Bovin *et al.*, 1999) and Simple Sequence Repeat (SSR) markers (Bhattramakki *et al.*, 2000). Among the different types of molecular marker systems available, SSR markers best satisfy the criteria of sufficient polymorphism, repeatability and cost effectiveness required for successful utilization in applied marker-based selection. In sorghum a reasonably large number of SSR markers have been developed (Brown *et al.*, 1996; Taramino *et al.*, 1997; Kong *et al.*, 2000; Bhattramakki *et al.*, 2000; Schloss *et al.*, 2002), often using the elite breeding line BTx623 as a source, and these are suitable for screening the existing sorghum RIL population to construct a genetic linkage map and to identify QTLs for shoot fly resistance and its component traits.

The analysis of genetic diversity and relatedness among individuals within a species or among different species or populations is a central task for many disciplines of biological science. Genetic diversity and phylogenetic studies were initially conducted using quantitative and qualitative traits, which are mostly morphological, using various statistical methods i.e. analysis of variance, covariance,

D^2 statistics and Metroglyph analysis. These analyses are mostly based on quantitative traits that are highly influenced by environmental effects and require tedious statistical procedures. Molecular markers are being widely used in various areas of plant breeding as important tools for evaluating genetic diversity and determining cultivar identity (molecular fingerprinting). Establishment of a molecular marker and phenotypic assessment database of crop germplasm will help breeders to trace down the origins and degrees of relatedness of many landraces and cultivars. Considering the potential of molecular markers crop breeders can extend their hands to use these to supplement other tools currently being used in their crop breeding program. In this present study, we used SSR markers to estimate the level of allelic differences among 91 sorghum accessions collected from different parts of the world and previously identified as resistant to one or more major insect pests of this crop, with the aim of assessing their genetic diversity.

DNA markers that are tightly linked to agronomically important genes can be used as a molecular tool for marker-assisted selection (MAS) in plant breeding (Ribaut and Hoisington, 1998). MAS involves using the presence/absence of a marker as substitute for or to assist in phenotypic selection, in a way that makes it more efficient, effective, reliable and cost effective compared to the phenotypic based selection in conventional plant breeding methodology. Host plant resistance can play pivotal role in integrated pest management. Sources of resistance to insect pests have long since been identified; however, these have not been used effectively in crop improvement programs because the levels of resistance available are either too low or it is only rarely possible to develop optimum levels of insect infestation to screen the test material. Use of biotechnological approaches can play a significant role in developing cultivars with resistance to insects. There is an urgent need for innovation in the improvement of phenotyping systems for assessing resistance to insect pests. Once accurate and precise phenotyping systems for insect resistance have been established, the molecular markers can be used in dissecting the genetic basis of resistance, identifying the location of underlying genes and the nature of their gene action. Such knowledge will significantly accelerate the introgression of insect resistance genes into high yielding cultivars. The final outcome of marker-assisted crop breeding will be the rapid production of improved varieties and at lower cost.

With this background, a research program 'Genetic diversity analysis, QTL mapping and marker-assisted selection for shoot fly resistance in sorghum [*Sorghum bicolor* (L.) Moench]', was therefore attempted with following objectives:

1. To assess genetic diversity by SSR markers in a set of insect resistant lines.
2. Phenotyping a set of RILs (296B × IS 18551) for components of resistance to shoot fly over seasons.
3. Identification of QTLs for shoot fly resistant component traits using the marker genotyping and phenotyping data of the RIL population derived from 296B × IS 18551 cross.
4. Introgression of shoot fly resistant component traits in agronomically superior genotypes using molecular marker-assisted selection.

REVIEW OF LITERATURE

CHAPTER II
REVIEW OF LITERATURE

2.1 Application of SSR markers in diversity analysis of sorghum insect resistant germplasm accessions

The present review covers the assessment of genetic diversity at a molecular level and its application in crop improvement in general and sorghum in particular.

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop of world providing food and fodder throughout the world (Dogget, 1988). It is a crop with extreme genetic diversity. Its adaptation to harsh environments, specifically its high levels of resistance to biotic stresses and tolerance to abiotic stresses, accounts for its success throughout the semi-arid regions of the world. It has numerous mechanisms that allow it to survive and still be productive in these conditions. Harlan and Dewet (1972) subdivided the cultivated sorghum into five morphologically distinct races: bicolor, guniea, caudatum, kafir, and durra. Intermediate races are designated, for example, as kafir-csudatum, durra-bicolor, etc. They speculated that the race durra and bicolor arose from the wild subspecies *aethiopicum*, that the kafirs arose from *ve. Ticilliflorum*, and that the guineas evolved from *arundinaceum*. Subraces or working groups (Murty and Arunachalan, 1967) describe some of the variation within races and intermediate races and often refer to commonly used groups by sorghum scientists as feteriatas, zera-zera, kaura, kaoliang, milo, sorgo, sudangrass, etc. A refinement of the working groups as they fit with and complement the Harlan and de Wet race classification has been proposed by Dahlberg (2000).

The germplasm pool of the genus *Sorghum* is characterized by abundant diversity. The immense morphological diversity of the cultivated races of sorghum had resulted from variable climate and geographical exposure in which its wild ancestors evolved, coupled with selection pressure imposed by the environment and the man during and after domestication. Many sources of exotic and unique germplasm have been discovered and utilized over the years for sorghum improvement. Traits such as grain yield, resistance to shoot fly, stem borer, midge and greenbug had been found and incorporated into current germplasm and it has resulted in tremendous improvement in crop adaptation, resistance to biotic stresses, tolerance to abiotic stresses, and food and fodder productivity.

Understanding and management of the natural variation within the domesticated cultivars and their wild relatives of a plant species is very important in the establishment of an efficient breeding program aimed at crop improvement. Exploiting natural variation is very important for several reasons. Genetic uniformity in crops is undesirable as it makes the crop vulnerable to epidemics and environmental disasters resulting in yield loss. Many wild relatives of crop plants contain genes conferring resistance to biotic stresses such as pests and diseases, and tolerance to abiotic stresses such as drought, cold, and salinity. When these traits are incorporated into economically important varieties, large losses in yields can be avoided. A plant breeder also aims at improving certain desired characters such as grain quality and yield for specific end use adaption. A pre-requisite for improving the overall plant characteristics is an understanding of the germplasm available for use in breeding, which in turn will allow a systematic sampling of the germplasm for breeding and conservation purposes. DNA markers have been used to quantify genetic diversity and determine phylogenetic relationships in several plant species (Clegg, 1991; Lee, 1998). Cluster analysis is useful for studying the relationships among closely related accessions while ordination (principal component analysis) provides a more complete representation of the relationship among major groups. Such an analysis is very useful for producing 'core' collections at the international centers (Virk *et al.*, 1995), which can represent most of the diversity in the germplasm collection and allow one to extrapolate conclusions to the entire collection.

Following domestication, genetic variation in crop plants has continuously narrowed due to continuous selection pressure for specific target traits, *i.e.*, yield and its attributes. This narrowing of genetic variation has render crops more vulnerable to disease and insect epidemics and jeopardized the potential for sustained genetic improvement over the long term (Harlan, 1989). This risk was brought sharply into focus in 1970 with the outbreak of southern corn leaf blight, which drastically reduced corn yields in USA, and was attributed to extensive use of a single system of cytoplasmic-genetic male sterility (Texas type) for hybrid seed production, which was unfortunately linked to disease susceptibility (Ulstrop, 1978). Thus, it is extremely important to study the genetic composition of the germplasm of existing modern day cultivars in comparison with their ancestors and related species. This will not only provide information on their phylogenetic relationship but also indicate where there are chances of finding new and useful genes, as the accessions with most distinct

DNA profiles are likely to contain a greater number of novel alleles. DNA profiling to make such sampling decisions is now underway in most crops. Many DNA markers, both specific as well as arbitrary, have been used so far for DNA fingerprinting of various classes of germplasm (Callow, 1997; Virk. *et al.*, 1997). AFLP markers are a new class of molecular marker that has gained popularity for the study of genetic polymorphism, especially in species where polymorphism is extremely rare using other types of marker systems. Pakniyat *et al.* (1997) used AFLP for studying variation in wild barley with reference to salt tolerance and associated eco-geography.

More recently the discovery and application of several more readily reproducible of polymorphism assays based on variation in the number of short tandemly repeated DNA sequences (*i.e.*,SSRs) has increased the utility of PCR-based molecular marker genotyping for genetic diversity and marker-assisted breeding at least in crops where the necessary investment to develop appropriate primers can be made. DNA simple sequence repeats are numerous and are highly polymorphic in plants (Morgante and Olivieri, 1993; Wang *et al.*, 1994; Rongwen *et al.*, 1995; Yang *et al.*, 1994). SSRs are a highly useful class of such PCR-based genetic markers. Although costly to develop relative to some other classes of genetic markers, once developed their analysis is both easy and inexpensive. They are co-dominant, occur in high frequency, and can display a high level of polymorphism even among closely related accessions. Their high information content and other favorable characteristics make them excellent genetic markers for many types of investigation including marker-assisted selection and fingerprinting of germplasm collections (Brown *et al.*, 1996). SSR markers are detected utilizing the polymerase chain reaction with pairs of unique DNA primer sequences flanking the repeated region. They have not only revolutionized mammalian genome analysis (Hearne *et al.*, 1992), but have also facilitated plant breeding and genetics. Recently, SSR marker technology has been developed and used for genome mapping and DNA fingerprinting in crop plant species such as rice (Wu and Tanksley, 1993), wheat (Roder *et al.*, 1998), barley (Saghai Maroof *et al.*, 1994), maize (Senior and Heun, 1993; Taramino and Tingy, 1996), sorghum (Brown *et al.*, 1996; Taramino *et al.*, 1997; Dean *et al.*, 1999; Bhattramakki *et al.*, 2000; Dje *et al.*, 1999, 2000; Kong *et al.*, 2000; Smith *et al.*, 2000; Ghebru *et al.*, 2002; Haussmann *et al.*, 2002; Schloss *et al.*, 2002),

Genetically mapped markers tagging specific genes of interest to plant breeders have been identified. Examples include resistance genes for blast and gall

midge (Nair *et al.*, 1995a; 1996) using RFLP- and PCR-based approaches in rice, and leaf rust resistance gene LR 28 in wheat (Naik *et al.*, 1998); QTLs for protein content in wheat (Prasad *et al.*, 1999) heterosis in rice (Nair *et al.*, 1995b), downy mildew resistance (Jones et al., 2002) and drought tolerance (Yadav *et al.*, 2002, 2004) in pearl millet.

Germplasm analysis to study genetic diversity is other important area in which a lot of efforts have been put for fingerprinting of crops like rice (Ramakrishna *et al.*, 1994; Gupta *et al.*, 1994), wheat (Sen *et al.*, 1997; Pujar *et al.*, 1999), pearl millet (Chowdari *et al.*, 1998) etc. are being carried out extensively. This information has potential in strategic planning of future crop breeding efforts to improve agricultural sustainability in the SAT. Information on the genetic diversity available within a crop species is important for selection of parental strains and in the prediction of hybrid performance especially in crops such as rice, sorghum and maize in which hybrids are commercially important. The various steps involved in hybrid breeding programs, such as making several crosses and screening the combination for superior performance and heterosis are very costly, laborious, and time consuming. Hence, if heterosis can be predicted before making the crosses, then the number of crosses to be performed and the progeny to be screened in field trials can be reduced considerably. Various investigators are trying to correlate genetic diversity, as quantified by DNA markers, to predict hybrid performance, in various hybrid-breeding programs because the level of genetic diversity between the parents has been proposed as a possible predictor of heterosis. Studies with maize (Godshalk *et al.*, 1990; Melchinger *et al.*, 1992) revealed that molecular marker analysis is useful for assigning maize inbreds to heterotic groups, but the RFLP based genetic distance cannot be used to predict hybrid performance, while in oats, Moser and Lee (1994) have shown that molecular marker based genetic distance could be a predictor of hybrid performance only for those crosses where the parents belong to the same heterotic group and can not be extended to crosses between different heterotic groups.

Smith *et al.* (1990) observed a significant relationship between parental genetic distance and F_1 performance with a simultaneous increase in sample size as well as the number of markers used for analysis. Stuber *et al.* (1992) reported a significant relationship between parental heterozygosity and hybrid yield when the number of parental inbred lines was increased. While Lanza *et al.* (1997) observed consistent correlation between grain yield and random amplified polymorphic DNA

(RAPD) marker-based genetic distance in maize, Martin *et al.* (1995) and Barbosa-Neto *et al.* (1996) were not able to establish any relationship between marker-based genetic distance and hybrid performance in wheat. In rice, Zhang *et al.* (1994, 1995) used eight lines representing a major portion of the elite rice germplasm used in the hybrid rice breeding programs in China, to determine the relationship between marker locus heterozygosity, performance, and heterosis. Their studies revealed that correlations between mid-parent heterosis and specific heterozygosity (based on positive markers) were large and may be useful for prediction of heterosis. If such correlations are confirmed using a larger sample size, then it can certainly aid in planning the most productive crosses in the hybrid-breeding program.

The results of Xiao *et al.* (1996a) involving crosses between four japonica and six indica elite inbred rice lines had indicated that genetic distance measures based on RAPDs and simple sequence repeats (SSRs) could be useful for predicting yield potential and heterosis of intra-subspecific hybrids, but not of inter-subspecific hybrids. Heterotic groups are not clearly defined in sorghum as in maize, studies using molecular markers cluster A/B-pairs and R-lines separately (Ahnert *et al.*, 1996); however the majority of RFLP patterns were common to both groups suggesting that A/B-pairs and R-line groups have not diverged to an extreme degree (Verling *et al.* 1994 and Ahnert *et al.* 1996). A close relationship between morphological markers and molecular markers with respect to cluster formation has been reported by many workers [*e.g.*, Virk *et al.* (1995) in rice and Bhattacharjee *et al.* (2002) in pearl millet]. Smith and Smith (1991) identified 47 maize hybrids using 80 RFLP probes. Wall *et al.* (1984) used zein protein markers to differentiate maize inbreds. Dallas (1988) identified rice cultivars by using RFLP markers identified using two human mini-satellites as probes.

Ghareyazie *et al.* (1995) assessed genetic diversity among 35 Iranian rice varieties by comparing these with two typical *indica* and three typical *japonica* varieties using PCR-based RFLP markers Virk *et al.* (1995) used RAPD markers to identify duplicate accessions in a rice germplasm bank. Taxonomists had traditionally used morphological markers to classify genetic resources in sorghum. The morphological traits used in the taxonomic classification of sorghum to different races are conditioned by a relatively small number of genes. However, more economically important traits, which are related to adaptation exhibiting enormous variability across sorghum germplasm, are often complex and quantitatively inherited. Hence

classifying germplasm accessions based on solely on a few discrete morphological characters would not necessarily provide an accurate indication of genetic divergence among the cultivated genotypes of sorghum (Menkir *et al.*, 1997). In sorghum, Tao *et al.* (1993) demonstrated the use of RFLP and RAPD markers to differentiate sorghum accessions and obtained different clusters according to their sub-specific groupings (*i.e.*, Durra, Zera-zera, Caud-Nig and Caffrorum). The result also indicated that individuals of similar taxonomic grouping but different geographic origin may be genetically less identical than previously considered and similar frequencies of polymorphism were obtained with RAPD with RFLP markers. Results of these experiment indicated that a high level of genetic uniformity exists within *Sorghum bicolor*. Deu *et al.* (1994) used RFLP markers and related allelic variation in these to racial differentiation among 94 sorghum germplasm accessions and breeding lines primarily of African origin. Oliveira *et al.* (1996) used RFLP, RAPD and Inter Simple Sequence Repeat (ISSR) markers in genetic diversity studies of 84 sorghum lines, and found that both racial characterization and geographic origin correlated with relatedness. Several workers have selected diverse parents fc crossing based on the genetic diversity revealed by RFLP and/or RAPD markers, and obtained close relationships between the levels of marker diversity observed and heterosis expressed by the F_1 hybrids (Smith *et al.*, 1990 and Xiao *et al.*, 1996a, in sorghum and rice, respectively). The advent of PCR-based molecular marker techniques such as RAPD (Williams *et al.*, 1990) has further facilitated analysis of the sorghum genome.

Pammi *et al.* (1994) identified conditions that allowed reproducible amplification of RAPD markers and tested them on 32 different genotypes of sorghum. Cui *et al.* (1995) compared the restriction fragment length patterns of 53 sorghum accessions from Africa, Asia, and USA and detected different levels of polymorphism according to source continent. Deu *et al.* (1995) assayed mtDNA variation using RFLP and showed a significant genetic differentiation among the cultivated sorghum crop. The *bicolor* and *guinea* races exhibited the highest variation while the *kafir* race had the least. The homogeneity of *kafir* may be due to its relatively recent domestication (Harlan and Dewet, 1972). Dje *et al.* (2000) evaluated the use of microsatellite markers to quantify genetic diversity within as well as among the accessions sampled from the world germplasm collection of sorghum. Considerable variation was found at 5 microsatellite loci analyzed, with an average number of alleles per locus equal to 2.4 within accessions and 19.2 in the over all

sample of 25 accessions. Results shows that microsatellite data are useful in identifying individual accessions with higher relative contribution to the overall diversity of the collection.

Grenier *et al.* (2000) evaluated the genetic diversity three subsets of around 200 accessions each from the world sorghum germplasm collection using 15 polymorphic microsatellite loci. The average allele richness of each subset was equivalent to 16.1, 16.3 and 15.4 alleles per locus for the subsets PCS (selective sampling based on quantitative characters), L (random sampling after stratification of the entire landrace collection), T (selection based on the geographical origin of the landraces and the traits under farmers selection), respectively. Average genetic diversity was estimated at 0.81 for the PCS subset, 0.77 for the L subset, and 0.80 for the T subset.

Smith *et al.* (2000) evaluated the potential ability of SSR technology for research product development, seed production quality assurance, and genetic resource conservation management for sorghum. Fifty genetically diverse elite sorghum inbreds with known pedigrees were used to compare the discrimination abilities of 15 SSR markers with 104 RFLPs. RFLP data allowed all lines to be uniquely identified except two lines that could not be distinguished by the molecular data. (As the set of lines used in the present study encompass a relatively broad array of germplasm diversity representing different geographical areas, maturity ranges, germplasm groups and inter-group crosses; even the very small set of these 15 SSR loci were able to uniquely identify these lines). The mean Polymorphism Information Content (PIC) values were 0.62 and 0.58 for RFLPs and SSRs, respectively. Correlations for pair-wise molecular profile distance with pedigree distance among the maintainer parents (B-lines) were 0.52 and 0.53 for RFLP and SSR data, respectively; and for male parents (R-lines) were 0.41 and 0.47 for RFLP and SSR data, respectively. This set of 15 SSR markers could be used to help the genetic resource conservation management in sorghum.

Ghebru *et al.* (2002) used sorghum SSR markers to characterize genetic diversity in 28 Eritrean sorghum landraces and compared this diversity to a representative sample of the world sorghum collection. Pools of SSR markers were sized and score on automated DNA sizing gels. A high level of diversity was observed among the Eritrean landraces compared to other sorghum germplasm, in both the number and size range of SSR markers. Individual landraces were found to

carry a high level of within-population diversity and heterozygosity, and between-populations diversity was equally high. Most of the Eritrean sorghum landraces evaluated clustered in a separate sub-group from the other sorghum germplasm included in this study. These results indicate that a great deal of germplasm diversity and genetic novelty are available in Eritrean sorghum and that SSR markers can contribute to the wise use of this diversity for sorghum improvement.

Jordan *et al.* (2003) investigated the value of molecular marker-based distance information to identify high yielding grain sorghum hybrid in Australia. Data from 48 trials were used to produce hybrid performance estimates for four traits (grain yield, height, maturity and stay green) for 162 hybrid combinations derived from 70 inbred parent lines. Each line was screened with 113 mapped RFLP markers. The Roger's distances between the parents of each hybrid were calculated from the marker information on a genome basis and individually for each of the ten linkage groups of sorghum. Some of the inbred parents were related so the hybrids were classified into 75 groups, with each group containing individual hybrids that showed a similar pattern of Roger's distance across linkage groups. Correlation between the hybrid group performance and hybrid group Roger's distances were calculated. A significant correlation was observed between whole genome based Roger's distance and grain yield (r=0.42). This association is too weak to be of value for identifying superior hybrid combinations. One reason for the generally poor association between parental genetic diversity and yield may be that important QTLs influencing heterosis are located in particular chromosome regions and not distributed evenly over the genome. Diversity on individual linkage groups was explored to predict hybrid performance and this detected two linkage groups explaining 38% of the total variation in hybrid performance for grain yield, while another model combining phenotypic trait data and parental diversity on a particular linkage group explained 71% of the total variation in grain yield and had potential for use in the selection of heterotic hybrids.

Monica *et al.* (2004) assessed the genetic diversity in elite sterility maintainer (B-lines) and fertility restoring (R-lines) sorghum inbreds as compared with a group of exotic and converted germplasm from world collections. A set of 100 SSR markers and 1357 AFLP marker with known map positions were utilized to determine genetic similarity in the groups of B-lines, R-lines, and US public inbreds. Cluster analysis of genetic similarity estimates revealed that the classification of sorghum inbreds is based on the sorghum working groups, Zera-zera, Kafir, Kafir-Milo, Durra and

Feterita. Cluster analysis failed to give a clear differentiation between B- and R-lines, suggesting that R- and B-lines do not represent well-defined heterotic groups in this set of public lines. By comparing the different classes of molecular markers (SSRs, AFLPs, combination of SSRs and AFLPs), it was determined that the distribution of markers and the coverage of the genomes by the markers did not affect the classification of genotypes.

Kamala *et al.* (2005) studied genetic and phenotypic diversity among 36 randomly selected downy-mildew resistant sorghum accessions, the former using 10 SSR marker loci and the latter using 20 phenotypic traits. The number of alleles (aj) at individual loci varied from 5 to 14 with an average of 8.8 alleles per locus. Nei's gene diversity (Hj) varied from 0.59 to 0.92 with an average of 0.81 per locus. High gene diversity and allelic richness were observed in races durra-caudatum (Hj=0.76, aj=4.3) and guinea-caudatum (Hj=0.76, aj= 3.8), and among accessions from east Africa (Hj=0.78, aj=7.2). The regions were genetically more differentiated than the races as indicated by Wright's F_{st}. The pattern of SSR-based clustering of accessions was more in accordance with their geographic proximity than with their racial likeness. This clustering pattern matched poorly with that obtained from phenotypic traits. The inter-accession genetic distance varied from 0.30 to 1.0 with an average of 0.78, while inter-accession phenotypic distance varied from 0.1 to 0.55 with an average 0.33. Eleven accession pairs had phenotypic distances more than 0.5 and genetic distance more than 0.7. These could be used as potential parents in sorghum downy mildew resistance breeding program (personal communication from Dr. S. Chandra, Principal Scientist, Statistics and Head, Bioinformatics Unit, ICRISAT).

Casa *et al.* (2005) quantified and characterized diversity in a panel of cultivated and wild sorghum with 98 SSR loci distributed through out the genome. In a panel of 104 accessions comprising 73 landraces and 31 wild sorghums. Evaluation of SSR polymorphism indicated that landraces retained 86% of the diversity observed in the wild sorghum. The landraces and wild accessions were moderately differentiated, but there were a little evidences of population differentiation among racial groups of cultivated sorghum. Neighbor-joining analysis showed that wild sorghums generally formed a distinct group and about half of the landraces tended to cluster by race indicating a history of gene flow among the various cultivated type or recent common ancestry.

2.2 Host plant resistance: mechanisms and inheritance of shoot fly resistance and its component traits in sorghum

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal crop of semi-arid regions of Asia, Africa, the Americas and Australia. Generally the lower yields in Asia and Africa are associated with pest damage. Nearly 150 insects species have been reported as pests on sorghum (Reddy and Davis, 1979; Jotwani *et al.*, 1980). Shoot fly is one of the major pests of sorghum in Asia and Africa. Adoption of chemical control methods is not economically feasible for most of the sorghum-growing farmers. Therefore, utilization of host-plant resistance is the most realistic approach to reduce losses caused by sorghum insect-pests. Even though genetic variability for shoot fly resistance is available in the sorghum germplasm, the level of resistance is not high and the available sources of resistance have poor agronomic features. The quantitative nature of resistance to this insect and a large environmental variation in its expression hinders genetic manipulation of shoot fly resistance by conventional plant breeding procedures. Resistance of plants to insects is the consequence of heritable plant characters that result in a plant being relatively less damaged than plants without those characters (Sharma, 1997). Many other studies have also revealed that a number of component traits are associated with shoot fly resistance.

The present review covers the areas of control of shoot fly, with main emphasis on host plant resistance, mechanisms and inheritance of resistance, and breeding for resistance. It also summarizes reports on molecular markers, QTL mapping and statistical techniques for mappings in general and for sorghum in particular.

2.2.1 Shoot fly control

Control of sorghum shoot fly can be achieved by early and/or timely sowing, increased seed rate, thinning and destroying the seedlings with deadhearts, crop rotations, fallowing and others methods like use of insecticides (Singh and Sharma, 2002). However, timely sowing depends on several factors like cropping system, rainfall, soil type and moisture status at sowing time, many of which are out of control of farmers. From previous studies (Jotwani *et al.*, 1970) it has been established that in *kharif* season shoot fly incidence and damage increases with delay in sowing date. Planting time studies during *kharif* season using high yielding cultivars showed that early *kharif* sowing with the onset of the southwest monsoon either avoided or

significantly reduced the incidence of damage by shoot fly (NRCS, 1998). This finding proved to be extremely useful for the widespread cultivation of high yielding cultivars possessing lesser levels of shoot fly resistance. In the case of *rabi* sorghum, advancing sowing dates gives better yield potential and efficient use of residual moisture under rainfed condition. However such plans for advancing *rabi* sorghum sowing are spoiled due to higher shoot fly incidence. For control of shoot fly in *rabi* sorghum, use of various insecticides like phorate, disulfan and carbofuran have been advocated. However, under high shoot fly pressure such attempts at chemical control fail. As far as biological control is concerned, more than 15 species of shoot fly predators have been recorded, but their predation potential has not been assessed under field conditions (Singh and Sharma, 2002).

2.2.2 Host plant resistance

The use of resistant varieties may offer the best and perhaps the only economical method of control of certain pests like sorghum shoot fly, because the control of insects on a crop of low value precludes the use of insecticides (Dhams, 1943). Painter (1951) defined resistance in plants to insect attack as the relative amount of heritable qualities of the plant that influence the ultimate degree of damage done by the insect. While according to Smith (1989), resistance of plants to insects enables a plant to avoid or inhibit host selection, inhibit oviposition and feeding, reduce insect survival and development, and tolerate or recover from injury by insect populations that would cause greater damage to other plants of the same species under similar environmental conditions.

2.2.2.1 Sources of resistance

The existence of resistance in sorghum to shoot fly was first reported by Ponnaiya (1951a), who identified resistant cultivars; most of them were from peninsular India. Subsequently, Rao and Rao (1956) and Jain and Bhatnagar (1962) evaluated 42 and 196 cultivars, respectively and selected a few promising resistance sources. The search for sources of resistance to shoot fly continued through field evaluation of thousands of entries of the World Sorghum Collection by the All India Coordinated Sorghum Improvement Project (AICSIP) during the 1960s (Singh *et al.*, 1968; Pradhan, 1971; Young, 1972) and by AICSIP and ICRISAT during the 1970s and 1980s (Jotwani, 1978; Rao *et al.*, 1978; Jotwani and Davies, 1980). Rao (1972) remarked that most of these identified resistance sources belong to the *maldandi* or

dagdi types of Indian winter sorghums or the *shallu* types usually grown mixed with *maldandi* or *dagdi* types, which consequently survived in small populations.

Several workers had screened sorghum germplasm for resistance to shoot fly considering the needs of the local breeding programs and identified resistance sources (Table 2.1). As the work on shoot fly resistance continues, several new sources are being put at breeders' disposal every year.

Frequency distributions of shoot fly reactions among sorghum germplasm accessions assessed for susceptibility to shoot fly revealed that out of 16694 accessions evaluated, 133 accessions showed high levels of resistance in the rainy season, but only 18 accessions showed high levels of resistance in the postrainy season (Sharma *et al.*, 2003). As far as taxonomic distribution is concerned, out of 1290 accessions showing some degree of resistance to shoot fly in the rainy season, most of the accessions belonged to the race *Durra* (471) or *Caudatum* (185). The geographic distribution of these sorghum germplasm accessions pointed out India as the main area of origin of accessions showing resistance to shoot fly in the rainy season, followed by Sudan and Nigeria. For postrainy season conditions, most of the resistant accessions originated from India, followed by Ethiopia, Sudan and Nigeria (Sharma *et al.*, 2003).

2.2.2.2 Mechanisms of resistance

All the three mechanisms of resistance suggested by Painter (1951) *viz.* ovipositional non-preference (Soto, 1974), antibiosis (Raina *et al.*, 1981), and tolerance/recovery resistance (Doggett *et al.*, 1970), are known to exist in sorghum for shoot fly resistance. The primary mechanisms of resistance to sorghum shoot fly have been observed to be non-preference for oviposition and perhaps a low level of antibiosis to the larvae (Young, 1972).

2.2.2.2.1 Ovipositional non-preference / antixenosis

Jain and Bhatnagar (1962) first reported ovipositional non-preference by shoot fly in resistant sorghum cultivars. Later several workers considered it as the primary mechanism of resistance in sorghum (Blum, 1967; Krishnananda *et al.*, 1970; Rangdang *et al.*, 1970; Jotwani *et al.*, 1971; Pradhan, 1971; Young, 1972; Soto, 1974; Narayana, 1975; Sharma *et al.*, 1977; Singh and Narayana, 1978; Singh and Jotwani, 1980a; Singh *et al.*, 1981; Sharma and Rana, 1983; Rana *et al.*, 1984; and Unnithan and Reddy, 1985).

Table 2.1. Sources of resistance to sorghum shoot fly, *Atherigona soccata*

Genotypes screened (Nos)	Season(s) of screening	Resistant Genotype(s)	Reference
214	Postrainy	N.J.993, N.J.995, N28/3, PB 2R, T-1 etc.	Ponnaiya, (1951a)
42	Postrainy		Rao and Rao (1956)
196	Rainy	IS 1056, IS 1150 and IS 2248	Jain and Bhatnagar (1962)
19	Rainy and post-rainy	IS 5566, IS 5285, IS 5623 and IS 5613	Krishnananda *et al.* (1970)
8	Rainy	IS 4522, IS 5210, IS 1061 and IS 1034	Jotwani and Srivastava (1970)
6	Rainy	IS 2312, IS 5641, IS 8316, IS 18557, IS 22133 etc.	Young (1972)
5	Greenhouse screening	IS 2123, IS 5470 and IS 1054	Soto (1974)
14	Late *kharif*	Jhalawar and Vallabhnagar	Kundu and Sharma (1975)
-	-	BP 53 (IS 1055)	Kundu, *et al.* (1977)
8	Late *kharif*	IS 2146, IS 4664, IS 5469 and IS 5490	Sharma *et al.* (1977)
45	Rainy	M 31-2B, MAL-B and XZM-2B	Lakshminarayana and Subba Rao (1978)
6	Greenhouse screening	IS 1054, IS 2269, IS 2123 and IS 5604	Singh and Narayana (1978)
17	Rainy, post-rainy and summer	IS 1054, IS 5469, IS 5490	Singh *et al.* (1978), Singh and Jotwani (1980a, b, c, d)
8	Late *kharif*	IS 2123, IS 5604, IS 5490 and IS 8315	Borikar and Chopde (1980,1981b, 1982); Borikar, *et al.* (1982a, b)

cont...

Genotypes screened (Nos)	Season(s) of screening	Resistant Genotype(s)	Reference
100	Rainy	PJ 3K, PJ 4K, PJ 6K, PJ 19K, PJ 20K, PJ 21K, M 35-1 ×X PJ 4R-22, M 35-1 × Improved Saoner, etc.	Mote *et al.* (1981); Bapat and Mote (1982a)
67	Rainy	*Sorghum purpureo-sericeum* and *S. versicolor*	Bapat and Mote (1982b); Mote (1984)
9	Late *kharif*	IS 4663, IS 4776, IS 5333, IS 8315, and PC 9	Khurana and Verma (1982, 1983, 1985)
43	Post-rainy	Improved Saoner, GM 2-3-1, and IS 3922	Salunkhe *et al.* (1982)
14000	Rainy, post-rainy, cage screening	IS 1034, IS 2123, IS 2312, IS 4646, IS 5604, IS 18366, IS 18368, IS 18369, IS 18471, IS 18551, etc.	Taneja and Leuschner (1985)
146	Rainy	E-303, E-501, E-502, E-503, E-601, B-641, 365A3, 367A1, 366B4, PS 18527, and PS 14523	Mote and Bapat (1983); Mote *et al.* (1983a, b)
22	Late *kharif*	IS 5642, IS 5490, IS 5469, and IS 4664	Sharma and Rana (1983)
8	Greenhouse screening	IS 5642, IS 3962, and IS 5613	Raina *et al.* (1984)
		PS 21171, PS 21318, PS 14093, PS 14103, and PS 14454	Agrawal and Abraham (1985)
18	Postrainy	IS 5490	Bothe and Pokharkar (1985)
32	Late *kharif*	CSV 6, SPV 13, SPV 70, CSH 7R, IS 5490, etc.	Kishore *et al.* (1985)
8	Late *kharif*	IS 2146, IS 4664, IS 5469, and IS 5490	Sharma and Rana (1985)
42	Rainy	E 303 and IS 2312	Shinde *et al.* (1985)

cont...

Genotypes screened (Nos)	Season(s) of screening	Resistant Genotype(s)	Reference
-	-	IS 2122, IS 2123, IS 4660, IS 5092, IS 5480, and IS 18551	Uniithan and Reddy (1985)
9	Rainy	E 201 to E 208, and E 303	Kishore (1986)
20	Rainy	IS 1082, IS 2146, IS 2312, IS 5470, IS 5622 and IS 5633	Mote *et al.* (1986)
8	Late *kharif*	IS5604, IS 5490, and IS 2146	Nimbalkar and Bapat (1987)
67	Rainy and post-rainy	IS 1456, IS 7094, and IS 12611	Jadhav *et al.* (1988)
20	Late *kharif*	IS 1054, IS 2123, IS 2312, IS 2146, IS 18551 etc.	Omori *et al.* (1988)
		IS 1054, IS 18551, IS 2123, and IS 5469	Singh and Verma (1988)
20	Rainy, late *kharif* and summer	IS 2205, IS 1054, IS 5469, IS 5619, IS 18557, IS 8320, S 386, and SPV 102	Patel *et al.* (1985); Patel and Sukhani (1990)
32	Rainy and post-rainy	P 24, E 302, 370 × 3660A, IS 1199 etc.	Dalavi *et al.* (1990)
205	Postrainy	IS 2312, IS 2191, IS 4516, IS 17596, IS 33714, and IS 33843	Balikai *et al.* (1998)
39	Rainy	PGN 1, PGN 8, PGN 19, PGN 20, PFGS 2, PFGS 8, PFGS 27 etc.	Kishore (2001)
16694	*Kharif, rabi*	IS 1034, IS 2146, IS 2205, IS 2312, IS 4664, IS 5604, IS 22121, IS 22144, IS 22145, IS 22148, IS 22149, IS 22196, and IS 18551	Sharma *et al.* (2003)

Jain and Bhatnagar (1962) screened 196 sorghum varieties from the World Collection to assess varietal resistance to shoot fly and reported significantly less oviposition on resistant varieties as compared with susceptible ones. Similar results were also reported by Blum (1969b), Jotwani *et al.* (1971) and Jotwani and Srivastava (1970). They also reported that the efficacy of this mechanism was not stable and that it breaks down under no choice conditions or under heavy shoot fly pressure. When geographic distribution was considered, degree of shoot fly preference was found to be more (55%) in temperate and comparatively less (33%) in Indian varieties (Singh *et al.*, 1981).

Behavioural responses of shoot fly showed that initial choice of a susceptible cultivar, CSH 1 was random, but that the duration of female stay on resistant germplasm accessions IS 2146, IS 3692 and IS 5613 was brief (Sharma and Rana, 1983). In addition, adult females laid eggs on non-preferred cultivars only after laying several eggs on alternate susceptible CSH 1 seedlings.

Raina *et al.* (1984) reported that in single choice tests, significant non-preference for oviposition was observed on IS 2146, IS 3962 and IS 5613. In another experiment where females were given no choice for an oviposition substrate but could escape into an outer cage, ovipositional non-preference was evident for five the seven test cultivars. IS 2146 and IS 3962 were consistently non-preferred for oviposition in both of these tests.

Singh and Jotiwani (1980a) and Borikar *et al.* (1982a) indicated that efficiency of this mechanism of resistance is not stable and it tends to breakdown under no choice conditions and under heavy shoot fly population pressure. Mote *et al.* (1986) reported that the leaves of the some sorghum cultivars resistant to shoot fly were pale green compared to dark green colour of the susceptible cultivars. Texture and width of the leaf were also important factors in selection of the oviposition substrate by the female fly. Narrowness and erectness of the leaves reduce oviposition substrate resulting in less egg laying and lower deadhearts incidence compared to plants having broad and drooping leaves. Genotypes ICSV 705, IS 1054, IS 2146, IS2206, IS 4663, IS 5613, PB 15881-3, IS 18551, and IS 2312 have been reported to display high levels of antixenosis for oviposition (<17 eggs seeding^{-1}) as compare to susceptible check Swarna (18.8 eggs seedling^{-1}) across Indian shoot fly screening locations (AICSIP, 2003). Kamatar and Salimath (2003) suggested that plants with eggs contributed directly to deadhearts incidence (%) and could be used as a criteria to select sorghum

resistant to shoot fly, while leaf colour, seedling vigor, glossiness, leaf width and seedling height contributed indirectly towards plant resistance. Wild sorghum germplasm accessions belong to Para-sorghum and Stiposorghum sections were immune to shoot fly damage, while Heterosorghum and Chaetosorghum accessions showed negligible damage and the test accession of section Sorghum exhibited susceptibility to shoot fly under multi-choice conditions (Venkateshwran, 2003).

2.2.2.2.2 Antibiosis

Antibiosis to shoot fly was reported by Jotwani and Srivastava (1970), Blum (1972), Soto (1974) and Sharma *et al.* (1977). Survival and development were adversely affected when shoot fly larvae were reared on resistant varieties (Jotwani and Srivastava, 1970; Narayana, 1975; Raina *et al.*, 1981; Unnithan and Reddy, 1985) compared with susceptible genotypes (Singh and Narayana, 1978). Growth and development were retarded, and the larval and pupal periods were extended by 8–15 days on resistant varieties (Singh and Jotwani, 1980b). Survival and fecundity were also better on highly susceptible varieties (Singh and Narayana, 1978), but adversely affected on resistant varieties (Taneja and Leuschner, 1985). Survival and longevity of females and fecundity were adversely affected when the larvae were reared on shoot fly resistant genotypes (Raina *et al.*, 1981). Larval and total growth indices were significantly lowered on resistant compared with susceptible varieties. The percentage pupation on resistant varieties was significantly lower compared with that on susceptible varieties (Dhavan *et al.*, 1993).

Raina *et al.* (1981) suggested that trichomeless cultivars accumulate more dew and stay wet longer. This situation would facilitate the movement of freshly hatched larvae to the base of central shoot. On the other hand, trichomed cultivars tend to dry faster, making the downward journey of larvae more difficult. The earliest work that reported to antibiosis as a possible mechanism of shoot fly resistance in sorghum was that of Ponnaiya (1951a,) He attributed this to early deposition of irregular shaped silica crystals in the resistant cultivars, which was confirmed by Blum (1968).

Raina (1985) reported that three different factors, individually or in combination, may contribute to the expression of antibiosis to shoot fly in sorghum: (i) trichomed cultivars hinder the movement of newly hatched larvae to the base of the whorl; (ii) resistant cultivars had greater silica deposits and lignification of cells, which nay restrict larval penetration to the base of the whorl; (iii) biochemical deficiencies or presence of chemical factors in resistant cultivars may adversely affect

the development and survival of larvae and reduce the fecundity of the resulting adults.

Stability parameters for IS 8315 and IS 2123 revealed that the level of oviposition will differ on these two resistant lines under different levels of infestation pressure but there will be relatively less mortality in these resistance sources than in more susceptible sorghum genotypes. This is probably indirect evidence of antibiosis resistance mechanisms present in these two genotypes (Borikar and Chopde, 1982). Some cultivars are preferred for oviposition; however, levels of infestation as measured by deadhearts production are low mainly due to antibiosis (Mote *et al.*, 1986). Lower larval survival on resistant genotypes as compared to a susceptible one has also been reported by Jadhav *et al.*, (1986). The mortality of the first instar was highest (96%) in the first 24 hours (Mowafi, 1967; Bushara, 1972; Zein el Abdin, 1981), which depends not only on the ability of the female to select a suitable oviposition site, but also difficulty in penetrating the leaf sheath, and covering the distance between the egg deposition site and the seedling growing point (Delobel, 1982). The larva growing on a resistant variety is typically sickly in appearance and smaller compared to that grown on susceptible varieties. No larval survival was observed on accessions of Stiposorghum and Heterosorghum (*Sorghum laxiflorum*) and Para-sorghum had relatively higher levels of deadhearts incidence, but there was no fly emergence (Venkateswran, 2003). These results indicated that along with the non-preference mechanism of resistance to shoot fly, a high degree of antibiosis is also present in different groups of wild *Sorghum* accessions. The resistance of sorghum to the sorghum shoot fly is largely a cumulative effect of non-preference and antibiosis mechanisms (Raina *et al.* 1981).

2.2.2.2.3 Tolerance / recovery resistance

Five shoot fly resistant and 2 shoot fly susceptible sorghum varieties were studied in order to evaluate the association between several plant traits and tiller survival both under field and stimulated conditions (Blum, 1969a). In both experiments, tillers of all resistant varieties grew faster than tillers of the susceptible ones and also infestation by shoot fly was delayed by 2 days in resistant varieties as compared with susceptible ones. This form of resistance has been referred to as tiller survival, while Doggett *et al.* (1970) referred to this phenomenon as recovery resistance. Similar results were also observed by Blum (1972).

Doggett (1972) pointed that synchronized tillering after the main shoot is killed, is a potential form of recovery resistance. In Africa, farmers actually prefer an initial infestation of their sorghum by shoot fly that led to profuse tillering and subsequently a good harvest. However, Indian sorghums were known to be non-tillering and any basal tillering was a consequence of failure of the main shoot to grow due to deadheart formation. However, the tillers of susceptible varieties continue to be attacked by sorghum shoot fly under outbreak conditions, thus resulting in failure to yield the harvestable heads (Sharma et al., 1977).

Raina (1985) opined that tolerance can be greatly influenced by growth conditions and thus may not always be predictable at various locations, particularly those with irregular rainfall patterns. Further, recovery resistance/tolerance does not appear to be an useful mechanism particularly when shoot fly populations progressively increase as the rainy season continues (Singh *et al.*, 1981,Singh and Rana, 1986).

Mote *et al.* (1985) observed that SPH 196 and SPH 325 were less susceptible to *A. soccata* at the initial stages of seedling growth and expressed the highest frequency of recovery resistance and hence grain yield among 14 sorghum hybrids tested. Tiller development consequent to deadheart formation in the main shoot and the subsequent survival and recovery of the sorghum plant depends in part on the level of primary resistance. Varieties with high recovery of resistance appeared to yield more under shoot fly infestation (Rana *et al.*, 1985).

2.2.2.3 Factors associated with resistance

Some seedling (physico-morphological) characters (Blum, 1968; Maiti and Bidinger, 1979; Raina, 1981; Maiti *et al.*, 1984), as well as some biochemical factors, are associated with shoot fly resistance in sorghum (Singh and Jotwani, 1980c; Patel and Sukhani, 1990). Resistant cultivars are usually tall with thin stems having long internodes and short peduncles. Also they typically have narrow glossy and yellowish-green leaves. These leaves possess trichomes on the abaxial surface, which act as physical barriers to movement of young maggots (Kishore *et al.*, 1985; Mote *et al.*, 1986). Colour of leaves, glossiness of leaves and presence of trichomes are prominent attributes conferring resistance to shoot fly in sorghum (Jadhav *et al.*, 1986). These factors have been studied in detail and hence are reviewed individually below.

2.2.2.3.1 Glossiness

The glossy trait, a characteristic of most of the winter (*rabi*) sorghum varieties of India (Blum, 1972; Rao *et al.*, 1978), is reported to be associated with shoot fly resistance (Blum, 1972; Bapat *et al.*, 1975; Maiti and Bidinger, 1979; Taneja and Leuschner, 1985; Omori *et al.*, 1988). Tarumoto (1980) reported a simple screening technique for identification of glossy cultivars among large germplasm sets. The difference between glossiness and non-glossiness can be detected by whether or not sprayed water adheres on leaf blades.

Maiti and Bidinger (1979) screened approximately 8000 lines from the world sorghum germplasm collection for resistance to shoot fly and observed that lines with trichomes on their abaxial surface were more resistant to shoot fly than lines lacking such trichomes. These resistant lines also had other distinctive characteristics, which were evident only in first 3 weeks of seedling growth: leaves tended to be more erect and narrower, with yellowish-green glossy appearance, which is termed as the 'glossy trait'. A systematic survey of the world germplasm collection indicated a low frequency of accessions with the glossy trait (only 495 of 17,536 germplasm accessions screened) and 84% of these lines where of Indian origin. While glossiness is clearly manifested in the seedling stage, it gradually disappears as the seedling grows and soil fertility does not affect its expression (Maiti *et al.*, 1984).

Taneja and Leuschner (1985) identified 42 lines that were consistently resistant to shoot fly, and out of these 42 lines, 37 were glossy. Further evaluation of these lines for shoot fly reaction in rainy and postrainy seasons revealed that shoot fly incidence was higher in non-glossy lines than glossy ones in the post rainy season. However, glossiness contributed less to shoot fly resistance during the rainy season. .

Glossiness of seedling leaves may possibly affect the quality of light reflected from leaves and influence the orientation of ovipositting shoot flies towards their host plant. Also glossy leaves might also influence host selection by means of chemicals present in the surface waxes or by altered permeability of such waxes to chemicals present in the leaves (Sharma, 1993). Most of the lines resistant to shoot fly exhibit the glossy leaf characteristic during the seedling stage. The intensity of leaf glossiness at the seedling stage is positively associated with level of resistance to shoot fly (Sharma and Nwanze, 1997). Both A- and B-line components of pairs SPSFR 9406, SPSFR 94034, SPSFR 94036 and SP 55301 were significantly less susceptible to

shoot fly than susceptible check CSH 1 and had glossiness scores of <3 (ICRISAT, 1999).

Kamtar and Salimath (2003) observed highly significant inverse relationships between seedling glossiness score and both deadhearts incidence and oviposition levels. The level of resistance to shoot fly was higher when both glossy and trichomes traits occurred together (Agarwal and House, 1982). The presence of trichomes and glossiness have independent and apparently additive effects in reducing the incidence of damage by shoot fly (Maiti *et al.*, 1980).

2.2.2.3.2 Trichomes

Levin (1973) described the role of trichomes in plant defense and pointed out that in numerous species there were negative correlation between trichome densities and insect feeding and oviposition responses, including nutrition of larvae.

Maiti and Bidinger (1979) identified 32 lines from 8000 sorghum germplasm lines with trichomes on abaxial surface of the seedling leaf blade. These accessions had fewer plants with deadhearts and lower ratios of plants with deadhearts to plants with eggs than 35 lines without trichomes. Maiti *et al.* (1980) observed that the presence of trichomes on the seedling leaf surface resulted in a lower frequency both of oviposition by shoot fly and subsequent larval damage. Resistant accessions IS 2146, IS 3962 and IS 5613 had high densities of trichomes on the abaxial leaf surface while susceptible hybrid CSH 1 was found to lack trichomes. However, under heavy shoot fly infestations, the density of trichomes appeared not to make any difference between preference and non-preference for a sorghum cultivar as a substrate for oviposition.

Three wild *Sorghum* species (*Sorghum versicolor, S. purpureosericeum,* and an unidentified wild genotype) amongst 57 entries covering different species were found to be immune to shoot fly (Bapat and Mote, 1982b). It vas observed that these immune entries all had high densities of trichomes on the lower s of their leaf blades, which contribute to their resistance.

Maiti and Gibson (1983) suggested that trichomes might be less effective during the rainy season than during the postrainy season, possibly because of physiological factors or more severe shoot fly attacks during late rainy season plantings. Biradar *et al.* (1986) reported that the intensity of trichomes on the adaxial surface was 2 to 6 times more than abaxial leaf surface. Although, the trichome density on the abaxial surface of the leaf have significant and negative correlation

with deadhearts, it has indirect effect on oviposition by sorghum shoot fly (Dhillon, 2004). Role of plant trichomes in insect resistance is through physical barrier in the movement of insects on the plant surface (Peter et al., 1995). Trichomes in sorghum deter penetration of the young shoot fly larvae in the whorl (Maiti et al., 1980). Jayanthi *et al.* (1999) observed that the expression of trichomes in hybrids depended on the type of parents involved and the seasion of testing. If the postrainyseason-adapted resistant male-sterile lines were involved, trichome expression in hybrids was lower in the rainy season than in the post rainy season.

Trichomes can act as an insect resistance mechanism by limiting the insects' contact with the plant. Such trichome can act as a physical barrier to insect movement. In addition, glandular trichomes can contribute to insect resistance by producing toxic compounds, which poison the insect through contact, ingestion, and/or inhalation, and by producing gummy, sticky or polymerizing chemical exudates, which impede the insect movement (Duffey 1986, David and Moorthy 1988).

2.2.2.3.3 Interaction of glossiness and trichomes

A study of four combinations–glossy leaf and trichomes, glossy leaf only, trichomes only, and neither – revealed that the mean deadhearts percentages were 60.7, 70.9, 83.5 and 91.3, respectively (Maiti and Bidinger, 1979). The glossy trait alone (mean of 71% deadhearts) seemed to be more effective in reducing deadhearts incidence than trichomes alone (84% deadhearts). However, the combination of both characters (61% deadhearts) was significantly superior to the mean of the two resistance component traits taken individually. Similarly Agarwal and House (1982) also reported that the level of resistance was greater when both the glossy and trichome traits occur together.

2.2.2.3.4 Seedling vigor

Blum (1972) reported that shoot fly-resistant lines grow faster than susceptible ones, while Dhillon (2004) found that shoot fly-susceptible lines initially grow faster and attracted by shoot fly for oviposition, resulting the early deadheart formation, however the resistant lines delays oviposition, resulting in less deadhearts. Singh and Jotwani (1980d) indicated that longer and narrow leaves and faster seedling growth as indicated by leaf sheath length (8.36 cm in CSH 1 compared to 12.36 cm in IS 5469) and seedling height (29.13 cm in CSH 1 compared to 39.33 cm in IS 5469), coupled with hardness of the leaf sheaths may be contributing towards resistance to shoot fly.

Khurana and Verma (1985) studied plant characters of nine sorghum lines (6 resistant to shoot fly and 3 susceptible) and concluded that faster growing resistant plants may remain in the favorable height for relatively lesser period as compared to the slow growing susceptible plants. Taneja and Leuschner (1985) observed that in the postrainy season, shoot fly incidence was higher in sorghum lines that were less vigorous at seedling stage; however, the same trend was not observed in the rainy season. Also, it was observed that fast seedling growth might prevent the first instar larva from reaching the seedling growing tip, although leaf margins may be cut without causing deadheart symptoms.

Jadhav *et al.* (1986) studied morphological plant characters in 158 sorghum entries for interaction with response to shoot fly measured in terms of deadhearts incidence and concluded that apart from the glossy trait and presence of trichomes, initial faster plant growth rate confers resistance to shoot fly in sorghum.

Karanjkar*et al.* (1992) observed positive relationships between vigor of the plant and its escape from shoot fly attack (The seedling vigor score was recorded under moderate level of shoot fly infestation). Singh (1998) concluded that rapid seedling growth and long, thin seedling leaves make plants less susceptible to shoot fly. Seedling vigor was significantly and negatively associated with deadhearts and oviposition (Taneja and Leuschner, 1985). (The rapid seedling growth of tolerant/resistant genotypes acts as escape mechanism against shoot fly infestation, hence less oviposition and in turn less deadhearts incidence). Regression analysis indicated inverse associations between seedling vigor score and deadhearts incidence and direct associations with oviposition incidence and egg count (Kamtar and Salimath 2003).

2.2.2.4 Inheritance of resistance

Blum (1969b) developed 8 hybrids (made from 2 shoot fly susceptible and 4 resistant sorghum lines) and their F_2 progenies. The parental lines and all F_2 populations were evaluated under three levels of shoot fly infestation. The F_2 data indicated that resistance was partially dominant when evaluated under low shoot fly population pressure, while when evaluated under high shoot fly population pressure, susceptibility appeared to be dominant.

Balakotaiah *et al.* (1975) conducted a genetic analysis of resistance to sorghum shoot fly based on large F_2 populations from a diallel mating system involving exotic, Indian, and derived lines as parents. Gene effects estimated from

generation means analysis exhibited predominance of additive gene effects for the inheritance of shoot fly resistance.

Sharma *et al.* (1977) conducted a diallel analysis involving four agronomically superior dwarf and four resistant varieties of sorghum to study inheritance of resistance to sorghum shoot fly (It is a 8x8 diallel (without reciprocals) consisting 4 agronomically superior lines + 4 resistant lines). Inheritance of resistance was reported to be quantitative as indicated by the prevalence of continuous variation in different generations and the intermediate resistance levels expressed in F_1 hybrids of resistant and susceptible parents. Resistance was mainly additive in nature. It was also observed that F_1 hybrids of susceptible and resistant parents were slightly more susceptible than the mid-parental value and thus, susceptibility appeared to be partially dominant.

Borikar and Chopde (1980) evaluated an F_1 diallel cross with 4 resistant and 4 susceptible parental lines, under 3 distinct levels of shoot fly infestation in rainy season. The magnitude of additive components of variance, as compared to dominance components, increased with increases in shoot fly population pressure. Sorghum susceptibility to shoot fly appears to be recessive under low insect population pressure but exhibits dominance under high shoot fly population pressure.

Rana *et al.* (1981) studied the behaviour of shoot fly resistance in the F_1, F_2, F_3 and advanced generations of crosses between resistant and susceptible parental lines. In this study the F_1 was observed to be almost intermediate between the two parents with an added heterotic advantage of lower deadhearts percentage. Resistance showed partial dominance under low to moderate shoot fly population but this relationship may shift under heavy infestation conditions. The resistance is polygenic in nature and governed by additive genes.

Halalli *et al.* (1982) reported that in a seven parent diallel cross, comprising four high-yielding varieties and three cultivars with varying levels of shoot fly resistance crossed in all possible combinations and evaluated in post rainy season. The inheritance of shoot fly resistance was found to be controlled by both additive and non-additive genes effects.

Halalli *et al.* (1983) evaluated advanced generations during kharif to estimate extent of variability, heritability and genetic advance for shoot fly resistance. Five BC_1F_3 progenies, one F_3 progeny, and 3 F_4 progenies were found to be significantly

more resistant than the most highly resistant parent, IS 5604, suggesting transgressive inheritance of the character.

Patel *et al.* (1984) studied combining ability for shoot fly resistance in an 8-parent diallel cross without reciprocals. They reported negative general combining ability (GCA) effects in resistant parent for percent deadhearts both in normal and late sowings, suggesting preponderance of additive genetic variance. Patel *et al.* (1985) observed that in both normal and late sown conditions; additive (D) as well as non-additive (H_1, H_2) components of genetic variance were significant for resistance to shoot fly.

Nimbalkar and Bapat (1992) evaluated an 8-parent diallel cross under three levels of shoot fly infestation to study combining ability and genetic components of variation for shoot fly resistance. They observed additive gene action for shoot fly resistance.

2.2.2.4.1 Heritability estimates

Rana *et al.* (1975) reported that differences between shoot fly susceptible and resistant progenies are established from the F_3 generation and the heritability estimate for shoot fly resistance was about 25%. Borikar and Chopde (1981a) analyzed an 8-parent diallel cross in the F_1 and F_2 generations to study the genetic architecture of shoot fly resistance and indicated that heritability for shoot fly resistance appears to be around 23 to 25 percent.

Halalli *et al.* (1983) screened advanced generation materials and reported that broad sense heritability was around 30%, indicating a large influence of environment on shoot fly resistance. A summary of inheritance studies for different shoot fly resistance traits and their genetics along with heritability values reported by different workers are presented in Table 2.2.

2.2.2.5 Breeding for resistance

Although the work on sorghum shoot fly resistance was initiated in the early 1950s, no attempts were made to incorporate resistance into a variety with good agronomic base. Only since the late 1960s have various sorghum research workers [including Blum (1965, 1967, 1968, 1969a, b) from Israel, Doggett *et al.* (1970) and Starks *et al.* (1970) from East Africa, Harwood *et al.* (1972) from Thailand, Rao *et al.*, (1974), Balakotaiah *et al.* (1975), Rana *et al.* (1975, 1981, 1985), and Agrawal and House (1982) from India made significant contributions in breeding for shoot fly resistance.

Table 2.2 Inheritance of factors associated with host plant resistance to sorghum shoot fly, *Atherigona soccata*

Factors	Breeding material used	Gene action involved	Reference
Glossiness and non-glossiness			
	P_1 (non-glossy), P_2 (glossy), F_1, F_2	Simple recessive	Tarumoto (1980)
	F_2 populations of crosses among non-glossy (*Gl*), glossy (*gl*) and true glossy (*tgl*)	Multiple alleles	Tarumoto (1983)
		Both additive and non-additive	Agrawal and Abraham (1985)
	Twelve rainy and post-rainy bred male-sterile lines and 12 diverse restorers and their F_1s	Non-glossiness dominant and trait expression stable across seasons	Jayanthi *et al.* (1999)
Presence or absence of trichomes and trichome density			
	8000 lines from World Collection of Sorghum	Presence of trichomes – single recessive gene	Maiti and Bidinger (1979)
	Segregating generations developed by crossing trichomed and trichomeless parental lines	Presence of trichomes – single recessive gene	Gibson and Maiti (1983)
	Segregating generations developed by crossing trichomed and trichomeless parental lines	Trichomes – a major factor for resistance; at least two additional loci other than that previously reported are also involved in resistance	Maiti and Gibson (1983)
	P_1 (resistant), P_2 (susceptible), F_1, backcross involving susceptible line as recurrent parent	Trichome density on abaxial surface governed by complementary type epistatis	Biradar *et al.* (1986)
	P_1 (resistant), P_2 (susceptible), F_1, F_2, BC_1, BC_2	Adaxial leaf surface – additive and dominance × dominance	Borikar and Chandurwar (1989)
		Abaxial leaf surface – additive and additive × dominance	
	Twelve rainy and postrainy season-adapted male-sterile lines and 12 diverse restorers and their F_1s	Low Trichome density – partially dominant	Jayanthi *et al.* (1999)

(Table 2.2 cont...)

Factors	Breeding material used	Gene action involved	Reference
Seedling height			
	P_1 (resistant), P_2 (susceptible), F_1, F_2, BC_1, BC_2	Additive	Sharma *et al.* (1977)
	P_1 (resistant), P_2 (susceptible), F_1, F_2, BC_1, BC_2	Predominantly non-additive	Borikar and Chopde (1981b)
Deadhearts incidence			
	Large F_2 population from a diallel mating system involving exotic, Indian and derived lines	Predominantly additive	Balakotaiah *et al.* (1975)
	P_1 (4 resistant), P_2 (4 susceptible), F_1, F_2, BC_1, BC_2	Predominantly additive	Sharma *et al.* (1977)
	Seven parent diallel (susceptible × resistant) along with F_2	Both additive and dominance effects responsible for resistance; two recessive genes govern resistance	Hallali *et al.* (1982)
	8 × 8 diallel (resistant × susceptible)	Additive	Patel *et al.* (1984)
	P_1 (resistant), P_2 (susceptible), F_1, F_2, BC_1, BC_2	Both additive and non-additive (dominance, additive ×additive, dominance × dominance)	Biradar and Borikar (1985)
	16 F_2 progenies from crosses between susceptible × resistant parent	Two duplicate recessive genes govern the resistance	Rana *et al.* (1985)
	P_1 (resistant), P_2 (susceptible), F_1, backcross involving susceptible line as recurrent parent	Both by additive and non-additive components	Biradar *et al.* (1986)
	Eight parent diallel (3 resistant and 5 susceptible parents)	Additive and additive × additive gene interaction	Nimbalkar and Bapat (1987)
	Seven parent half-diallel analysis	Both additive and non-additive	Dhabholkar *et al.* (1989)

(Table 2.2 cont...)

Factors	Breeding material used	Gene action involved	Reference
	Eight (resistant) by eight (susceptible) diallel set evaluated under three levels Of shoot fly infestation	Additive	Nimbalkar and Bapat, (1992)
	Seven parent diallel trial involving resistance and susceptible lines	Both additive and non-additive gene action are responsible for resistance	Elbadawi *et al.* (1997)
Ovipostion non-preference			
	Eight F_1s derived from 2 susceptible and 4 resistant parents, F_2 under three distinct levels of shoot fly infestation	Non-preference was partially dominant under low shoot fly infestation; under high insect population pressure, preference appeared dominant	Blum (1969b)
	Take from book	Under low shoot fly population non-preference was dominant, while susceptibility was dominant under high infestation	Blum (1972)
	F_1s derived in line (7) × tester (11) mating design involving exotic and stable derivatives of exotic × Indian crosses	Additive	Rao *et al.* (1974)
	P_1 (resistant), P_2 (susceptible), F_1, F_2, BC_1, BC_2	Resistance additive; non-additive component negligible	Balakotaiah *et al.* (1975)
	Four resistant (P_1), four susceptible parents (P_2), F_1, F_2, BC_1, BC_2	Predominance of additive genetic variance	Sharma *et al.* (1977)
	Eleven sorghum cultivars (resistance) and 5 diverse (4 *milo* and 1 *durra* cytoplasm) male sterile lines and their F_1s	Ovipostion preference dominant	Kulkarni *et al.* (1978)
	F_1 diallel set derived from four tall resistant and four improved dwarf susceptible lines, under three distinct levels of infestation	Importance of additive component increased with increase in insect population	Borikar and Chopde (1980)

(Table 2.2 cont...)

Factors	Breeding material used	Gene action involved	Reference
	Four high yielding susceptible lines and 3 lines with varying level of resistance and their F_1s; under high shoot fly infestation	Both additive and non-additive and one group of dominant genes controls susceptibility	Halalli *et al.* (1982)
	16 F_2 progenies of crosses between susceptible × resistant parents	Single recessive gene governs non-preference	Rana *et al.* (1984)
	Eight parent diallel (3 resistant and 5 susceptible parents)	Both additive and non-additive gene actions involved, but predominance of non-additive gene action	Nimbalkar and Bapat (1987)
	Seven parent (resistance × susceptible) half diallel	Non-additive component responsible	Dabholkar *et al.* (1989)
Recovery resistance			
	Diallel set from crossing of varieties having high, intermediate and low levels of recovery resistance	Additive gene action important, but specific and epistatic effects also significant	Starks *et al.* (1970)
	Four resistant (P_1), four susceptible parents (P_2), F_1, F_2, BC_1, BC_2	Tiller survival and plant recovery not independent, but functions of genotypic differences for oviposition non-preference	Sharma *et al.* (1977)
	F_1 diallel set, derived from four tall resistant and four improved dwarf susceptible lines, under three distinct levels of infestation	Both additive and non-additive components significant, but importance of additive component higher	Borikar and Chopde (1981a)
	Four high yielding susceptible lines and 3 lines with varying level of resistance and their F_1s; under high shoot fly infestation	Both additive and non-additive gene effects involved, but importance of additive component is more	Halalli *et al.* (1982)

Blum (1965, 1967) improved the resistance of M 35-1 by two cycles of mass selection and successfully incorporated the resistance of the selected line into an adapted line with good agronomic characteristics using the pedigree method. Doggett *et al.* (1970) utilizing the recovery resistance mechanism available in the cultivar Namatera in crosses to elite line Serena developed high yielding lines possessing recovery resistance, by adopting the backcross method. Based on the largely additive genetic variance for recovery resistance, Doggett *et al.* (1970) established random mating populations for a long-term recurrent selection program.

In Thailand, Harwood *et al.* (1972) tried to improve the shoot fly resistance in material adapted to local conditions using resistance sources like IS 5604, IS 5383 and IS 4567. Three different approaches were taken: i) crossing these resistance sources with locally adapted varieties; ii) crossing these resistance sources with male-sterile seed parents of released hybrids, and iii) intermating the resistance sources. There was limited success because of various problems encountered due to undesirable characters of the resistance sources, which were photosensitive, tall and susceptible to mold and rust.

In 1968, breeding for resistance to shoot fly started in India (Vidyabhushanam, 1972). Initially the shoot fly resistant and popular local postrainy season variety M 35-1 was crossed with five susceptible lines, *viz.*, CK 60B, 2219B, IS 84, IS 3691 and 3675B (parents of released rainy season-adapted hybrids). Since the recovery of desirable F_2 segregates was very low, the F_1 hybrids were backcrossed to dwarf shoot fly susceptible lines. Selection of plants from the backcross progenies was done for two generations. When tested under heavy shoot fly infestation, the selected progenies proved to be highly susceptible.

Rao *et al.* (1974) recommended that due to superiority of hybrids of over parents and additive nature of inheritance, it could advantageously be capitalized in hybrids and line development programs. It was also opined (Balakotaiah *et al.*, 1975 and Sharma *et al.*, 1977) that the resistant × resistant crosses did not exhibit an improvement over the parents indicating no diversity among resistant lines. They have also concluded that resistance is due to gradual accumulation of desirable alleles rather than due to the presence of one or major genes.

Balakotaiah *et al.* (1975) observed that the characteristic way in which the seedling mortalities due to shoot fly gradually decreased from 65% to 23% in the order exotics, exotic × exotic, exotic × derivative, exotic × Indian, derivative × Indian,

Indian × Indian and Indian confirms that shoot fly resistance was due to gradual accumulation of desirable alleles rather than due to one or two major genes.

Rana *et al.* (1975) opined that transfer of resistance to shoot fly, which is primarily due to ovipositional non-preference, from the tall and generally late Indian varieties to dwarf, semi-dwarf and early-maturing forms is apparently feasible since inheritance appears to be largely additive. It was also suggested that the selection of resistant progenies, which exhibit seedling mortalities one standard deviation below the population mean under reasonable levels of infestation.

Sharma *et al.* (1977) also reported that resistance to shoot fly was due to the gradual accumulation of resistant genes of small effect, rather than being largely due to one or two major genes. They also reported positive associations of resistance with seedling height and performance *per se* of resistant varieties for oviposition incidences, seedling height, effective tillers percentage, plant recovery and yield per plant This necessitated selection of dwarf and high yielding plants from resistant families of susceptible × resistant crosses. It was opined that under such circumstances, where absolute resistance is lacking and threshold levels of resistance depend on shoot fly population, low intensity selection pressure should be applied under reasonable levels of infestation (50-80% shoot fly deadhearts on susceptible controls). Selection for effective tillers or plant recovery *per se* seems to be unnecessary, being a function of deadheart formation in the main shoot. Kulkarni *et al.* (1978) proposed that a shoot fly resistance hybrid breeding program should include dwarf female parents having some degree of resistance combined with Indian tall local resistant parents.

In a study of an eight-parent diallel cross (with 3 resistant and 5 susceptible lines) Borikar and Chopde (1981a, b) observed that additive variance a sizeable proportion of total genetic variance for shoot fly resistance, despite the presence of non-additive components for traits like plant recovery and eggs/plant. It was opined that a varietal breeding programme, through the exploitation of this fixable component by adopting a biparental cross approach, will be rewarding.

Rana *et al.* (1985) opined that in the absence of an immune source of resistance, a moderate level of resistance could be build-up in high yielding background. It was further inferred that breeding for resistance to shoot fly is a slow process, which requires several cycles of crossing to combine higher levels of resistance with yield potential and grain quality.

Singh and Rana (1986) observed that the behavior of resistance in F_1, F_2 and F_3 and advanced generations suggested the possibility of gradual improvement in resistant × intermediate and intermediate × intermediate crosses, where intermediate represents the high-yielding derivatives of resistant × susceptible crosses. By adopting such selection criteria in temperate × tropical crosses, which signifies susceptible × resistant crosses, it was possible to improve the level of resistance and develop a number of high-yielding varieties with adequate levels of shoot fly resistance.

2.2.2.6 Stability of resistance

Host plant immunity to shoot fly attack being absent, the level of 'deadhearts' symptoms in susceptible and resistant varieties varies with seasons, years and infestation levels (Singh and Rana, 1986). This has made it difficult to identify stable sources of resistance amongst the available pool of resistance sources.

Singh *et al.* (1978) conducted a stability study on 15 promising resistant varieties, identified on basis of preliminary screening of the world collection of sorghum, in six environments representing three crop growing seasons and two locations. It was noticed that most of the genotypes tested were consistent in their shoot fly reactions, but IS 1054, IS 5469 and IS 5490 were found to be the most stable. Borikar and Chopde (1981a) also reported that IS 5490, IS 5469 and IS 5490 × IS 5604 exhibited high degrees of resistance and greater phenotypic stability under three different shoot fly populations.

Chundurwar and Borikar (1983) evaluated 50 F_6 derivatives of shoot fly resistant × susceptible crosses under four levels of infestation to study their stability for resistance. Only five genotypes revealed deadheart incidence levels (%) at par with resistant control entry IS 168, regression coefficients less than unity and nonsignificant deviation from regression; indicating superiority of these genotypes over this control in respect to the stability of their shoot fly resistance. Among the germplasm lines tested in Indian Coordinated trials, IS 1082, IS 2146, IS 4664, IS 5470, IS 5566, PS 144454, PS 18061-3, PS 18822-4, PS 21318, PS 22121 and SPV 491 showed greater stability of resistance to shoot fly than IS 1054 (AICSIP, 1984).

Chundurwar *et al.* (1992) evaluated 32 sorghum genotypes to study genotype × environment interaction for shoot fly reaction in 4 different sowing dates. The high magnitude of environmental variance indicated that the level of shoot fly population played a major role and genotypes like IS 2146 and IS 5566 exhibited a high degree of stability for shoot fly resistance.

2.3 Molecular marker studies

2.3.1 Sorghum SSR markers

Simple sequence repeat- (SSR-) containing clones isolated from both bacterial artificial chromosome (BAC) and enriched genomic-DNA (gDNA) libraries and database sequences that contain SSRs were the sources for the sorghum SSRs mapped by Bhattramkki *et al.* (2000). Targeted isolation of SSR loci using BAC clones as proposed by Cregan *et al.* (1999) is likely to be the most efficient method for placing SSR loci in specific target genomic regions. BTx623 (Frederiksen and Miller, 1972) is the reference genotype used for sorghum molecular marker genotyping and it was the source of DNA used to construct the enriched libraries and the two sorghum BAC libraries that are currently available (Bhattramakki *et al.*, 2000). PCR primers for the amplification of DNA fragments containing SSRs from sorghum were successfully developed through three different approaches by Brown *et al.* (1996) and it was reported that sorghum fragments can be amplified using at least some maize SSR primers (Brown *et al.*, 1996).

Map locations have been published for nearly 300 sorghum SSR loci having primer sequences in the public domain (CT Hash, pers. comm.). Bhattramakki *et al.*, (2000) reported map location of 46 SSR loci based on previously reported primer sequences (Taramino *et al.*, 1997; Tao *et al.*, 1998a; Kong *et al.*, 2000) and 113 SSR loci (including four SSR-containing gene loci) based on novel primer sequences. These SSR marker loci were incorporated into pre-existing RFLP-based maps of Xu *et al.* (1994) (Kong *et al.*, 1997) and Peng *et al.* (1999) (Bhattramakki *et al.*, 2000). The number of SSR loci available per sorghum linkage group ranged from 8 to 30. Eight SSR loci that, although monomorphic among the 18 survey accessions, have high degree of homology to known genes (Bhattramakki *et al.*, 2000) remained to be mapped. The average number of alleles detected per locus at the polymorphic loci was 3.88. $(AG/TC)_N$ and $(AC/TG)_N$ repeats comprised the majority of these SSRs (52%) and 91% of the dinucleotide SSRs at these loci (Bhattramakki *et al.*, 2000). The estimated average probability that two accessions in a working group, would have different alleles at a locus ranged from 0.88 to 0.67 depending upon the working group to which the accessions belong (Kong *et al.*, 2000). In addition, the number of alleles per locus was positively correlated ($r = 0.68$, which is significant at the 1% level of probability) with the number of repeated units at the locus in BTx623, the strain from which the SSRs were originally isolated (Kong *et al.*, 2000). This

confirms that many *Sorghum bicolor* SSR loci are sufficiently polymorphic to be useful in marker-assisted breeding programs (Kong *et al.*, 2000). First complete genetic linkage map of sorghum, comprised of ten linkage group putatively corresponding to the ten gametic choromosome of Sorghum bicolor and Sorghum propinquum.The map includes 276 RFLP loci, predominately detected by pstl-digested Sorghum bicolor genomic probes, segregating in 56 F_2 progeny of a cross between Sorghum bicolor and Sorghum propinquum. The remarkable level of DNA polymorphism between these species will facilitate development of a high density genetic map (Chittenden et al 1994) Schloss (2002) Reported, the RFLP probes sequence were evaluated for presence of simple sequence repeat (SSRs) and 60SSRs (*Xcup* series) were developed and assayed in an array of sorghum germplasm comprising inbreed, land races and wild relatives. The sequence information and SSR loci generated through this study will be valuable in gene discovery, marker assisted selection, diversity and pedigree analysis.

2.3.2 Linkage maps in sorghum

Genetic studies of morphological traits in sorghum began early this past century and Doggett (1988) summarized genetic linkage of morphological and physiological mutants involving 49 loci. To date over 200 morphological and agronomically important markers have been identified (Berhan *et al.*, 1993); however, only nine linkage groups could be established with these markers and these consisted of only 2–10 loci (Pereira *et al.*, 1994). The biggest linkage group consisted of ten linked morphological marker loci (Doggett, 1988). Sorghum genome mapping based on DNA markers began in the early 1990s and since then several genetic maps of sorghum have been developed with large numbers of DNA-based markers including RFLPs, AFLPs and SSRs. Where opportunities have permitted, morphological marker loci have been integrated into these molecular marker-based genetic linkage maps. These maps will be useful in advanced breeding and genetic studies.

The construction of the first DNA-based sorghum linkage map was done using the RFLP technique with heterologous maize probes (Hulbert *et al.*, 1990). Later several more RFLP-based linkage maps of *S. bicolor* have been constructed (Binelli *et al.*, 1992; Whitkus *et al.*, 1992; Berhan *et al.*, 1993; Chittenden *et al.*, 1994; Pereira *et al.*, 1994; Ragab *et al.*, 1994; Xu *et al.*, 1994; Dufour *et al.*, 1997; Tao *et al.*, 1998a; Peng *et al.*, 1999, Haussmann *et al.*, 2002; Bowers *et al.*, 2003). Similarly, the RFLP maps of Xu *et al.* (1994) and Peng *et al.* (1999) have been improved with addition of

over 100 SSR markers (Kong *et al.*, 1997; Bhattramakki *et al.*, 2000), while that of Dufour *et al.* (1997) has been augmented with AFLP markers (Boivin *et al.*, 1999). Recently high-density genetic maps using AFLP, RFLP and SSR markers (Menz *et al.*, 2002) and RFLP probes (Bowers *et al.*, 2003) have been reported. These high-density integrated maps will accelerate genome mapping and comparative mapping activity in sorghum and other related grass species. The characteristics of different sorghum genetic maps are given in Table 2.3.

2.3.3 Marker-trait associations

Quantitative characters have been a major area of genetic study for over a century because they are a common feature of natural variation in populations of all eukaryotes (Kearsey and Farquhar, 1998). First attempts at studying them stemmed from the work of Galton (1889) on man before the rediscovery of Mendelian inheritance of quantitative characters through the pioneering work of Fischer (1918), which has been followed up by Wright (1934), Mather (1949) and Falconer (1989) to the new era. Despite these studies, the number of genes and their interactive effects controlling the expression of quantitative traits are poorly understood.

The basic concept of associating genetic markers with quantitative traits was first proposed by Sax (1923). Since then there has been great interest in genetic dissection of quantiative variation. Geneticists have recognized the potential use of linkages between quantitative genes and QTL for studying the nature of quantitative genetic variation (Sax, 1923; Lindstrom, 1926, 1931; Waxelson, 1933; Everson and Schaller, 1955;and Thoday, 1961) Unfortunately the relatively small numbers and sometimes-deleterious nature of qualitative marker genes was extremely limiting for linkage studies with quantitative genetic variation (Bubeck *et al.*, 1993).

Analysis of biochemical and DNA markers in crosses between parents that differ for a quantitative trait can be used to find markers linked to genes controlling the quantitative traits or QTLs (Gale and Witcombe, 1992). In plants the first attempts to use markers to perform genome-wide analysis of quantitative variation used allozymes (Tanksley *et al.*, 1982; Edwards *et al.*, 1987). Later RFLPs were used as DNA markers (Beckmann and Soller, 1983; Lander and Botstein, 1989), but these were followed by PCR markers such as RAPDs, microsatellites and AFLPs that were cheaper, safer and provided more marker data per unit of DNA (Westman and Kresovich, 1997). These polymorphic markers provided the framework maps with which the polygenes/QTLs could be located (Kearsey and Farquhar, 1998).

Table 2.3 Characteristics of different sorghum genetic linkage maps published to date.

Reference	Parents	Size and type of population	Markers	Genome length (cM)*	LG	Probe sources
Hulbert *et al.*, 1990	Shanqui Red × M91051	55 F_2	37 RFLPs	283R	8	Maize
Binelli *et al.*, 1992	IS 18729 × IS 24756	149 F_2	21 RFLPs	440U	5	Maize
Whitkus *et al.*, 1992	IS 2482C × IS 18809[3]	81 F_2	85 RFLPs, 7 isozymes	949H	13	Sorghum, maize
Berhan *et al.*, 1993	Shanqui Red × M91051	55 F_2	96 RFLPs	709R	15	Maize
Pereira *et al.*, 1994	CK60 × PI229828[2]	78 F_2	201 RFLPs	1530U	10	Sorghum, maize
Chittenden *et al.*, 1994	BTx623 × *S.propinquum*[1]	56 F_2	276 RFLPs	1445U	10	Sorghum, rice, oat
Xu *et al.*, 1994	IS 3620C × BTx623[3]	50 F_2	190 RFLPs	1789K	14	Sorghum, maize
Ragab *et al.*, 1994	BSC35 × BTx631	93 $F_{2:3}$	71 RFPLs	633H	15	Sorghum, maize
Lin *et al.*, 1995	BTx623 × *S.propinquum*[1]	370 F_2	202 RFLPs	935K	11	Sorghum
Tuinstra *et al.*, 1996	Tx7078 × B35	98 RILS	150 RAPDs, 20 RFLPs	Ca1580R	17	Maize, sorghum
Dufour *et al.*, 1997	IS 2807 × 379[3]	110 F_5 RILs	145 RFLPs, 4 cloned genes, 2 morphological markers	977H[a]	13	Maize, sugarcane
	IS 2807 × 249[3]	91 F_5 RILs	123 RFLPs, 4 cloned genes, 1 morphological markers	878H[a]	12	Maize, sugarcane
	Composite map of the two populations		183 RFLPs, 3 cloned genes, 2 morphological markers	1095H	13	Maize, sugarcane
Taramino *et al.*, 1997	CK60 × PI229828[2]	68 F_2	201 RFLPs, 7 SSRs	1575U	10	Maize, sorghum
Tao *et al.*, 1998a	QL39 × QL41[3]	120 F_5 RILs	155 RFLPs, 8 SSRs	1400U	21	Sorghum, cereals
Ming *et al.*, 1998	BTx623 × *S.propinquum*[1]	56 F_2	328 RFLPs	1750K[b]	10	Sorghum, cereals

cont...

Reference	Parents	Size and type of Population	Markers	Genome Length (cM)*	LG	Probe sources
Bovin *et al.*, 1999	IS 2807 × 379[3]	110 F_5 RILs	298 RFLPs, 137 AFLPs	1899H	11	Sorghum, cereals
Crasta *et al.*, 1999	B35 × RTx430	96 $F_{6:7}$ RILs	142 RFLPs	1602K	14	Sorghum, cereals
Peng *et al.*, 1999	BTx623 × IS 3620C[3]	137 F_{6-8} RILs	323 RFLPs	1347K	10	Sorghum, cereals
Bowers *et al.*, 2000	BTx623 × *S. propinquum*[1]	65 F_2	2399 loci based on 1925 RFLPs	1200U	10	Sorghum, cereals
Kong *et al.*, 2000	BTx623 × IS 3620C[3]	137 F_{6-8} RILs	11 RFLPs, 33 SSRs	1287K	10	Sorghum, cereals
Bhattramakki *et al.*, 2000	BTx623 × IS 3620C[3]	137 F_{6-8} RILs	354 RFLPs, 143 SSRs	1406K	10	Sorghum, cereals
Tao *et al.*, 2000	QL39 × QL41[3]	152 F_5 RILs	281 RFLPs, 25 SSRs	1871U	14	Sorghum, cereals, sugarcane
Xu *et al.*, 2000	B35 × Tx700	98 F_7 RILs	162 RFLPs	837H	10	Sorghum. maize
Bennetzen *et al.*, 2001	Framework map derived from comparison of the maps of Kong *et al.* (2000), Peng *et al.* (1999), Pereria *et al.* (1994) and Berhan *et al.* (1993)		154 RFLPs, 34 SSRs, 10 morphological markers	1450U	10	Sorghum, cereals
Klein *et al.*, 2001b	RTx × Sureno	125 F_5 RILs	44 SSRs, 85 AFLPs, 1 morphological marker	970K	10	Sorghum
Haussmann *et al.*, 2002	IS 9830 × E 36-1	225 $F_{3:5}$ RILs	125 AFLPs, 45 SSRs, 14 RFLPs, 3 RAPDs	1265H	10	Sorghum
	N 13 × E 36-1	226 $F_{3:5}$ RILs	158 AFLPs, 54 SSRs, 16 RFLPs	1410H	12	Sorghum
	Composite map of the two populations		339 AFLPs, SSRs, RFLPs and RAPDs	1424H	11	Sorghum

Cont...

Reference	Parents	Size and type of population	Markers	Genome length (cM)*	LG	Probe sources
Menz *et al.*, 2002	BTx623 × IS 3620C[3]	137 F_{6-8} RILs	336 RFLPs, 136 SSRs, 2454 AFLPs	1713K	10	Sorghum, cereals
Bowers *et al.*, 2003	BTx623 × *S. propinquum*[1]	65 F_2	2512 RFLPs	1059K	10	Sorghum, cereals, *Saccharum*, *Arabidopsis*

[a] Analyzed with Joinmap, MAPMAKER/EXP was used in all other maps

[b] Total map length does not include LG E

[1] Inter-specific cross; [2] inter-subspecific cross; or, [3] intra-specific cross involved in development of mapping population

* H, K = Map distances estimated using the mapping functions of Haldane (1919) and Kosambi (1944), respectively; U = mapping function not specified; R = Recombination frequency (%)

Several statistical approaches have been developed for detecting and quantifying the strength of these associations between markers and traits (Soller and Brody, 1976; Edwards *et al.*, 1987; Lander and Botstein, 1989; Knapp, 1989). The ability to detect a QTL with a marker is a function of the magnitude of QTL's effect on the character, the size of mapping population being studied and the recombination frequency between the marker and the QTL (Tanksley *et al.*, 1989). The realized QTL effect is a function of how large an effect the QTL has and how tightly it is linked to the marker or flanking markers (Gale and Witcombe, 1992). There are, however dangers associated with the establishment of breeding programs based on correlations of marker genotypes with quantitative traits before the identified factors (QTLs) have been tested in several genetic backgrounds and evaluate for associated effects on other characters of agronomic or economic importance (Tanksley and Hewitt, 1988).

It is well understood by plant breeders that genotype × environment (G×E) interactions exist for many quantitative traits, suggesting that general conclusions about QTLs, particularly those with small effects detected on the basis of single environments and single populations could lead to erroneous decisions. The use of QTL identification by breeders also will be influenced by the consistency of QTL regions across the germplasm (Bubeck *et al.*, 1993). One challenge of plant breeding is to take advantage of favorable direct effects of QTLs, while maximizing favorable environmental interactions and minimizing unfavorable ones (Bubeck *et al.*, 1993).

A greatly abbreviated list of agronomic traits subjected to marker-based mapping and QTL analysis includes 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 (Tanksley and Hewitt, 1988) and yield (Stuber *et al.*, 1987).

2.3.4 Statistical techniques for QTL analysis

QTL analysis is predicated on looking for associations between the quantitative trait and the marker alleles segregating in the mapping population. It has two essential stages: the mapping of the markers and association of the trait with the markers. Both of these require accurate data and statistical software (Kearsey and Farquhar, 1998). The basic theory underlying marker mapping has been available since the 1920s (Mather, 1938), but has to be extended to handle hundreds of markers simultaneously.

The availability of computer software packages has made this much easier (Young, 2001).

The traditional approach (Soller and Brody, 1976; Tanksley *et al.*, 1982; Edwards *et al.*, 1987) for detecting a QTL in the vicinity of a marker involves studying single genetic markers one at a time. However, if the QTL does not lie at the marker locus, its phenotypic effect diminishes relative to the true effect of the QTL as the distance (recombination frequency) increases between the marker locus and the QTL (Edwards *et al.*, 1987; Lander and Botstein, 1989). To overcome this, Knapp (1989) developed an approach that utilizes pairs of markers in a sequential manner and estimates the phenotypic effect of the QTL and its significance in the region bracketed by the two markers in each pair. Lander and Botstein (1989) reported development of such a method of mapping QTLs, interval mapping using LOD scores. Intervals between adjacent pairs of markers along a chromosome are scanned and the likelihood profile of a QTL being at any particular point in each interval is determined; or to be more precise, the log of the ratio of the likelihoods (LOD) of there being one QTL *vs.* no QTL at a particular point is determined (Lander and Botstein, 1989). An alternative approach using multiple regression was developed by Haley and Knott (1992). It often produces very similar results to LOD mapping both in terms of accuracy and precision, but has the advantages of speed and simplicity of programming. Tests of significance and confidence intervals can be obtained. Tanksley and Nelson (1996) advise that the statistical detection of QTLs is likely to depend not only on the type of population utilized, but is also likely to depend on the intra-locus and inter-locus interactions of the segregating QTLs.

For most mapping projects the most widely used genetic mapping software is MAPMAKER (Lander *et al.*, 1987). MAPMAKER is based on the concept of the LOD score, "the log of odds ratio" (Morton, 1955). The popularity of MAPMAKER is based on the ease with which it performs multipoint analysis of many linked loci (Young, 2001). The computer program JOINMAP is especially suited to relate one's map to those derived from other mapping populations (Stam, 1993).

To apply linkage maps to QTL analysis, MAPMAKER/QTL has been written to carry out simple interval mapping (SIM) QTL analysis using mathematical models and interfaces very much like the original MAPMAKER program (Lander and Botstein, 1989). Other programs like QTL Cartographer (Basten *et al.*, 1998) provide very much the same type of analysis. QTL analysis can also be performed by using

composite interval mapping (CIM) with the PLABQTL software as described by Rami *et al.* (1998) or with QTL Cartographer. For large-scale use of linkage information in a marker-assisted breeding, a program like Map Manager (Manley and Cudmore, 1998) helps to keep track of marker data in the population of interest. Hypergene (Young and Tanksley, 1989) or Graphical Genotyper (GGT) can help to display graphical genotypes. The program qGENE seeks to bring all of these important DNA marker tools together into single package (Nelson, 1997).

2.3.5 QTL mapping in sorghum

Numerous studies to identify QTLs for agronomically important traits have been conducted in sorghum and QTLs have been identified for a wide array of important traits (Table 2.4). This work has been important in improving our understanding of the genetic inheritance of specific traits and the best breeding approaches for them (Rooney, 2004). Adoption of other molecular technologies is important and is being tested. Markers detected for simply inherited traits such as maturity, height and fertility restoration have been identified and tested for the applicability of MAS schemes. These tests have had varying degrees of success. QTLs have been identified for drought stress (pre- and post-flowering), grain mold resistance, grain yield, and grain quality. Coulibaly (2002) was unsuccessful in using QTL markers to introgress post-flowering drought stress from donor parent B35 to several elite inbreds. Franks (2003) had limited success in using markers flanking QTLs for grain mold resistance to enhance grain mold resistance: they were effective in progenies with the exact same pedigree in which the QTLs were mapped, but they were not effective in any other population. The potential remains for the use of markers for simply inherited traits for introgression or pyramiding of traits, but there have been no reports published to document their use in sorghum (Rooney, 2004).

Table 2.4 Summary of qualitative and quantitative trait loci identified in sorghum

Trait	Reference
Drought tolerance (pre- and post-anthesis)	Tuinstra *et al.* (1996, 1997), Crasta *et al.* (1999), Subudhi *et al.* (2000), Tao *et al.* (2000), Xu *et al.* (2000), Coulibaly (2002); and Haussmann *et al.* (2003)
Anthracnose resistance	Boora *et al.* (1998) and Mehta (2002)
Rust resistance	Tao *et al.* (1998b)
Head smut resistance	Oh *et al.* (1994)
Downy mildew resistance	Gowda *et al.* (1995) and Oh *et al.* (1996)
Maturity	Lin *et al.* (1995) and Childs *et al.* (1997)
Height	Lin *et al.* (1995) and Pereria and Lee (1995); Klein *et al.* (2001a)
Yield and components	Pereria *et al.* (1995), Tuinstra *et al.* (1997), Rami *et al.* (1998) and, Sanchez-Gomez (2002),
Grain quality and mold resistance	Rami *et al.* (1998), Klein *et al.* (2001a), and Franks (2003)
Leaf blight resistance	Boora *et al.* (1999)
Fertility restoration	Klein *et al.* (2001b)
Pre-harvest sprouting resistance	Lijavetzky *et al.* (2000)
Striga resistance	Haussmann *et al.* (2004)
Greenbug resistance	Agrama *et al.* (2002),Katsar *et al.* (2002), Nagaraj *et al.* (2005)_
Midge resistance	Tao *et al.* (2003)
Shoot fly resistance	Folkertsma *et al.*(2005) unpublished; Sajjanar (2002), Deshpande (2005)
Tillering	Paterson *et al.* (1995)
Seed size and dispersal	Paterson *et al.* (1995)

2.3.6 QTL mapping for insect resistance in cereals

Like other quantitative traits, inheritance of resistance to a number of insects in cereals is polygenic (Khush and Brar, 1991). Phenotypic selection for such traits is difficult. Selection based on markers could theoretically ease the manipulation of such traits without affecting other agronomic traits. Molecular mapping experiments for quantitative insect resistance in maize, sorghum, rice, wheat and barley have been conducted and the details are presented in Table 2.5. The mapping population types generally used were $F_{2:3}$, RILs and doubled haploid lines (DHLs). The size of populations used varies between 71 (RILs) and 475 ($F_{2:3}$). Significant Q×E interaction was observed for resistance to corn borers in terms of leaf feeding rates (Jampatong *et al.*, 2002; Bohn *et al.*, 1996; Bohn *et al.*, 1997 and Groh *et al.*, 1998). This indicates the influence of environment on the expression of resistance traits.

Taking cognizance of the low power of QTL detection for small sample sizes (<300) found in simulation studies (Utz and Melchinger, 1994), several reasonably large sized RIL mapping populations have been developed in sorghum at ICRISAT, Patancheru. These are being screened for resistance to shoot fly, midge and stem borer.

Among the cereals, extensive QTL mapping experiments were done in maize for resistance to different species of corn borers. A commonly held view is that maize is exceptionally polymorphic, due to its highly cross-pollinated nature. A sufficiently large number of polymorphic RFLP loci can be found for maize in intraspecific crosses in contrast to many other crops where interspecific crosses are used to overcome lack of marker polymorphism within the cultigen. In addition, large numbers of RFLPs that have already been mapped in maize genome are publicly available (Bohn *et al.*, 1996). In case of sorghum, sorghum RFLP linkage maps (Subudhi and Nguyen, 2000) and an integrated SSR and RFLP linkage map (Bhattramakki *et al.*, 2000) are available (Haussmann *et al.*, 2002). These in turn have been supplemented by AFLP markers (Menz *et al.*, 2002) and RFLP markers from a wide array of graminaceous crop species (Bowers et al., 2003) to provide high density base maps for sorghum. The markers on these maps are of potential use in mapping sorghum genome regions associated with resistance to shoot fly, stem borer and midge.

Table 2.5 QTL mapping ... for insect resistance in cereals

Crop	Pest	Cross	Mapping population	Size of mapping population	No. of environments evaluated	Component character(s)	No. of QTLs identified	Q × E interaction	Reference
Maize	European corn borer (*Ostrinia nubilalis* Hübner)	B73 × B52	$F_{2:3}$	300	Two locations	Tunnel length ECB tunneling	7	Non-significant	Schon *et al.* (1993)
		B73 × B52	RILs	200	Four	Leaf feeding rates	9	-	Cardinal *et al.* (2001)
		B73Ht × Mo47	$F_{2:3}$	244	Three	Tunnel length Tunnel length	9	Significant	Jampatong *et al.* (2002)
					Three	Tunnel length	7	Significant	Krakowsky *et al.* (2002)
		De811 × B73	$F_{2:3}$	147	Three		7	-	Krakowsky *et al.* (2004)
		De811 × B73	RILs	191	Three		10	Significant	Cardinal and Lee (2005)
	Sugarcane corn borer (*D.saccharalis*)	CML131 × CML67	$F_{2:3}$	171	Two	Leaf feeding rates	10	Significant	Bohn *et al.* (1996); Bohn *et al.* (1997)
	Southwestern corn borer (*Diatrea grandiosella* Dyar)	CML131 × CML67	$F_{2:3}$	171	Two seasons	Leaf feeding	6	Significant	Bohn *et al.* (1997)
					Three seasons	Leaf feeding	9		
		CML131 × CML67	RILs	187	One season	Protein concentration	5	Significant	Groh *et al.* (1998)
				145	One season	Leaf toughness	7	-	
			$F_{2:3}$	475	-	Leaf feeding rates	7	-	
		Ki3 × CML139	RILs	158	Two seasons	Leaf feeding Leaf toughness	8	-	
		Ki3 × CML139	RILs	145	One season		2	Significant	

(Table 2.5 contd...)

Crop	Pest	Cross	Mapping population	Size of mapping population	No. of environments evaluated	Component character(s)	No. of QTLs identified	Q × E interaction	Reference
Sorghum	Greenbug (*Schizaphids graminum* Rond.)	GBIK × Redlan	RILs	95	-	Resistance and tolerance to Biotype	9	-	Agrama *et al.* (2002) Katsar *et al.* (2002)
	Shoot fly (*Atherigona soccata* Rond.)	BTx623 × IS 18551	RILs	252	Three	Glossiness Trichome density (upper surface) (lower surface) Oviposition Dead hearts	4 3 4 3 6	Significant Significant Significant Significant Significant	Sajjanar (2002) and Folkertsma *et al.* (2005; unpublished)
		296B × IS 18551	RILs	-	In progress	"	-	-	
	Stem borer [*Chilo partellus* (Swinoe)]	ICSV 745 × PB 15881-3	RILs	-	In progress	Different components of resistance	-	-	Unpublished, ICRISAT
		ICSV 745 × PB 15520	RILs	-	In progress	"	-	-	"
Rice	Brown plant hopper (*Nilaparvata lugents*)	IR64 × Azucena	DHLs	131	-	Mechanisms of resistance Ovicidal response (grade of watery	7	-	Alam and Cohen (1998)
		Asominori × IR24	RILs	71	-	lesions and egg mortality)	2	-	Yamasaki *et al.* (2000)

(Table 2.5 contd...)

Crop	Pest	Cross	Mapping population	Size of mapping population	No. of environments evaluated	Component character(s)	No. of QTLs identified	Q × E interaction	Reference
Rice	Brown plant hopper (*Nilaparvata lugents*)	B5 × Minghui 63	$F_{2:3}$	250	-	Mechanisms of resistance (antixenosis, antibiosis and tolerance)	2	-	Huang *et al.* (2001)
		Lemont × Teqing	RILs	160	-	"	7	-	Xu *et al.* (2002)
		IR64 × Azucena	DHLs	94	-	"	6	-	Soundararajan *et al.* (2004)
	Yellow stem borer [*Scirpophaga incertulas* (Walker)]	Co43 × W1263	F_2	-	-	Deadhearts and white-ears	-	-	Selvi *et al.* (2002)
	Green leafhopper (*Nephotettix virescens* Distant)	Taichung65 × ARC10313	F_{10} RILs	125	-	Antibiosis	4	-	Wang *et al.* (2004)
Barley	Cereal aphids	Harrington × TR306	DHLs	150	-	Aphid density	2	-	Moharrami pour *et al.* (1997)

Correlating a genetic map to the physical map would be highly valuable to plant geneticists for map based cloning of genes responsible for a particular QTL. Recently, an attempt has been made to locate molecular markers (*umc*105*a* on the short arm of chromosome 9, *csu*145*a* on the long arm) that flank QTLs for resistance to sugarcane corn borer (SCB) and southwestern corn borer (SWCB) in maize (Sadder and Weber, 2002). It was suggested that further polymorphic DNA sequences have to be identified before attempting to isolate these QTLs.

2.3.6.1 Shoot fly resistance component traits QTL mapping in sorghum

Sajjanar (2002) and Folkertsma *et al.* (2005 unpublished) genotyped 252 recombinant inbred lines (RILs) of a (BTx623 × IS 18551)-derived mapping population using 109 SSR markers. The genetic linkage map was constructed using JOINMAP versions 2.0 and 3.0, resulting in the formation of 10 linkage groups with a total map length of 1468 cM. QTL analysis using PlabQTL revealed the presence of 28 QTLs detected at least in two of three screening environments (four QTLs for seedling glossiness score, two QTLs for seedling vigor I, five QTLs for seedling vigor II, two QTLs for abaxial leaf surface trichome density, three QTLs for adaxial leaf surface trichome density, two QTLs for shoot fly oviposition incidence 14 days after seedling emergence (DAE), one QTL for shoot fly oviposition incidence21 DAE, four QTLs for shoot fly deadhearts incidence 21 DAE, three QTLs for shoot fly deadhearts incidence 28 DAE, and one QTL for seedling height I). Markers have been identified closely linked to the four deadhearts resistance QTLs. They will be used in marker-assisted backcrossing programs at ICRISAT and MAU-Parbhani.

Deshpande (2005) genotyping 213 RILs of 296B × IS 18551 mapping population using 114 SSR markers. The genetic linkage map has been constructed using Mapmaker/EXP 3.0 with the LOD threshold value at 3.0 and linkage distance (cM units) calculated using the Haldane (1919) mapping function. Markers were mapped in 10 linkage groups with a total map length of 2165.8 cM. QTL analyses performed using composite interval mapping (PlabQTL version 1.1) revealed the presence of 13 QTLs detected across two environments for important shoot fly resistant traits including seedling glossiness score (4 QTLs), seedling vigor score I (2 QTL), seedling vigor score II (1 QTL), deadhearts incidence (%) 28 DAE (1 QTL), seedling height I (1 QTL), seedling height II (1 QTL), trichome density of upper leaf blade surface (1 QTL), trichome density of lower leaf surface (2 QTLs).

2.4 Marker-assisted selection (MAS)

This section gives a detailed literature overview of different topics that deal with the study of marker-assisted selection in general, and for disease and insect resistance in crops in particular.

Marker-assisted selection (also referred as 'marker-assisted breeding') may greatly increase the efficiency and effectiveness of plant breeding compared to conventional breeding methods. Once markers that are tightly linked to genes or QTLs of interest have been identified, prior to field evaluation of large numbers of plants, breeders may use specific DNA marker alleles as diagnostic tools to identify plants carrying the target genes or QTLs (Michelmore, 1995; Ribaut *et al.*, 1997; Young, 1996). The advantages of MAS include a) substituting for complex field trials (that need to be conducted at particular times of year or at specific locations, or are technically complicated) with molecular tests helps in saving time and eliminating unreliable phenotypic evaluation associated with field trials due to environmental effects; b) selecting genotypes at seedling stage; c) gene 'pyramiding' or combining multiple genes simultaneously; d) avoiding the transfer of undesirable or deleterious genes ('negative linkage drag'; this is of particular relevance for introgression of genes from wild species); e) selecting for traits with low heritability; and f) testing for specific traits where phenotypic evaluation is not feasible (e.g., quarantine restrictions may prevent exotic pathogens to be used for screening).

In MAS the tight linkage of markers to a gene if interest is exploited for indirect selection of traits in a breeding programme. Two pre-requisites for adopting MAS in plant breeding programs are:

1. one or more marker loci tightly linked to the gene of interest, and
2. a population that is polymorphic for the marker(s) and gene of interest, which are in extreme linkage disequlibrium.

There are at least three possible approaches to applying MAS in plant breeding:

(a) selection based on markers alone with no measurements of phenotype;

(b) simultaneous selection on markers and phenotype; and

(c) two-stage selection with the first stage involving use of markers to select among the genotypes and second involving phenotypic selection among the previously selected genotypes.

The potential efficiency of marker-aided selection depends on the heritability of the trait, the proportion of genetic variance explained by the markers, and the selection method used.

MAS an important plant breeding tool in which molecular biology can be applied to transfer traits from donor parents to recurrent parents. MAS has been a useful tool for facilitating rapid generation advancement in case of application of QTLs in breeding programs (Lande and Thompson, 1990; Knapp, 1994 and 1998). Gimelfarb and Lande (1995) presented detailed analysis of the relationship between genetic markers and QTLs in the process of MAS. Mohan *et al.* (1997) concluded that MAS could be used to pyramid major genes, including disease and insect resistance genes, with the ultimate goal of producing crop cultivars with larger numbers of desirable traits. A study conducted by Eatington *et al.* (1997) assessed the usefulness of marker-assisted effects estimated from early generation testcross data for predicting later generations testcross performance.

MAS can be used to pyramid several segregating resistance genes into single host cultivars where hybrids are possible Witcombe and Hash (2000) have described how practically to strategically deploy resistance genes in a potentially more durable manner has been previously been practiced, by exploiting the ability of MAS to introgress multiple resistance genes into a common hybrid seed parent background, and then intermating the products to produce agronomically uniform cultivars that segregate for multplie resistance genes. The frequency of genotypes having resistance alleles at several loci increases greatly in both the seed parent and its hybrids when the overall frequency of resistance alleles in the maintainer line(s) increases.

The ability to manipulate genes responsible for quantitative traits is a prerequisite for sustained improvement in crop plants. MAS in pedigree, backcross and population improvement breeding methods is especially useful for traits that are otherwise difficult or impossible to deal with by conventional means alone (Hash and Bramel-Cox, 2000). There has been an implicit expectation that marker-based QTL analyses will make it easier and faster for breeders to manipulate these traits (Soller and Beckmann, 1983; Tanksley, 1983), but this expectation has often not been realized—in large part because of the emphasis in research on model systems and subsequent difficulties in extrapolating from such models to more complex (and less well understood) applications.

The development of linkage maps with abundant markers in a wide range of crop species was accelerated by development of newer and simpler DNA marker systems like RAPDs (Williams *et al.*, 1990), AFLPs (Vos *et al.*, 1995) and SSRs, also known as microsatellites (Akkaya *et al.*, 1992). Scientists soon began to believe that the promise of MAS originally proposed by Sax (1923) and Thoday (1961) might soon become a reality (Young, 1999). Analyzing plants at the seedling stage, screening multiple characters that would normally be epistatic with one another, drastically minimizing linkage drag, and rapidly recovering a recurrent parent's genotype in genomic regions distant from genes that are the target of introgression were some of the potential advantages of MAS (Tanksley *et al.*, 1989).

In order to tag any gene of interest with selection fidelity of 99%, Tanksley (1983) observed that it would be necessary to have marker loci spaced at 20-cM intervals throughout the genome. Selection can be exerted for a number of markers simultaneously, which will have the effect of selecting for QTLs with positive effects on the quantitative trait of interest (Paterson *et al.*, 1988). However, one of the major drawbacks is that when the linked marker used for selection is some distance away from the gene of interest, this permits crossovers to occur between the marker and the target gene. This produces a small percentage of false positives/negatives in the screening process (Mohan *et al.*, 1997). Therefore, in the final analysis, the success of MAS will depend on identifying highly polymorphic marker(s) as close to the target gene as possible to ensure its/their utility across many breeding populations (Mohan *et al.*, 1997).

For efficient MAS some additional QTL mapping steps have been suggested by Young (1999):

1) repetition of phenotyping over several years and locations,
2) repetition of combined genotyping and phenotyping in a larger sibling population,
3) repetition in genetically unrelated populations, and
4) detailed analysis in marker-generated near-isogenic lines (NILs) that isolate the effects of individual QTLs.

Marker-aided selection has been well demonstrated in traits that are largely controlled by major genes, such as blast resistance (Hittalmani *et al.*, 1995), gall midge resistance (Nair *et al.* 1995a) and semi dwarfism (Cho *et al.*, 1994) in rice. However, the utility of MAS in manipulating quantitative traits was presented by Dudley (1993) in his paper on the potential of molecular markers in manipulation of genes affecting

quantitative traits. Stuber *et al.*, (1987) reported the exploitation of MAS in quantitative traits manipulations for maize improvement, demonstrating the effectiveness of marker-based techniques for identifying and locating QTLs and for detailed genetic investigation of quantitative trait variation. He reported more precise mapping of QTLs in several plant populations and multiple trait associations within specific genomic regions. Stuber (1994) demonstrated the transfer of QTLs using MAS for improving the yield level of maize inbred lines.

Breeding of insect and disease resistance and tolerance to abiotic stresses has become a worldwide issue for crop improvement. To identity the insect/disease reaction of breeding materials, plants must be inoculated artificially or naturally or in specific environments where the biotic stress exists. Artificial inoculation may be impractical when the insect pest or disease is under quarantine control. However, evaluation of plant response to different insects or diseases or different biotypes/strains/races of the same stress agents is often very difficult. Using molecular markers associated with each of the stress responses will help select for resistance to multiple insect pests, plant diseases, or variants of these without inoculation or creating the specific screening environment required for conventional phenotypic screening. Similarly, plant response to multiple biotic stresses can be predicted simultaneously using molecular markers associated with tolerance or sensitivity to these stresses. There are several successful examples of u 'ng MAS to select for resistance to biotic stresses in rice. For example, Hittalmani *et al.* (2000) used marker-assisted selection to combine three rice blast resistance genes (*Pi1*, *Pi2*-5, *Pi*-TA) into a single genotype. For *Pi2*-5 a single marker was used where as flanking markers were used for the other two targeted host plant resistance genes. MAS was effective in developing a resistance gene pyramid in line containing all three resistance genes. The product breeding line with this resistance gene pyramid had a broader resistance spectrum than lines with only one of the three resistance genes. Huang *et al.* (1997) pyramided four bacterial blight resistance genes (*Xa4*, *Xa5*, *Xa13* and *Xa21*) using PCR-based markers. Sanchez *et al.* (2000) transferred three bacterial blight resistance genes into a susceptible rice line possessing desirable agronomic characters. Ribaut *et al.* (1999) identified five QTLs for drought tolerance that were stable over across several drought stress environments, and successfully transferred these to an elite but drought-sensitive line in maize. Shen *et al.* (2000) at IRRI reported that after QTLs affecting root parameters were identified using a rice double haploid population

derived from cross IR 64 × Azucena, a marker-assisted backcrossing program was started to transfer the alleles of Azucena (a drought tolerant upland rice variety) at four QTLs for deep roots (mapping on rice chromosomes 1, 2, 7, 9) from selected DH lines into IR 64. The resulting breeding products showed significant improvement of root mass and root length. Marker-assisted selection for QTLs controlling the stay-green trait (a component of terminal drought tolerance) in sorghum is in progress at ICRISAT-Patancheru (Hash *et al.*, 2003). Six QTLs of relatively large effect from donor parent B35, which have been independently mapped by two or more groups of earlier workers, are targeted in this program, with agronomically elite and genetically diverse sorghum varieties R16, ICSV 111, IRAT 204 and ISIAP Dorado as recurrent parents.

Molecular marker based QTL analysis in tomato demonstrated that QTLs isolated from wild germplasm can improve phenotype of c mmercial varieties for many economic characters (de Vicente and Tanksley, 1993; Eshed and Zamir, 1994; Zamir and Eshed, 1998) as a result of which specific QTLs for increased yield and soluble solids were transferred to cultivated tomato varieties. Xiao *et al.* (1996b) demonstrated that wild rice species *O. rufipogon* carries favorable alleles at two QTLs, which increase grain numbers per plant and thus have potential to substantially increase yield of rice. A slow growing wild relative of the cultivated tomato, *Lycopersicon pennellii,* has been observed to have genes for increased rate of dry matter accumulation and 'soluble solids' concentration.

2.4.1 Efficiency of marker-assisted selection

The analytical approached of Lande and Thompson (1990) focused on first generation selection. Succeeding studies have focused on the efficiency of MAS over several successive generations using computer simulations (Zhang and Smith, 1992, 1993; Gimelfrab and Lande, 1994a,b, 1995; Wittaker et al., 1995). Results from these studies showed that MAS could be more efficient than purely phenotypic selection in quite large populations and for traits with relatively low heritabilities. The simulations also showed that additional genetic gain provided by MAS, when compared with purely phenotypic selection, rapidly decreased when several successive cycles of selection had occurred, and that MAS may become less efficient than phenotypic selection in the long term. This situation becomes more acute when the effects associated with markers are not reevaluated at each generation. MAS was as effective as phenotypic selection for developing populations with diverging grain yield (Stuber

and Edwards,1986). MAS with an index of 34 sweet corn traits was as effective as phenotypic selection (Edwards and Johnson, 1994). In common bean, Schneider et al. (1997) used five RAPD markers for MAS of yield in a drought-stressed environment. MAS improved yield performance by 11 percent and 8 percent under stress and non-stress conditions, respectively, but the conventional selection for yield failed to improve performance under stress.

The efficiency of the backcross method in transferring QTLs will be governed by the magnitude of linkage drag and correct identification of QTL-marker associations during the process of QTL mapping. The current QTL mapping technology maps QTLs within genomic segments of 15-20 cM, which increases the probability of linkage drag. Further, large numbers of false positive associations (Type I error) are more important than failure to identify the real associations (Type II error), because the marker-based selection in the former case becomes an exercise in futility (Dudley, 1993). Another error (Type III) emerging from detection of significant association of a QTL with a wrong marker is even more serious. QTLs mapped with stringent levels of significance and high threshold values that are based on the size of the genome, using fully-saturated genetic maps will be more expensive to generate, but are expected to improve the efficiency of marker-assisted QTL transfer through backcrossing.

Hospital *et al.* (1997) used computer simulations to study the efficiency of MAS based on an index combining the phenotypic value and molecular score of individuals. They observed that in the first generation the relative efficiency (RE) of expected efficiency of MAS over the expected efficiency of purely phenotypic selection generally increases with 1) larger population size, 2) lower heritability values of the target trait, and 3) high type-I error risk. Their studies showed that higher efficiency of MAS for fixation of favorable alleles at QTLs with large effects in early generations is balanced over successive generations by a higher rate of fixation of unfavorable alleles at QTLs with small effects in later generations. This explains why MAS may become less efficient than phenotypic selection in the long-term. MAS efficiency therefore depends, at least in part, on the genetic determination of that trait.

The efficiency of MAS generally reduced with increasing distance between markers. So, the optimal distance recommended between two adjacent markers flanking a particular target QTL is about 5-10 cM (Hospital *et al.*, 1997). However,

the efficiency of marker-assisted selection is less efficient than the phenotypic selection in the long-term (Hospital *et al.*, 1997) if there is linkage between favorable alleles of large effect and unfavorable alleles of small effect in the genomic region(s) subject to marker-based foreground selection.

Knapp (1998) presented estimates of the probability of selecting one or more superior genotypes by MAS to estimate its cost efficiency relative to phenotypic selection. The frequency of superior genotypes among the selected progeny increases as the selection intensity increases. Van Berloo and Stam (1998) assessed the effectiveness of MAS compared to phenotypic selection, showing that MAS appears particularly promising when dominant marker alleles are present at the QTL and linked in coupling phase. Uncertainty in estimated QTL map positions reduces the benefits of MAS.

Young (1999) pointed out that despite innovations like better marker systems and improved genetic mapping strategies, most marker associations are not sufficiently robust for successful MAS. Charmet *et al.* (1999) studied the accuracy of QTL location determination, showing that it greatly affects selection efficiency. MAS for QTLs have recently started to be applied to the genetic improvement of quantitative characters in several crops such as tomato (Lawson *et al.*, 1997; Bernacchi *et al.*, 1998), maize (Graham *et al.*, 1997), and barley (Han *et al.*, 1997; Toojinda *et al.*, 1998).

Hospital and Charcosset (1997) provided a general framework for the optimization of the use of molecular markers in backcross breeding programs aimed at introducing one to several superior QTL into a recipient line. Using at least three markers per QTL allows a good control of the donor chromosome segment over several generations. When several target alleles are monitored simultaneously, background selection among the limited number of individuals resulting from the foreground selection step accelerates the increase in genomic similarity with the recurrent parent with only limited increase in the cost. Frisch *et al.* (1999b) determined the number of marker data points (MDP) required in background selection, the size of the population to be used and compared a two-stage selection procedure (one background and one foreground selection step), with alternative selection procedures (one foreground and two or three background selection steps). They concluded that as the number of selection processes increases, the number of MDP required decreases.

Moreau *et al.* (2000) evaluated the relative efficiency of MAS in the first cycle of selection through an analytical approach taking into account the effect of experimental design (population size, number of trials and replication/trial) on QTL detection. They concluded that expected economic returns of MAS compared to the phenotypic selection decrease with increases in the cost of genotyping..Bunyamin *et al.* (2003) reported MAS for complex traits in common bean using an index based on QTL-linked markers and ultramtric genetic distance(from a cluster analysis) between lines and a target parent. A comparison of the mean seed yield of the top five lines selected by different schemes demonstrated that the highest yielding group was selected on the basis of a combination of phenotypic performance and high QTL-based index, followed by groups identified by high QTL-based index, conventional selection, and low QTL-based index, respectively. The study showed that use of a QTL-based index in conjugation with the ultra metric genetic distance to the targeted parent would enable a plant breeder to select lines that retain important QTLs in a desirable genetic background. Therefore this type of MAS would be expected to be superior to phenotypic selection.

2.4.2 General consideration for all trait categories in marker assisted selection

2.4.2.1 Gene introgression

Gene introgression involves the introduction of a target gene into a productive recipient line or cultivar. Gene introgression can be used in both backcrossing and intercrossing programs. By using DNA markers to identify recombinants, introgressed chromosome segments might be "trimmed" to minimal size, reducing the extent to which the recurrent genotype is disrupted by undesirable alleles closely linked to genes controlling the target trait (Tanksley and Rick, 1980). It is often critical in plant breeding that allelic substitution be precise so that only the target gene and the shortest possible segment of the linked chromosome are transferred from the donor parent to the recipient parent, the latter of which is usually a cultivar or inbred line with very good combining ability. To reduce false positives in MAS, markers used for foreground selection must be tightly linked to the gene/QTL controlling the target trait, and flanking markers or multiple markers around the region can be used simultaneously. A three-marker system, with three markers located on a short chromosomal block of a few (<5) cM, will be desirable in such cases (Zhang and Huang, 1998). The marker in the middle, preferably intragenic or co-segregating with the target gene, will be used in foreground selection to indicate the presence of the

target gene in the selection process. The marker on each side will be used to indicate the absence of the chromosome segment from the donor parent (negative selection), that is, selection for recombination between the target gene locus and the marker locus. For genes that have been cloned, the marker in the middle can be developed from the cloned gene or gene sequence. This system will be very useful when the target gene is only available in wild species and linkage drag is proven to be associated with the chromosome segment to be introgressed.

The first such study employed a cross between the wild rice relative *Oryza rufipogon* and the Chinese *indica* hybrid 'V20'/'Ce64' (Xiao *et al.*, 1998). Although the *O. rufipogon* accession was phenotypically inferior for all 12 traits studied, transgressive segregation was observed for all traits, and 51% of the QTL detected had beneficial alleles from *O. rufipogon*. By MAS and field selection, an excellent CMS restorer line ('Q661') carrying one of the major QTLs for yield components was developed. Its hybrid, 'J23A'/'Q661', out-yielded the check hybrid by 35% in a replicated trial for the second rice crop in 2001 (Yuan, 2002).

2.4.2.2 Whole genome selection

MAS can also be practiced at the whole genome level. DNA marker-based whole genome selection or "background selection" can be used to accelerate recovery of recurrent parent genotype in the backcrossing process for breeding improved parental lines. Compared to a conventional backcross program that usually takes five to seven generations to recover most of the recurrent parental background, MAS may save two to four backcross generations in the transfer of a single target allele (Tanksley *et al.*, 1989; Hospital *et al.*, 1992; Fisch *et al.*, 1999). Combined with selection for target traits, whole genome selection allows the breeder to simultaneously transfer targeted traits through backcrossing. (Combined foreground and background selection allows the breeder to save a few generations for transferring a single QTL, but simultaneous transfer of multiple traits remains a very difficult and expensive exercise) using MAS. It is probably more cost-effective to transfer multiple QTLs in parallel single-QTL backcrossing programs and then use a complex series of crosses of the single-QTL introgression lines to pyramid the QTLs).

As genetic mapping information accumulates from different mapping populations, it will be possible to establish a complete profile for all the genes associated with a specific trait or trait category. Whole genome selection can be used to select the best trait/gene combinations based on selection for each of the target loci

for which position in the genome is known. It is possible to select the best cassette of marker alleles for any trait and/or trait combination.

To transfer the bacterial blight resistance gene *Xa*21, 128 RFLP markers, evenly distributed across the 12 rice chromosomes, were used to recover the genetic background of 'Minghui 63', a widely used parent (Chens *et al.*, 2000). MAS was also be used by the same group to improve '6078', an elite restorer line with high yield potential by transferring *Xa*21 from IRBB21 (Chens *et al.*, 2001).

2.4.2.3 Selection for multiple genes/traits

MAS provides opportunities for simultaneous selection of multiple traits/genes. In some cases, multiple pathogen races or insect biotypes must be used to identify plants for multiple resistances, but in practice this may be difficult or impossible because different genes may produce similar phenotypes that cannot be distinguished from each other. Marker-trait associations can be used to simultaneously select multiple resistances for different disease races and/or insect biotypes, and pyramid them into a single line through MAS. To find a CMS restorer in rice through testcrossing and progeny testing, a candidate male parent has to be testcrossed with a CMS line and male fertility of the resulting hybrid progeny assessed to find out if the candidate male parent has fertility restoration ability. However, sterility in the testcross hybrid could result from the absence of either fertility restoration genes or wide compatibility genes or both when an intersubspecific cross is involved. MAS can be used to distinguish between these two different causes of sterility. Hybrid rice provides an advantage over inbred cultivars because dominant genes and/or QTLs with favorable effects from both parents that can be integrated into one hybrid. An integrated breeding program including MAS was initiated in China to improve elite hybrid rice.

2.4.2.4 Integrated genetic mapping and MAS

In many cases, genetic mapping results obtained from specific crosses cannot be used for MAS for the same traits in different crosses. There are three reasons for this phenomenon. First, quantitative traits are usually controlled by many genes. Genes are only segregating at the loci where two parents are genetically different and thus can be mapped using the population from these two parents. For a randomly selected mapping population, the parents will have a strong chance to share identical alleles at some of the genetic loci. There is a high probability that segregating genes already mapped in one mapping population are not segregating in a second mapping population. Second, mapping population parents could have alleles that are different

from those of elite breeding populations. Interactions among these multiple alleles will modify marker-trait associations when different allele combinations are considered. Third, G×E interaction could make the establishment of marker-trait association depend on specific environments. One of the best ways to avoid these limitations is to integrate the genetic mapping for a trait with improvement of that trait in an elite background, *i.e.*, identify the marker-trait associations from a breeding population so that they can easily be used for MAS of the same population. This is critical for quantitative traits, which are genetically controlled by many genes and interact with environments. Advanced backcross QTL analysis proposed by Tanksley and Nelson (1996) to accelerate the process of molecular breeding is one of the approaches that can be used for this purpose.

2.4.2.5 Cost benefit analysis of MAS

The cost of using the 'tools' of MAS in applied plant breeding programs is a major consideration. The cost of using MAS compared to conventional plant breeding varies considerably between studies. Dreher *et al.* (2003) indicates that the cost effectiveness of MAS needs to be considered on a case-by-case basis. Factors that influence the cost of utilizing markers include: inheritance of the trait, method of phenotypic evaluation, field/glasshouse and labour costs, and the cost of resources.

In some cases, phenotypic screening is cheaper compared to marker-assisted selection (Bohn *et al.*, 2001; Dreher *et al.*, 2003). However, in other cases, phenotypic screening may require time-consuming and expensive assays, and the use of markers will then be preferable-especially in private-sector breeding programmes where reducing the time required to achieve a unit gain in varietal performance can help make a company's products more competitive in the market. Some studies involving markers for disease resistance have shown that once markers have been developed for MAS, it is cheaper than conventional methods (Yu *et al.*, 2000). In other situations, phenotypic evaluation may be time-consuming and/or difficult and therefore using markers may be cheaper and preferable (Dreher *et al.*, 2003; Young, 1999; Yu *et al.*, 2000). An important consideration for MAS, often not reported, is that while markers may be cheaper to use, there is a large initial cost in their development. An estimate for the cost to develop a single marker was AUD $100,040 (Langeridge *et al.*, 2001).

MATERIALS AND METHODS

CHAPTER III
MATERIALS AND METHODS

3.1 Application of SSR markers in diversity analysis of sorghum insect resistant germplasm accessions

3.1.1 Plant material:

Ninety-one sorghum genotypes were selected for the present study. These include elite open-pollinated varieties, hybrid parental lines, recurrent parents used in marker-assisted backcrossing programs at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Patancheru for the stay-green trait (a component of post-flowering drought tolerance), and germplasm accessions exhibiting resistance to sorghum shoot fly, spotted stem borer, and sorghum midge. They are currently used in breeding programs at the ICRISAT, Patancheru and/or at the National Research Center for Sorghum (NRCS), Rajendranagar, Hyderabad and breeding programs of state agricultural universities in India. Seeds of these accessions have been maintained by the ICRISAT germplasm unit (Appendix I).

3.1.2 Ninety six-well plate mini-prep genomic DNA extraction:

Details on the preparation of solutions and buffers used in DNA extraction are presented in Appendix II.

Lysis buffer (3% CTAB) was preheated to 65°C in a water bath before the start of tissue sample collection.

Steel balls (2 per tube), pre-chilled in the freezer at –20°C for about 30 minutes, were added to plastic extraction tubes.

Leaf strips 6-cm long were collected (final weight 30 mg) from one-week-old seedlings of each germplasm accession, cut in pieces (1 mm length) and transferred to tubes.

A. Grinding and extraction:

1. 450 µl of preheated (65°C) CTAB buffer was added to each tube containing leaf tissue samples.
2. Tissue sample grinding was conducted using the Sigma Geno-grinder at 500 strokes per minute for 2 minutes. Grinding was repeated until the color of the sample solution became pale green.

3. After grinding, the tube box was fixed in a locking device and incubated at 65°C in a water bath for 10 minutes with manual shaking at regular intervals.

B. Solvent extraction:

1. 450 µl of mixed chloroform:iso-amyl alcohol (C:IAA=24:1) was added to each tube and centrifuged at 6200 rpm for 10 minutes.
2. After centrifugation, the aqueous layer (approximately 300 µl) was transferred to a fresh tube.

C. Initial DNA precipitation:

To each tube 0.7 volume (approximately 210 µl) of cold isopropanol was added and the samples were kept at –20°C for 10 minutes.

The box of 96 tubes was then centrifuged at 6200 rpm for 15 minutes using the box centrifuge.

Supernatant was decanted from each tube and crude DNA pellets were allowed to air dry (minimum 20 minutes).

D. RNAse treatment:

200 µl of low salt $T_1E_{0.5}$ buffer (Tris-HCL 1: EDTA 0.5) and 3 µl of RNAse (stock 10 mg/µl) were added, mixed properly and incubated at 37°C for 30 minutes (can be kept overnight at room temperature).

E. Solvent Extraction:

200 µl of phenol:chloroform:iso-amyl alcohol (P:C:IAA=25:24:1) was added, mixed well, and centrifuged at 5000 rpm for 5 minutes.

After transferring the aqueous layer to a fresh tube, this step was repeated with the chloroform:iso-amyl alcohol mixture (C:IAA=24:1).

F. DNA precipitation:

To the tubes containing aqueous layer (1/10th of its total volume approximately) sodium acetate (from 3M stock) and 2 volumes (300 µl) of 100% ethanol were added, mixed, and the tubes subsequently kept at –20°C for 5 minutes.

Following this brief incubation the box of tubes was centrifuged at 6200 rpm for 10 minutes.

G. Ethanol Wash:

After centrifugation the supernatant was carefully decanted. In order to remove excess salts, 200 µl of 70% ethanol was added to the pellet followed by centrifugation at 6200 rpm for 5 minutes.

H. Final re-suspension:

Supernatant was decanted and the pellets were allowed to air dry for one hour.

Dried pellets were re-suspended in 100 to 150 µl of TE buffer and kept at room temperature to dissolve completely (approximately one hour).

Dissolved DNA samples were kept in a refrigerator at 4°C.

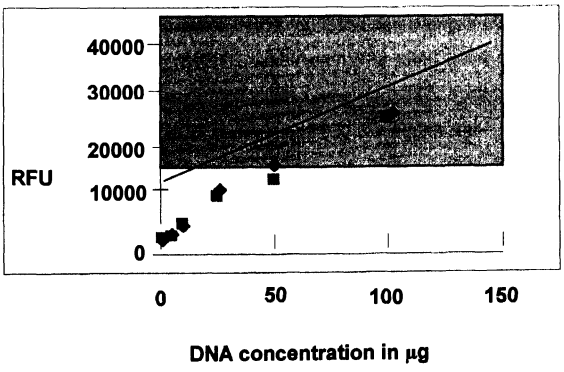
3.1.3 Checking DNA quality and DNA concentration

The DNA quality was checked using 1.2% ready-made agarose gels (Amersham Biosciences). For this, 1 µl of DNA solution was mixed with 1 µl of orange dye and 8 µl of distilled water and the mixture loaded into a well on the 1.2% ready to run agarose gel. The gel was run for 10 minutes, after which the quality was checked under UV. A smear of DNA indicated poor quality whereas a clear band indicated good quality. Samples of poor quality were re-extracted.

The DNA concentration was assessed using a Spectrafluor Plus spectrophotometer after staining the DNA with Picogreen™ (1/200 dilution). Based on the Relative Fluorescence Units (RFU) values and using the standard curve, DNA concentrations were calculated. The DNA was diluted to a final concentration of 2.5ng/µl. Figure presents a calibration curve where

DNA concentration = -2.78273+0.002019*RFU.

Standard curve showing the linear relationship between RFU and DNA concentration.



3.1.4 Primer selection:

For assessment of genetic diversity of the 91 sorghum genotypes includes in this study. 21 SSR primer pairs were used including pairs from the *Xcup* series (15 primer pairs), the *Xtxp* series (4 primer pairs), and for both *Kaf* and *Xgap*84. These primer pairs detect 21 SSR marker loci that had carefully selected based on the following criteria:

- Markers should be mapped at different loci on different sorghum linkage groups.
- The markers should display a range of allele sizes in prior publications.

Primer sequences for the markers used in this study have been described in the following publications: the *Xtxp* markers by Bhattramakki *et al.* (2000) and Kong *et al.* (2000), the *Xgap* 84 markers by Brown *et al.* (1996), the *kaf* marker by Taramino *et al.* (1997), and the *Xcup* markers by Schloss *et al.* (2002). Seven groups of three primer pairs were formed. Each group contained three pairs of primers with the forward primer of the first pair labeled with 4,7,2',4',5',7'-hexachloro-6-carboxyfluorescein (HEX), the forward primer of the second pair labeled with 6-carboxyfluorescein (6-FAM), and one primer of the third pair labeled with 7',8"-benzo, 5'-fluoro-2',4,7-trichloro-3-carboxyflourescein (NED) (Table 3.1).

3.1.5 PCR Amplification:

Polymeric chain reaction (PCR) amplification of each SSR loci was performed in a total reaction mixture volume of 5 µl containing sorghum genomic DNA, PCR buffer (Applied Biosystems), dNTPs, $MgCl_2$ (Applied Biosystems), forward primer (Applied Biosystems) labeled with HEX, NED, or FAM dye phosporamidites (Applied Bio-systems), reverse primer (MWG), and Ampli *Taq* Gold DNA polymerase (Applied Bio-systems) in an Applied Bio-systems Gene Amp PCR system 9700 thermo-cycler using a "Touch Down" PCR technique. PCR conditions were previously optimized for each primer pair using a grid of nine reactions. Three different sets of PCR conditions were used for PCR amplification (Table 3.2).

The initial DNA denaturation at 94°C for 15 min, to activate the *Taq* polymerase, was followed by 10 cycles with the following profile: denaturation for 15 sec at 94°C, annealing for 20 sec at 61°C (the annealing temperature was decreased by 1°C for each cycle) and extension for 30 sec at 72°C. This was followed by 31 cycles with the following profile: denaturation at 94°C for 10 sec, annealing at 54°C

Table 3.1: Fluorescent-labeled sorghum SSR primers (Applied Biosystems) used in sorghum diversity study.

SSR locus	Label	LG[1]	Repeat motif	Primer Sequences	References
*Xcup*32	NED	A	$(AAAAT)_4$	**F:** ACTACCACCAGGCACCACTC **R:** GTACTTTTTCCCTGCCCTCC	Schloss *et al.* (2002)
*Xcup*61	HEX	A	$(CAG)_7$	**F:** TTAGCATGTCCACCACAACC **R:** AAAGCAACTCGTCTGATCCC	Schloss *et al.* (2002)
*Xcup*14	HEX	A	$(AG)_{10}$	**F:** TACATCACAGCAGGGACAGG **R:** CTGGAAAGCCGAGCAGTATG	Schloss *et al.* (2002)
*Xcup*11	NED	A	$(GCTA)_4$	**F:** TACCGCCATGTCATCATCAG **R:** CGTATCGCAAGCTGTGTTTG	Schloss *et al.* (2002)
*Xtxp*320 (phyB)	NED	A	$(AAG)_{20}$	**F:** TAAACTAGACCATATACTGCCATGATAA **R:** GTGCAATAAGGGCTAGAGTGTT	Bhattramakki *et al.* (2000)
*Xcup*63	NED	B	$(GGATGC)_4$	**F:** GTAAAGGGCAAGGCAACAAG **R:** GCCCTACAAAATCTGCAAGC	Schloss *et al.* (2002)
*Xcup*69	FAM	B	$(ATGCG)_4$	**F:** ACAGCACCAAGGTGAAGGAC **R:** ATGTAGGGCACCAGCTTCAC	Schloss *et al.* (2002)
*Xgap*84	HEX	B	$(AG)_{14}$	**F:** CGCTCTCGGGAGAATGA **R:** TAACGGACCACTAACAAATGATT	Brown *et al.* (1996)
*Xcup*62	HEX	C	$(GAA)_6$	**F:** CGAGAAGATCGAGAGAACCC **R:** TGAAGACGACGACGACAGAC	Schloss *et al.* (2002)
*Xcup*60	NED	C	$(CGGT)_4$	**F:** GTATGCATGGATGCCTGATG **R:** GCGAGGGTATGTAGCTCGAC	Schloss *et al.* (2002)
*Xcup*06	HEX	C	$(CTGC)_4$	**F:** GGCAGTAGCAGGCGTTTAAG **R:** AACTGAATCAGGTCATGGGC	Schloss *et al.* (2002)
*Xtxp*114	FAM	C	$(AGG)_8$	**F:** CGTCTTCTACCGCGTCCT **R:** CATAATCCCACTCAACAATCC	Bhattramakki *et al.* (2000)
*Xcup*53	HEX	C	$(TTTA)_5$	**F:** GCAGGAGTATAGGCAGAGGC **R:** CGACATGACAAGCTCAAACG	Schloss *et al.* (2002)
*Xcup*37	HEX	D	$(AG)_9$	**F:** CCCAGCCTTCCTCCTGATAC **R:** GTACCGACTCCAATCCAACG	Schloss *et al.* (2002)

Table 3.1 cont...

SSR locus	Label	LG[1]	Repeats	Primer Sequence	References
Xtxp40	FAM	E	$(GGA)_7$	**F:** CAGCAACTTGCACTTGTC **R:** GGGAGCAATTTGGCACTAG	Kong *et al.* (2000)
Xcup28	NED	F	$(TGAG)_5$	**F:** GGTGTGAGACTGTGAGCAGC **R:** TATAGCACGGTTGTTGTGCC	Schloss *et al.* (2002)
Xcup02	NED	G	$(GCA)_6$	**F:** GACGCAGCTTTGCTCCTATC **R:** GTCCAACCAACCCACGTATC	Schloss *et al.* (2002)
Xcup07	FAM	I	$(CAA)_8$	**F:** CTAGAGGATTGCTGGAAGCG **R:** CTGCTCTGCTTGTCGTTGAG	Schloss *et al.* (2002)
Xcup52	FAM	J	$(AATT)_5$	**F:** CTCCTCGCCGTCATCATC **R:** TAAAGAGAAACGCAGGCAGG	Schloss *et al.* (2002)
XSbKafGK1	FAM	J	$(AAC)_9$	**F:** AGCATCTTACAACAACCAAT **R:** CTAGTGCACTGAGTGATGC	Tamarino *et al.* (1997)
Xtxp15	FAM	J	$(TC)_{16}$	**F:** CACAAACACTAGTGCCTTATC **R:** CATAGACACCTAGGCCATC	Kong *et al.* (2000)

[1]Sorghum linkage group designations following the system of Peng *et al.* (1999), Subudhi and Nguyen (2000), and Menz *et al.* (2002), which have the following relationships with sorghum chromosome designations assigned by Kim *et al.* (2005):
A= SBI-01, B= SBI-02, C= SBI-03, D= SBI-04, E= SBI-07, F= SBI-09, G= SBI-10, H= SBI-08, I= SBI-06, J= SBI-05

Table 3.2: PCR protocols used for amplification with labeled SSR primers in sorghum diversity study.

Protocol No: 7

SSR locus	Primer (1 pM)/µl	$MgCl_2$ (1 mM)/µl	dNTP (0. 375 mM)/µl	DNA (1.25 ng)/µl	Enzyme (0.2 U)/µl	Buffer (1X)/µl	Water (µl)
Xcup07	97	97	36.375	0.5	19.4	48.5	138.225
Xcup14	97	97	36.375	0.5	19.4	48.5	138.225
Xtxp114	97	97	36.375	0.5	19.4	48.5	138.225
Xgap84	97	97	36.375	0.5	19.4	48.5	138.225

Protocol No: 4

SSR locus	Primer (0.5 pM)/µl	$MgCl_2$ (0.75 mM)/µl	dNTP(0.5 mM)/µl	DNA (1.25 ng)/µl	Enzyme (0.25 U)/ µl	Buffer (1X)/µl	Water (µl)
Xcup28	48.5	72.75	48.5	0.5	24.25	48.5	194

Protocol No: 5

SSR locus	Primer (0.5 pM)/µl	$MgCl_2$ (1 mM)/µl	dNTP (0.25 mM)/µl	DNA (2.5 ng)/µl	Enzyme (0.2 U)/µl	Buffer (1X)/µl	Water (µl)
Xcup62	48.5	97	24.25	1	19.4	48.5	150.35
Xcup32	48.5	97	24.25	1	19.4	48.5	150.35
Xtxp40	48.5	97	24.25	1	19.4	48.5	150.35
Xcup61	48.5	97	24.25	1	19.4	48.5	150.35
Xcup02	48.5	97	24.25	1	19.4	48.5	150.35
Xcup52	48.5	97	24.25	1	19.4	48.5	150.35
XSbKafGK1	48.5	97	24.25	1	19.4	48.5	150.35
Xcup60	48.5	97	24.25	1	19.4	48.5	150.35
Xcup06	48.5	97	24.25	1	19.4	48.5	150.35
Xcup53	48.5	97	24.25	1	19.4	48.5	150.35
Xcup63	48.5	97	24.25	1	19.4	48.5	150.35
Xcup69	48.5	97	24.25	1	19.4	48.5	150.35
Xcup37	48.5	97	24.25	1	19.4	48.5	150.35
Xcup11	48.5	97	24.25	1	19.4	48.5	150.35
Xtxp320 (phyB)	48.5	97	24.25	1	19.4	48.5	150.35
Xtxp15	48.5	97	24.25	1	19.4	48.5	150.35

for 20 sec, extension at 72°C for 30 sec. After these 31 reaction cycles the extension at 72°C was prolonged for 20 min. Subsequently, the PCR product samples were stored at 4°C. Then 1 µl (for FAM- and HEX-labeled PCR products) to 1.5 µl (NED-labeled PCR products) was transferred to a 96-well ABI plate containing 7 µl formamide, 0.3 µl ROX size standards, and 4.2 µl Double Distilled Water. The remainder was PCR-amplified for an additional 6 cycles with the following profile (denaturation at 94°C for 10 sec, annealing at 54°C for 20 sec, extension at 72°C for 30 sec). Samples were then stored in –20°C until further use.

3.1.6 Electrophoresis

a) Non-denaturing polyacrylamide gels

One µl of loading buffer was added to 3.0 to 3.5 µl of each PCR sample. Two µl of this buffered PCR product was then loaded on each lane of a 96-track 6% non-denaturing polyacrylamide gel containing 29:1 acrylamide/bisacrylamide, 10X TBE, and water. In addition, four wells were loaded with a 100 bp size standard to ensure proper sizing of the amplified fragments. The gel was run at 600 V of constant power in 0.5X TBE for 3 h, using a BioRAD gel sequencing apparatus.

3.1.7 Silver staining

After PCR product separation by PAGE, the gel was placed in water for 5 min, soaked in 0.1% CTAB for 20 minutes with gentle shaking, incubated in 0.3% ammonia for 15 min, and placed in silver nitrate solution (0.1% silver nitrate, 1M NaOH and 25% ammonia) for 15 min with gentle shaking. After incubation in this silver nitrate solution, the gel was placed in developer (30 g sodium carbonate and 0.4 ml formaldehyde in 2 liters of water) with gentle shaking until bands became visible, rinsed in water for 1 min to stop the staining reaction, and placed in fixer (30 ml glycerol in 2 liters of water) for a few seconds.

After silver staining the PAGE gels, the size(s) (base pairs) of the most intensely amplified specific bands or alleles for each SSR marker were estimated based on migration relative to the 100 base pair (bp) DNA ladder (consisting of fragments ranging from 100 to 1000 bp). The presence (1) or absence (0) of each PCR fragment was scored for each of the 91 genotypes.

3.1.8 ABI Prism 3100 genetic analyzer

PCR products of each group of 3 primer pairs were pooled post-PCR. Because of the different signal intensities of the fluorophores, 1 µl (in case of FAM- and HEX-

labeled PCR products) to 1.5 µl (in case of a NED-labeled PCR products) was added to a mix of 7 µl formamide, 0.3 µl ROX size standards, and 4.2 µl Double Distilled Sterilized water (total volume 15 µl). The samples were denatured for 5 min at 94°C and cooled on ice. The plate with the samples was then centrifuged 1 min at 760 rpm (Eppendorf) and stored at –20°C until separation on the ABI 3100 or ABI 3700 capillary electrophoresis DNA sequencing machines.

96-well plates (96 genotypes × three primers) were placed in the ABI 3100 or ABI 3700 machine. The samples were separated using the following protocols:

- ABI 3100: dye-set "D", run module "SSR 20 minutes", and analysis module "GSHD Analysis". The fragments were separated in a 36-cm capillary array, using POP_4 as a carrier.
- ABI 3700: dye-set "D", run module "GeneScan2-POP6DefaultModule", and analysis module "GSHD Analysis". The fragments were separated in a 50-cm capillary array, using POP_6 as a carrier.

After completion of the run, the peak patterns were sized using Gene Scan. Presence or absence of allelic fragments were scored using the Genotyper software.

3.1.9 Data Analysis

SSR data was analyzed for both PAGE and ABI PCR product separation methods. Clear and distinct amplification products were scored as '1' for presence and a '0' for absence of bands. The NTSYS (Numerical Taxonomy and Multivariate Analysis System) program was used for cluster analyses. The data was used to generate Jaccard's similarity coefficients based on SSR bands. The Jaccard's coefficients between each pair of accessions were then used to construct a dendrogram using the un-weighted pair group method with arithmetic averages (UPGMA).

3.2 Phenotyping of RILs 296B × IS 18551 for components of resistance to sorghum shoot fly

3.2.1 Material

The experimental material consisted of a set of 259 Recombinant Inbred Lines (RILs) ($F_{7:8}$), derived from a cross between two sorghum-inbred lines, *viz.*, 296B (susceptible to shoot fly) and IS 18551 (resistant to shoot fly). Table 3.3 elaborates salient features of these two parental lines. The RIL population progenies along with both parents were used for phenotyping and genotyping.

Table 3.3 Salient features of parental lines of RIL mapping population

Parents	Salient features
296B	Derived from *Aispuri*. Semi-compact earhead, white grain, foliage tan coloured. Leaves of seedling are non-glossy with no trichomes. Highly susceptible to shoot fly.
IS 18851	Originates from Ethiopia, race *Durra*. Earhead with straw coloured grain and glumes larger than 296B. Leaves of seedling are light green, glossy, narrow and pointed upward with dense trichomes on both sides of the leaf balde. Resistant to shoot fly. Very tall at maturity.

3.2.2 Development of mapping population

The RILs were produced at ICRISAT, Patancheru. After the initial cross between 296B and IS 18551, a single F_1 plant was selfed. The resulting F_2 seeds were sown and F_2 plants were selfed. The F_3 seeds were sown head-to-row, each F_3 plant was selfed and from each head-to-row a single plant was randomly chosen to provide the seeds for the next generation. This, modified single-seed-descent method, where each line is maintained through selfing a single randomly selected plant, was repeated for 3 to 4 generations, up to F_7. During RIL development the plant material, recommended protection measures were taken to protect the plants against shoot fly and other insects. Bulked seed was harvested from randomly selected F_6 plants to produce 259 F_7 recombinant inbred lines (RILs). Each F_7 line represents the individual F_2 plant from which it is derived. The details on pedigrees of 259 $F_{7:8}$ RILs of cross 296B × IS 18551 is given in Appendix III (Fig. 3.1).

3.2.3 Evaluation of RILs for resistance to sorghum shoot fly, *Atherigona soccata*

Screening of the RIL for shoot fly resistance was carried out at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh. A total of 300 lines (259 RILs + 14 repeated checks of each of 296B and IS 18551, and a standard check, CSH 9 repeated 13 times), were sown on 16th August during the 2002 *kharif* season (E_1). For early *rabi* season (E_2), a total of 270 entries

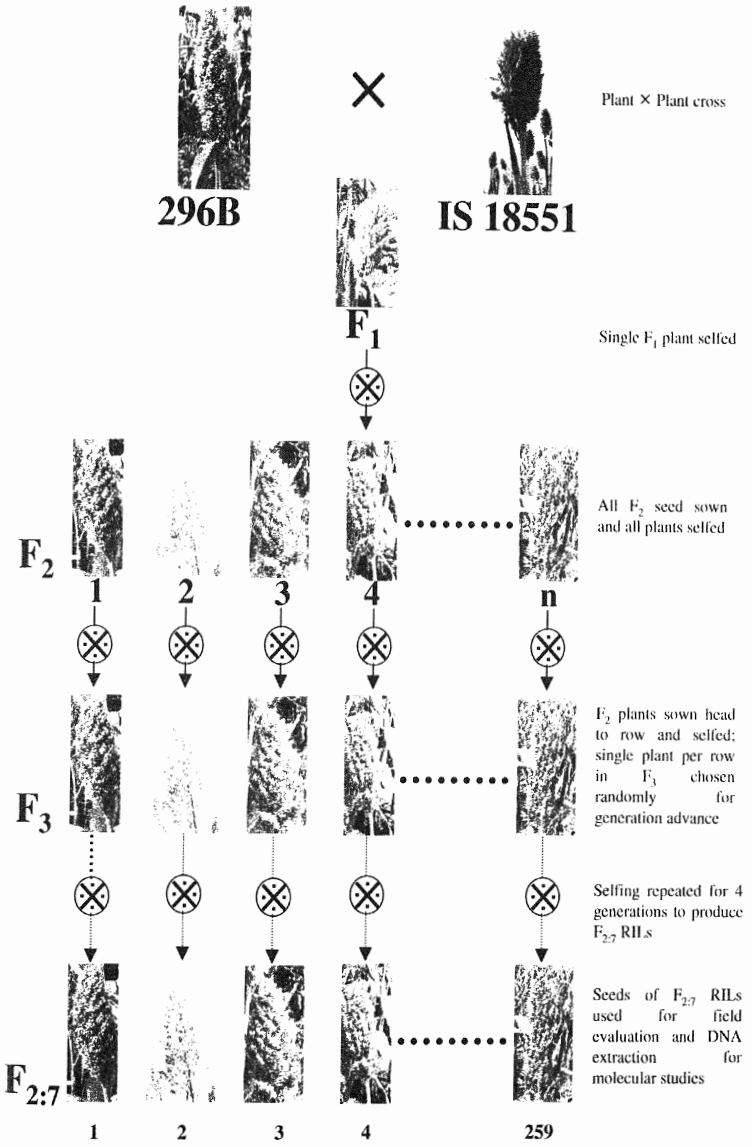


Fig 3.1. Schematic diagram of RIL development procedure

(259 RILs + 4 times repeated checks of each of 296B and IS 18551 + standard check CSH 9 repeated 3 times), were sown on 16th October 2004. The two screening environments are referred as E_1 (late *kharif*) and E_2 (early *rabi*). The test material was planted in balanced α design, with 75 cm and 10 cm inter- and intra-row spacing, respectively. In the late *kharif* and *rabi* seasons, each entry was grown in two-row plots of 2 m length in four and three replications, respectively.

Shoot fly infestation was quite high during the *kharif* season. During the 2004 *rabi* season, the shoot fly infestation was relatively low. To ensure uniform and optimum shoot fly infestation under field conditions, the interlard-fish meal technique (Sharma *et al.*, 1992) was followed to screen for resistance to shoot fly (Plate 3.1).

3.2.3.1 Observations

Observations were recorded on leaf glossiness, trichome density on abaxial and adaxial surface of leaf, percent plants with eggs and deadhearts, time to 50% flowering, plant height, recovery resistance, aphid damage score, and grain yield in each plot during the 2002 *kharif* and 2004 *rabi* seasons. Observations were also recorded on leaf pigmentation, seedling vigor, seedling height, midge damage score, and agronomic performance during the 2004 *rabi* season.

3.2.3.1.1 Glossiness

Leaf glossiness was recorded during early morning hours when reflection of light is maximum (Plate 3.2a). Intensity of leaf glossiness was recorded visually on a scale of 1 to 5 (1 = pale green, shiny, narrow, and leaves pointed upwards, and 5 = broad, dull green, and drooping leaves) at 7 days after emergence (DAE) (Sharma et al., 1997).

3.2.3.1.2 Seedling vigor

Seedling vigor (a combination of height, leaf growth, and robustness) was evaluated on a 1 to 5 scale at 9 DAE (1 = plants with maximum height, leaf expansion and robustness, and 5 = plants with minimum growth, little leaf expansion, and poor adaptation) (Sharma et al., 1997).

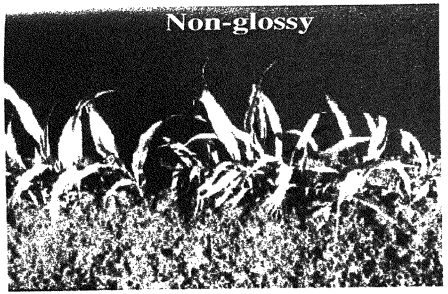
3.2.3.1.3 Seedling height

Seedling height (cm) was measured from the base of the plant to the tip of the top-most completely opened leaf on three randomly selected plants from each plot at 20 DAE.

3.2.3.1.4 Plant stand

The total number of plants in each plot was determined at 20 DAE. This number was used to calculate the percentage of oviposition and deadhearts incidence.

Plate 3.1: Interlard fish-meal technique used to field screen the test material for resistance to sorghum shoot fly, *Atherigona soccata*.



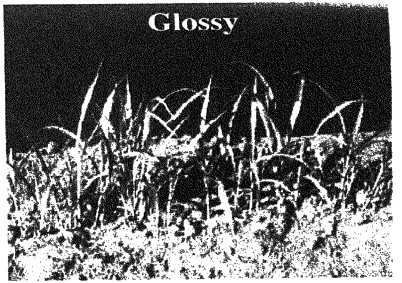
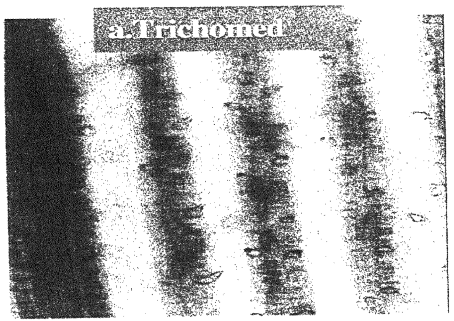


Plate 3.2a: Glossy leaf trait associated with resistance to sorghum shoot fly, *Atherigona soccata*.



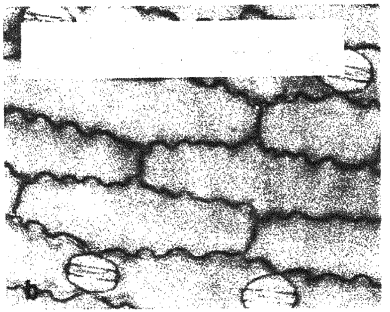


Plate 3.2b: Trichomes on the leaf surface; a = trichomed. b = non-trichomed.

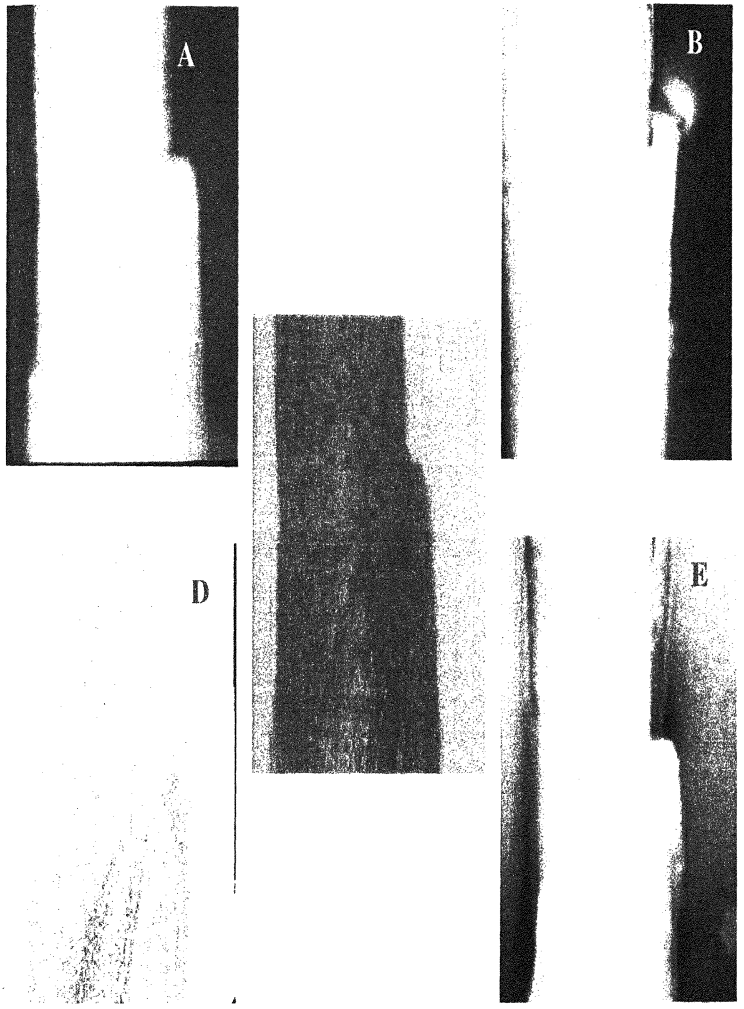


Plate 3.3: Plumule and leaf sheath purple pigmentation scores of sorghum genotypes at 5 days after seedling emergence. A = 1 (dark pink). B = 2 (fair pink). C = 3 (light pink). D = 4 (very light pink). E = 5 (green).

tillers and panicles similar to the main stem, and 9 = less than 10% plant with uniform height tiller and panicles similar to the main stem panicles of non-damaged plants.

3.2.3.1.12 Agronomic score

Agronomic performance of the test material was evaluated at crop maturity on a scale of 1 to 5 based on panicle length, and production potential, where 1 = good productive potential, and 5 = poor agronomic performance, poor productive potential and poor adaptation to agro-climatic conditions.

3.2.3.1.13 Midge damage

Midge damage was evaluated at crop maturity on 1 to 9 scale, where, 1 = < 10% midge damaged spikelets, 2 = 11 to 20% midge damaged spikelets, 3 = 21 to 30% midge damaged spikelets, 4 = 31 to 40% midge damaged spikelets, 5 = 41 to 50% midge damaged spikelets, 6 = 51 to 60% midge damaged spikelets, 7 = 61 to 70% midge damaged spikelets, 8 = 71 to 80% midge damaged spikelets, and 9 = > 81% midge damaged spikelets.

3.2.3.1.14 Aphid damage

Aphid damage was evaluated at crop maturity on a 1 to 9 scale, where, 1= few aphids present with no apparent damage to the leaves and 9= heavy aphid density on infested leaves (Table 3.4)

Table 3.4 Aphid ratings in relation to percentage

Aphid density/injury rating	Aphid density/injury %
1	1-10
2	11-20
3	21-30
4	31-40
5	41-50
6	51-60
7	61-70
8	71-80
9	> 80

3.2.3.1.15 Grain yield

All the mature panicles from each plot were harvested in bulk and threshed together, and expressed the yield as gram per plot.

3.2.4 Statistical analysis

3.2.4.1 Phenotyping data analysis

3.2.4.1.1 Analysis of variance (ANOVA)

The analyses of variance for phenotypic data sets were performed using the residual maximum likelihood algorithm (ReML). which provides the best linear unbiased predictors (BLUPs) of the performance of tested genotypes (Patterson and Thompson, 1971). ReML estimates the components of variance by maximizing the likelihood of all contrasts with zero expectation. For each trait and for each entry, the predicted means were calculated with entries as fixed effects for both individual environment (season) and across screening environments (seasons) analyses; replications, error, and entry × replication interactions as random effects in individual screening environment analyses; and replication, error, entry × replication, and entry × environment interactions as random effects in the across screening environments analysis.

Entry means were estimated by generalized least squares with weights depending on the estimated variance components according to Paterson (1997). The data was analyzed using the GenStat (6^{th} edition) package (Payne, 2002).

3.2.4.1.2 Estimates of broad-sense heritability (h^2) on of pr ,geny-mean basis

Broad-sense heritability h^2 (progeny-mean basis) was estimated across RILs in each of the two screening environments for all candidate resistance component traits as well as for the traits measured at crop maturity. It is the ratio of total genotypic variance to phenotypic variance and was calculated following Falconer (1989) for the data recorded in individual environments, E1 and E2:

$$h^2 = \frac{V_g}{V_p}$$

Broad-sense heritability estimates across the screening environments were computed by the formula,

$$h^2 = \frac{V_g}{V_g + V_{ge} + V_e}$$

where,

h^2 = broad-sense heritability

V_g = genotypic variance

V_p = phenotypic variance

V_e = environmental variance

V_{ge} = G × E interaction variance

3.2.4.1.3 Superiority of RILs over the parents (transgressive segregation)

The calculation of superiority of RILs over parents for shoot fly resistance and other traits were worked out using following formula:

S1 = (RIL-P1)/P1

S2 = (RIL-P2)/P2

where,

S1 = superiority to P1 (296B)

S2 = superiority to P2 (IS 18551)

P1 = Mean of parent 1 (296B)

P2 = Mean of parent 2 (IS 18551)

The information obtained was used to estimate the proportion of transgressive segregants in the RIL population (based on means across the two screening environments); RILs showing phenotypic characteristics with values lying outside the parental limits for shoot fly resistance components as well as other traits were considered transgressive segregants.

3.2.4.1.4 Test of significance of means

To test whether the difference between means of each parent and mean of RILs is small enough to accept the null hypothesis, i.e., $\overline{X1} = \overline{X2}$, the t-test was applied and calculations were made using the formula given by Singh and Chaudhary (1996):

$$t = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

where, $S_1^2 = \frac{\Sigma (X_{i1} - \overline{X_1})^2}{n_1 - 1}$

$$S_2^2 = \frac{\Sigma (X_{i2} - \overline{X_2})^2}{n_2 - 1}$$

The calculated value of 't' was compared with the table value of 't' to test its significance at $(n_1 + n_2) - 2$ degrees of freedom.

3.2.5 Marker data analysis

3.2.5.1 Occurrence of non-parental alleles

This population was found to contain unexpectedly large number of progeny with non-parental alleles. Around 18% of the total population (46 RILs out of the 259-entry RIL population) was detected to be carrying non-parental alleles. These entries were discarded from the dataset used as input for the linkage and QTL mapping analyses.

3.2.5.2 Information on genetic linkage map used for QTL mapping

The QTL mapping was done by utilizing the skeleton map developed by Deshpande (2005). The linkage map of 296B × IS 18551 based RIL population had 111 SSR marker loci mapped over 11 linkage groups (Fig 3.2). The linkage groups varied in length from 22.9 cM to 385.2 cM with a total linkage map length of 2165.8 cM.

3.2.5.3 QTL analysis

A total number of 213 RIL progenies from the cross 296B × IS 18551 were used for marker trait associations. The BLUPs of these 213 RILs derived from the cross 296B × IS 18551 were used for QTL analyses. QTL analyses were performed using

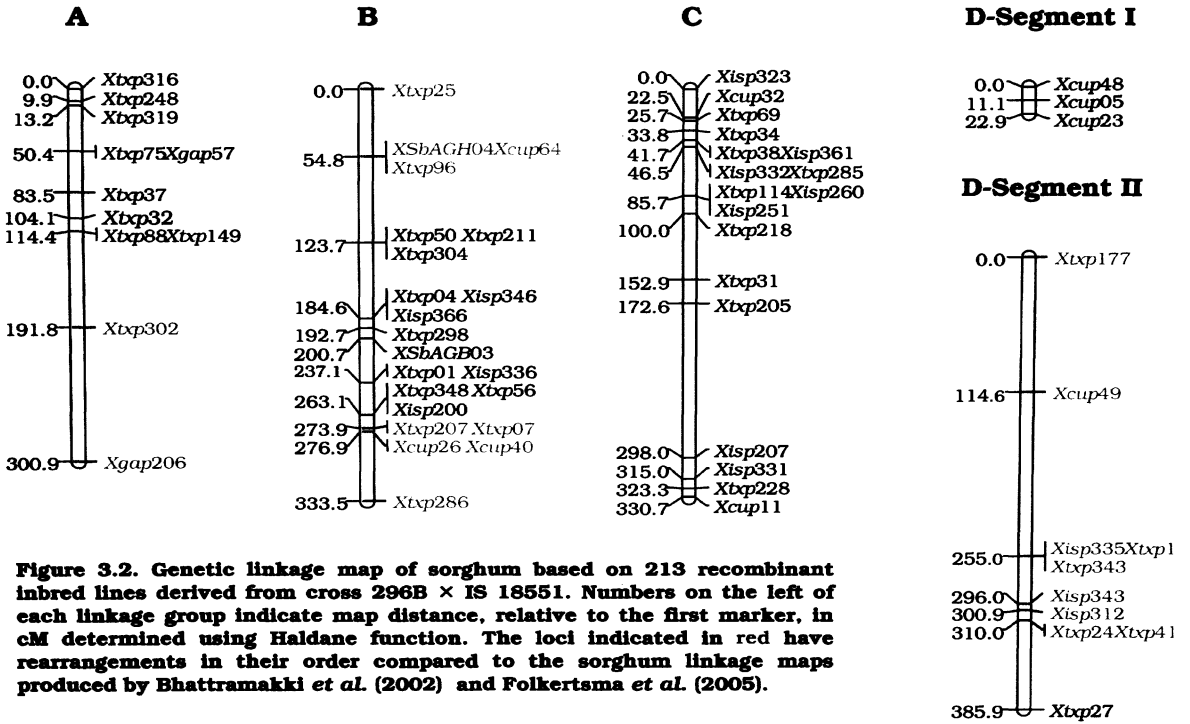
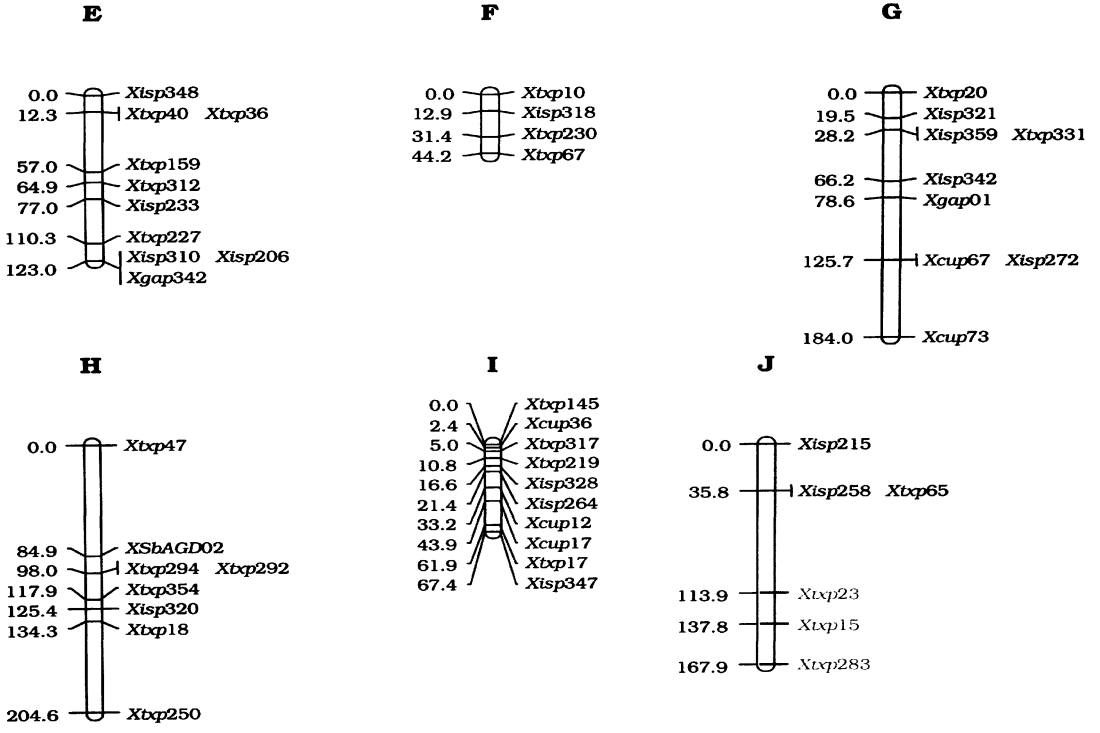


Figure 3.2. Genetic linkage map of sorghum based on 213 recombinant inbred lines derived from cross 296B × IS 18551. Numbers on the left of each linkage group indicate map distance, relative to the first marker, in cM determined using Haldane function. The loci indicated in red have rearrangements in their order compared to the sorghum linkage maps produced by Bhattramakki *et al.* (2002) and Folkertsma *et al.* (2005).

Fig. 3.2. Cont..


E
0.0 Xisp348
12.3 Xtxp40 Xtxp36
57.0 Xtxp159
64.9 Xtxp312
77.0 Xisp233
110.3 Xtxp227
123.0 Xisp310 Xisp206
Xgap342
F
0.0 Xtxp10
12.9 Xisp318
31.4 Xtxp230
44.2 Xtxp67
G
0.0 Xtxp20
19.5 Xisp321
28.2 Xisp359 Xtxp331
66.2 Xisp342
78.6 Xgap01
125.7 Xcup67 Xisp272
184.0 Xcup73
H
0.0 Xtxp47
84.9 XSbAGD02
98.0 Xtxp294 Xtxp292
117.9 Xtxp354
125.4 Xisp320
134.3 Xtxp18
204.6 Xtxp250
I
0.0 Xtxp145
2.4 Xcup36
5.0 Xtxp317
10.8 Xtxp219
16.6 Xisp328
21.4 Xisp264
33.2 Xcup12
43.9 Xcup17
61.9 Xtxp17
67.4 Xisp347
J
0.0 Xisp215
35.8 Xisp258 Xtxp65
113.9 Xtxp23
137.8 Xtxp15
167.9 Xtxp283

composite interval mapping (CIM) (Jansen and Stam, 1994; Zeng, 1994). Required computations were performed using PlabQTL Version 1.1 (Utz and Melchinger, 2000), which performs CIM by employing interval mapping using a regression approach (Haley and Knott, 1992) with selected markers as cofactors. Markers to serve as cofactors were identified using stepwise regression with an F-to-enter and an F-to-delete threshold value of 3.5. The presence of a putative QTL in an interval was tested using a critical LOD threshold as determined by PlabQTL using the Bonferroni χ^2 approximation (Zeng, 1994) corresponding to a genome-wise type I error of 0.25. Since the mapping population used in the present study was constituted of RILs, the additive model 'AA' was employed for analyses in which additive × additive epistatic effects were included. The point at which the LOD score had the maximum value in the interval was taken as the estimated QTL position. QTLs detected in different environments were treated as common if their estimated positions were within 20 cM of each other and their estimated effects had identical sign. The proportion of phenotypic variance explained by a single QTL was estimated as the square of the partial correlation coefficient. Estimates of the additive effect of each detected QTL, the total LOD score, and the total proportion of phenotypic variance explained jointly by all detected QTL were obtained by fitting a multiple linear regression model that simultaneously included all detected QTL for the trait in question. QTL × environment interaction was analysed over all three environments as described by Utz and Melchinger (2000). The proportion of genetic variance explained by the QTL was adjusted for QTL × environment interactions to avoid overestimation. After the QTL analysis with PlabQTL, the QTLs identified for components of resistance were assigned to the linkage groups based on linkage positions of markers on the linkage map developed by Bhattramakki *et al.* (2000).

3.2.5.4 QTL analysis for a single environment

To localize and characterize QTLs controlling components of resistance to shoot fly, the combined phenotypic and molecular data were analyzed with PlabQTL (Utz and Melchinger, 1996). Interval mapping using multiple regression approach with flanking markers (CIM *i.e.* composite interval mapping) was followed according to the procedure described by Haley and Knott (1992). Since the mapping population used in the present study constitutes RILs, the additive model AA was chosen for analysis in which additive × additive effects were included.

The LOD score was calculated from the F-value for the multiple-regression (Haley and Knott, 1992) as

LOD = n ln (1 + p*F /DFres)*0.2171

where,

p = number of parameters fitted;

F ratio = SSR(full) – SSR (red) / p MSE(full)

where;

SSR(full) = sum of squares for regression with full model, i.e. with QTL and cofactors

SSR(red) = sum of squares for regression with reduced model, i.e. without the QTL

MSE(full) = SSE/DFE = residual mean square (full model)

P MSE = number of estimated QTL effects

DFres = number of degrees of freedom for residual sum of squares in multiple regression;

The percentage of phenotypic variance explained by a putative QTL (R^2%) was calculated. This is based on the partial correlation of the putative QTL with the observed variable, adjusted for cofactors (Kendall and Stuart, 1961). In the simultaneous fit, the cofactors are ignored and only the putative QTLs initially detected and their estimated positions were used in multiple regressions to obtain the final estimate of the additive effects and percentage of phenotypic variation for a particular trait that could be explained by the QTL(s). The adjusted $R^{\wedge 2}$% (adjR^2%), the finally explained portion of the phenotypic variance, was estimated according to Hospital *et al.* (1997). The additive effect was calculated as half the differences between genotypic values of two homozygotes (Falconer, 1989):

Additive effect = (Parent P2 - Parent P1)/2

3.2.5.5 QTL analysis across the environments and Q × E interaction

The analysis was done with PLABQTL (following the same procedure described above) to identify QTLs for the traits using BLUPs across the two screening environments and for each of the two individual screening environments. The occurrence of additive × additive interaction was tested for significance by adding digenic epistatic effects to the additive effects in the model. The Q × E interaction for shoot fly resistance was estimated by a fitted model to the adjusted entry means of each environment as described by Bohn *et al.* (1996). A simultaneous analysis with all detected putative QTLs was performed for each screening environment. The

results were obtained in the form of tables showing ANOVA and the estimated effects.

The additive effects were obtained for all detected putative QTLs for each environment as well as across the environments. The estimated MS (Q × E) were calculated from the difference of the fits of the data from individual environments and across environments. These values were tested for significance with a Sequentially Rejective Bonferroni F-test (SRBF).

3.3. Marker-assisted selection for shoot fly resistance traits in sorghum

3.3.1. Background of marker-assisted selection for shoot fly resistance and component traits in sorghum

Screening for the shoot fly resistance and component traits in three environments of 252 recombinant inbreds lines (RILs) of BTx623 × IS 18551 mapping population was done, while genotyping using 109 SSR markers was undertaken to explore the genomic regions associated with shoot fly resistance (Sajjanar, 2002, Folkertsama *et al 2005.* unpublished). The genetic linkage map was constructed using Joinmap version 2.0 and 3.0 resulting in the formation of 10 linkage groups, with total map length of 1468 cM. QTL analysis using PlabQTL version 1.1 revealed the presence of 28 QTL detected at least in 2 of the 3 environments (4 for leaf glossiness, 2 for seedling vigor I, 5 for seedling vigor II, 2 for abaxial leaf surface trichome density, 3 for adaxial leaf surface trichome density, 2 for oviposition at 14 DAE, 1 for oviposition at 21 DAE, 4 for deadhearts at 21 DAE, 3 for deadhearts at 28 DAE, and 2 for seedling height I). Closely linked markers were identified for the four deadhearts QTLs, which can be used in a marker-assisted backcrossing program. In the present study efforts are being made to transfer the deadhearts QTLs into elite sorghum breeding lines developed at Sorghum Research Station (SRS), M.A.U., Parbhani by marker-aided selection using the closely linked markers. The markers associated with shoot fly resistant traits are listed below (Table 3.5)

Table 3.5 Target genomic regions, linked SSR markers and associated shoot fly resistance QTLs for marker-assisted selection (Folkertsma *et al.*, unpublished).

Linkage group	Associated SSR markers	QTLs co-localized with genomic regions
A	*Xtxp*75, *Xtxp*37	Deadhearts I, Oviposition I
E	*Xtxp*40, *Xtxp*312	Deadhearts I, Oviposition I
G	*Xixp*263, *Xgap*01, *Xtxp*141	Glossiness, Trichome density upper and lower leaf surface, Seedling vigor II, Oviposition I and II, and Deadhearts I and II
J	*Xisp*258, *Xtxp*65, *Xtxp*15	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II

3.3.2 Plant material

Elite sorghum breeding lines were obtained from SRS, M.A.U., Parbhani (personal communication with Dr S S Ambekar, Sorghum Breeder, SRS, M.A.U., Parbhani), while donor parents were obtained from ICRISAT, Patancheru, A.P.,India (personal communication with Dr B V S Reddy, Principal Scientist, Sorghum Breeder, ICRISAT).

Recurrent parents: PMS 28B, PMS 20B, KR 192

Donor parents (ICRISAT): IS 18551 (shoot fly resistant parent), RIL 153, RIL 189, and RIL 252 derived from the BTx623 × IS 18551 shoot fly resistance mapping population.

3.3.3 Salient features of parental line used in backcross program

Recurrent parents

PMS 28B: Maintainer line developed from ICSB 94040B × MS 296B. The special feature of this line is its long panicle with a large number of primaries and secondaries. It is kharif adapted, tan type, good combining ability, juicy white midrib, compact panicle, medium seed size and is susceptible to shoot fly (Plate 3.4).

PMS 20B: This A_2 cytoplasm maintainer line, is derived from a cross between MS 296B (A_2) and SPV 900. It has bold seed with yellow endosperm. The panicle is oblong with a large number of secondaries. It is *rabi*-adapted with non-tan plant type and medium height. It is agronomically superior, has good combining ability, juicy white midrib, awns and is susceptible to shoot fly (Plate 3.4).

KR 192: This is a mid-tall restorer line derived from cross SPV 544 × SPV 462. It has good combining ability for yield and yield-contributing characters. It can be grown in *kharif, rabi* and *summer* seasons. It has broad leaves, white juicy midrib, and pearly white grain color. Panicles are compact and awnless with medium-bold grains having 50 percent of the grain surface covered by glumes. It is grain mold resistant and shoot fly susceptible (Plate 3.4).

Donor parents

IS 18551 (resistant parent): Origin from Ethiopia, race *durra*, panicles with straw-colored grain and large glumes. Leaves of seedlings are light green, shiny, narrow and pointed upwards with dense trichomes. Resistant to shoot fly, very tall at maturity, agronomically poor, four shoot fly resistance QTLs mapped (Plate 3.4).

RIL 189: This RIL was selected from the shoot fly resistant population (BTx623 × IS 18551). It is mid tall, has non-tan foliage, juicy white midrib, glumes partially covered, narrow upward pointing leaves, awnless, high number of trichomes and glossy, three shoot fly resistance QTLs mapped.

RIL 252: Derived from shoot fly resistant mapping population BTx623 × IS 18551, mid tall, narrow upward leaves, juicy white midrib, panicle like IS 18551, white grain, awnless, high trichome density and glossy, three shoot fly resistance QTLs mapped.

RIL 153: A mid tall, juicy white midrib, medium to high trichome density, medium glossy, and three shoot fly resistance QTLs mapped.

3.3.4 Parental genotyping with SSR markers

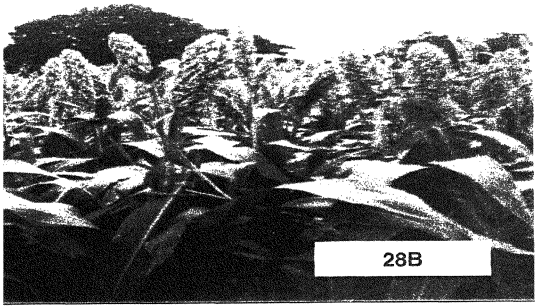
Approximately ten to twelve seeds/set of each of the parents were sown individually in small pot. Staggered sowing was employed in two sets to ensure nicking of flowering period between donor and recurrent parents. The sowing was done at ICRISAT, Patancheru during second and fourth week of August 2003.

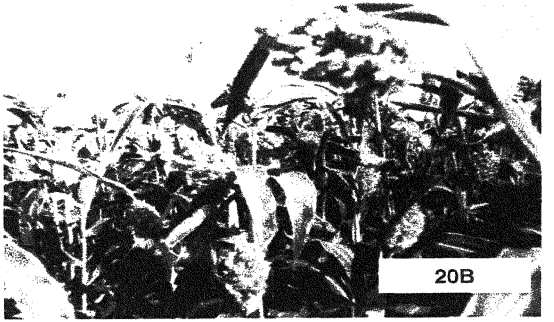
3.3.5 DNA extraction

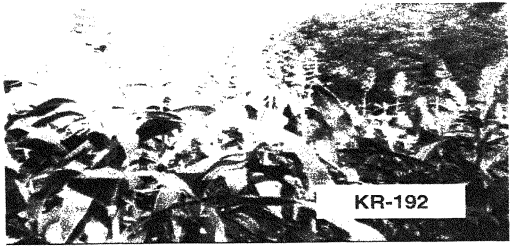
DNA from parental plants was extracted from individual one-week-old seedlings by using a modified CTAB method (Mace *et al.*, 2003) as described in section 3.1.2.

3.3.6 Selection of the markers

A set of eleven sorghum SSR markers linked to targeted shoot fly QTLs (Table 3.6) from four linkage group (Fig 3.3) was used for PCR amplification using DNA from recurrent and donor parents as templates in order to identify polymorphic SSR markers among recurrent and donor parents.







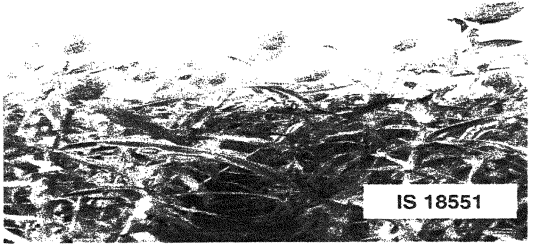


Plate 3.4: Recurrent and donor parents used in marker-assisted selection for shoot fly resistance and its component traits.

Table3.6 Fluorescent labeled sorghum SSR primers (Applied Biosystem) used for foreground selection in marker-assisted breeding for shoot fly resistance

SSR locus	Linkage group	Label F- or R-	Primer sequence Forward (F)	Tm	Reverse (R)	Tm	SSR repeat motif
Xtxp37	A	F-Hex	AACCTAAGAGGCCTATTTAACC	56.5	ACGGCGACTCTGTAACTCATAG	58.4	(TC)23
Xtxp75	A	F-Fam	CGATGCCTCGAAAAAAAAACG	55.9	CCGATCAGAGCGTGGCAGG	63.1	(TG)10
Xtxp312	E	F-Ned	CAGGAAAATACGATCCGTGCC/	63.0	GTGAACTATTCGGAAGAAGTTTGGAC	64.0	(CAA)26
Xtxp40	E	F-Fam	CAGCAACTTGCACTTGTC	53.7	GGGAGCAATTTGGCACTAG	56.7	(GGA)7
Xixp10362	E	F-Hex	CCTTCGTGTTTGGAAAGTT	-	CCGGTTGGATGAGAAGTA	-	AT/CT/GT
Xtxp141	G	F-Ned	TGTATGGCCTAGCTTATCT	55.0	CAACAAGCCAACCTAAA	47.9	(GA) 23
Xisp10263	G	F-Fam	TATCTTCTCCGCCCTTTC	-	TAAGNGCCAAGGGAATG	-	CA/CTG
Xgap1	G	F-Fam	TCCTGTTTGACAAGCGCTTATA	-	AAACATCATACGAGCTCATCAATG	-	(AG)6
Xtxp15	J	F-Fam	CACAAACACTAGTGCCTTATC	55.9	CATAGACACCTAGGCCATC	56.7	(TC)16
Xtxp65	J	F-Hex	CACGTCGTCACCAACCAA	56.0	GTTAAACGAAAGGGAAATGGC	55.9	(ACC)4(CCA)3CG(CT)8
Xisp10258	J	F-Ned	GCAGGACCGGATAGAGAT	-	ATCCCGGAATGATGAAGT		CAA/CCG

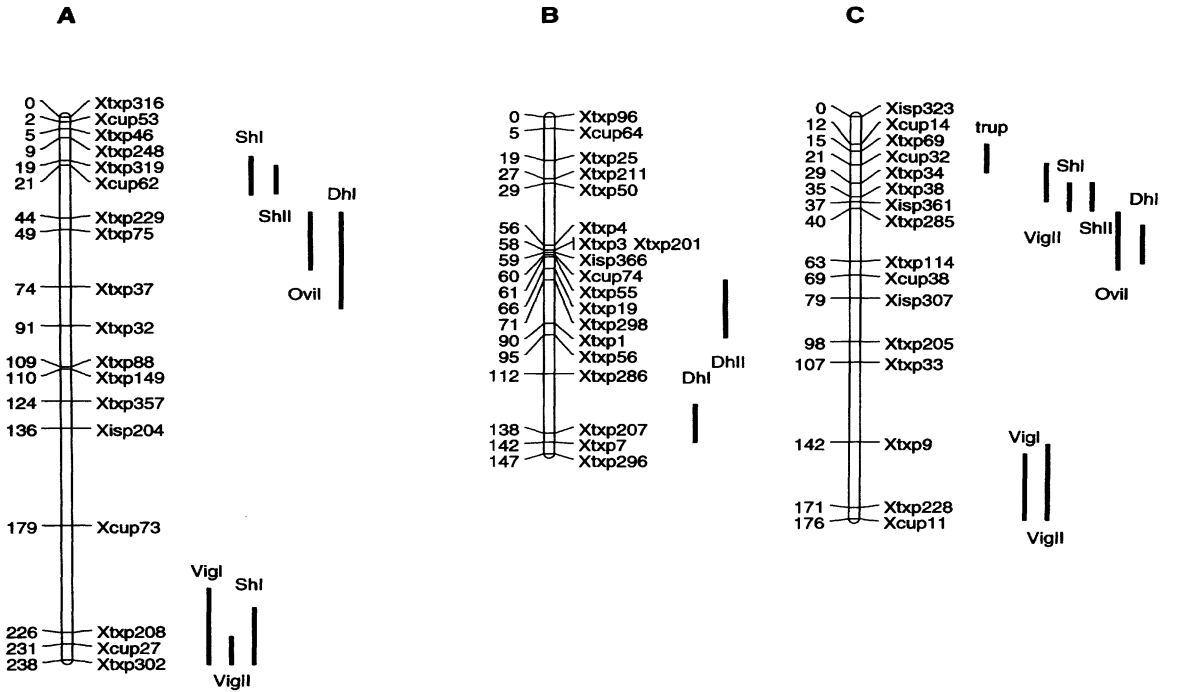


Fig 3.3: QTL positions of shoot fly resistance component traits for 252 recombinant inbred lines derived from cross BTx623 × IS 18551, based on analysis across two screening environments.

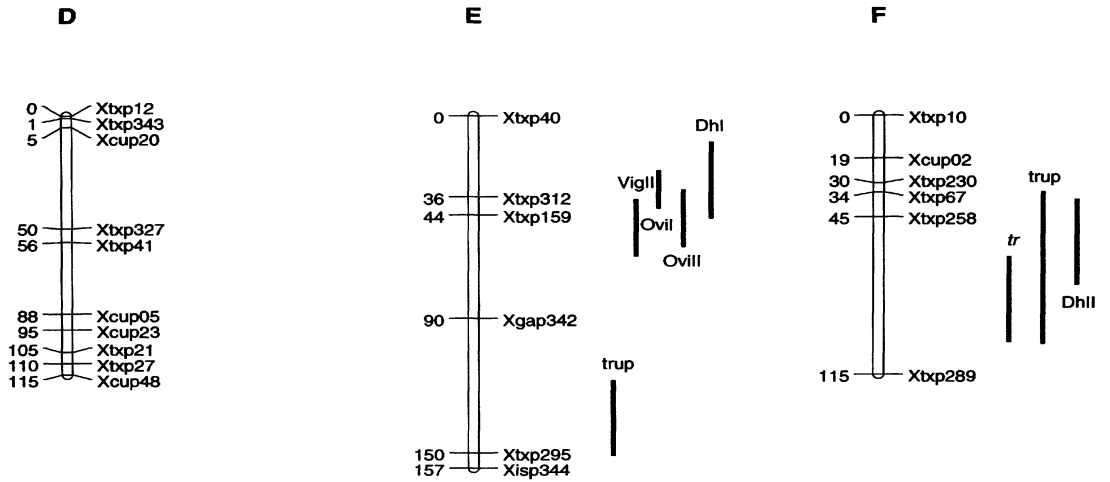


Fig. 3.3: QTL positions of shoot fly resistance component traits for 252 recombinant inbred lines derived from cross BTx623 × IS 18551, based on analysis across two screening environments.

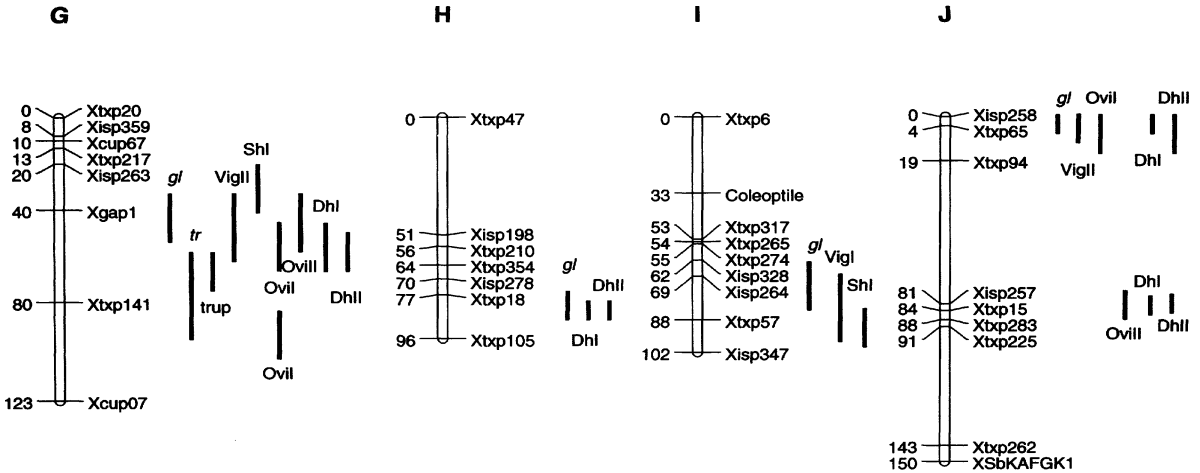


Fig. 3.3: QTL positions of shoot fly resistance component traits for 252 recombinant inbred lines derived from cross BTx623 × IS 18551, based on analysis across two screening environments.

3.3.7Amplification of SSR markers

PCR reactions were conducted in 384 wells plates in a PE 9700 Perkin Elmer (Norwalk Conn.) DNA thermocycler. The reactions were performed in volumes of 5µl using four different PCR protocols and a touchdown PCR program. Reaction mixture contains 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.25-2.5 ng of DNA, 2pm of forward and reverse primer, 1mM $MgCl_2$, 80-100 µM of each dNTP and 0.1 units of *Taq* DNA polymerase. PCR conditions were previously optimized for each primer using a grid of nine reactions Smith *et al.*, (1995). Three different protocols were used for PCR amplification i.e. protocol 5, 7, and 4.

The touch down PCR program consisted of initial denaturation for 15 min at 94°C and then 10 cycles of denaturation for 10 sec at 94°C, annealing at 61-52°C for 20 sec, the annealing temperature for each cycle is reduced with 1°C, and extension at 72°C for 30 sec; 35 cycles (for SSRs screened using PAGE) and 31 cycles (for SSRs screened using the ABI sequencer) of denaturation for 10 sec at 94°C, annealing at 54°C for 20 sec and extension at 72°C for 30 sec. The last PCR cycle was followed by a 20 min extension at 72°C to ensure amplification to equal lengths of both DNA strands.

If the parents showed PCR product polymorphism of more than 5 bp, then PCR products were separated on 6% non-denaturing PAGE (Poly Acrylamide Gel Electrophoresis) gels and silver stained using the modified procedure of Tegelstrom (1998). If the polymorphism between the parents is less than 5 bp, then PCR products were separated by capillary electrophoresis using a ABI Prism 3700 (Perkin Elmer) sequencing. For this purpose fluorescent-labeled primes were used. All ABI primers were screened with the help of Dr. Rolf Folkertsma, PDF, ICRISAT.

3.3.8 Non-denaturing PAGE (Poly Acrylamide Gel Electrophoresis)

1 µl of loading dye (orange red + EDTA + NaCl + Glycerol) was added to 3 µl of PCR product. From this mixture, 2µl of the sample was loaded into a well of the 6% non-denaturing PAGE gel.

The gel was prepared using

52.5 ml of double distilled water; 7.5 ml of 10X TBE buffer; 15.0 ml of Acrylamide:Bis-acrylamide (29:1) solution 450 µl of Ammonium Per Sulphate (APS); and 100 µl of TEMED.

Along with the samples, the 100 bp marker ladder (50 ng/µl) was also loaded in the first and last lanes of the gel to ensure proper sizing of amplified PCR fragments. Most of the markers used allowed clear differentiation of donor and recurrent parent alleles. The gel was run at 550V of constant power in 0.5x TBE buffer for 3 hours using a BioRad sequencing gel apparatus.

3.3.9 Silver staining

After running of PAGE gels for the required time, the gels were developed by silver staining.

Sequential steps involved in silver staining

The gel was placed in

- water for 5 min
- 0.1% CTAB solution for 20 min (2 g in 2 lit of water)
- 0.3% ammonia solution for 15 min (26 ml of 25% ammonia solution in 2 lit of water)
- 0.1% silver nitrate solution for 15 min (2 g of silver nitrate + 8 ml of 1 M NaOH in 2 lit of water and add ammonia solution up to the solution becomes colorless)
- water for few sec
- Developer (30 g of sodium carbonate + 400 µl of formaldehyde in 2 lit of water)

After developing the bands gels were rinsed in water for 1 minute and placed it in fixer (30 ml glycerol in 2 lit of water) for a few seconds.

Continuous shaking is required throughout the silver staining procedure.

After silver staining of the PAGE gels, the size (base pair) of the intensely amplified specific bands or alleles for each SSR marker was estimated based on its migration relative to the 100 bp DNA ladder (fragments ranging from 100 bp to 1000 bp) and presence or absence of parental alleles were scored.

3.3.10 Data collection and analysis

Scoring of the gels

The silver stained bands of amplified PCR products in the gels were scored as A, B, H, OFF and "-" based on their pattern compared with those of the parents. "A" was defined as the homozygote for the allele from the recurrent parent (28B/20B/KR 192), "B" was defined as the homozygote for the allele from donor parent IS 18551/RIL 189/RIL 252/RIL 153, "H" was defined as the heterozygote (presence of both recurrent and donor parent alleles), "OFF" was defined as an allele observed from unknown source, and "-" was a missing sample. During parental polymorphism studies, amplified PCR products were scored as "A" and "B", while for the backcrossing program amplified PCR products were scored as "A", "B" and "H".

In parental genotyping the PCR product was run on the automatic DNA sequencer (ABI 3700) and the amplified allele size was scored. Polymorphic SSR markers, that flanked targeted QTL of each of four linkage group, were identified. Based on parental allelic size the pure homozygous plants were selected and tagged at seedling stage and these plants advanced for crossing to produce F_1 hybrids (plant to plant crosses). A detailed list of the numbers of plant genotyped and numbers of pure plants selected for crossing is presented below (Table 3.7).

Table 3.7 Summary of SSR markers used to identify pure parental plants employed for F_1 hybrid production

Name of parent	Plant numbers	No. of plants geno-typed	No. of SSR markers used	No. of parental type plants selected for crossing
20B	SP 1-12	12	*Xtxp*75, *Xtxp*37, *Xtxp*15, *Xtxp*225, *Xtxp*65,	09
	SP 177-186	10	*Xisp*10258, *Xtxp*141, *Xgap*1, *Xisp*10263,	09
			*Xtxp*312, *Xtxp*40, *Xisp*10362	
28B	SP 133-135	03	*Xtxp*75, *Xtxp*37, *Xtxp*15, *Xtxp*225, *Xtxp*65,	03
	SP 287-296	10	*Xisp*10258, *Xtxp*141, *Xgap*1, *Xisp*10263,	08
			*Xtxp*312, *Xtxp*40, *Xisp*10362	
KR 192	SP 141-152	12	*Xtxp*75, *Xtxp*37, *Xtxp*15, *Xtxp*225, *Xtxp*65,	05
	SP 297-306	10	*Xisp*10258, *Xtxp*141, *Xgap*1, *Xisp*10263,	06
			*Xtxp*312, *Xtxp*40, *Xisp*10362	
IS 18551	SP 109-120	12	*Xtxp*75, *Xtxp*37, *Xtxp*15, *Xtxp*225, *Xtxp*65,	11
	SP 267-176	10	*Xisp*10258, *Xtxp*141, *Xgap*1, *Xisp*10263,	08
			*Xtxp*312, *Xtxp*40, *Xisp*10362	
RIL 189	SP 153-164	12	*Xtxp*75, *Xtxp*37, *Xtxp*15, *Xtxp*225, *Xtxp*65,	05
	SP 307-316	10	*Xisp*10258, *Xtxp*141, *Xgap*1, *Xisp*10263,	06
			*Xtxp*312, *Xtxp*40, *Xisp*10362	
RIL 153	SP 85-96	12	*Xtxp*75, *Xtxp*37, *Xtxp*15, *Xtxp*225, *Xtxp*65,	04
	SP 247-256	10	*Xisp*10258, *Xtxp*141, *Xgap*1, *Xisp*10263,	04
			*Xtxp*312, *Xtxp*40, *Xisp*10362	
RIL 252	SP 165-176	12	*Xtxp*75, *Xtxp*37, *Xtxp*15, *Xtxp*225, *Xtxp*65,	06
	SP 317-326	10	*Xisp*10258, *Xtxp*141, *Xgap*1, *Xisp*10263,	05
			*Xtxp*312, *Xtxp*40, *Xisp*10362	

In the present study we have tried to produce seven hybrids by using three recurrent parents and four donor parents, as listed below.

1. 28B (288) × RIL 189 (312)
2. KR 192 (304) × IS 18551 (267)
3. 20B (186) × RIL 252 (318)
4. 20B (179) × RIL 153 (248)
5. KR 192 (300) × RIL 252 (319)
6. 28B (293) × IS 18551 (268)
7. 28B (292) × RIL 153 (252)

3.3.11 Testing of hybridity with SSR markers

Staggered sowing was employed to ensure nicking of flowering period. The sowing was done in two sets between the second and fourth weeks of April 2004. Five and fifteen seeds each of seven hybrids and three recurrent parents, respectively, were sown individually in pots for each set of sowing. DNA was extracted from one-week-old seedling leaf tissue and genotyping of each F_1 plant was undertaken with four SSR markers flanking the targeted QTLs in each linkage group. Heterozygous plants in the five hybrid populations were chosen for each targeted QTLs at the seedling stage and these plants were advanced by crossing to produce BC_1F_1 seed. A detailed list of numbers of plant genotyped, markers used, numbers of selected heterozygous plants, and numbers of backcrosses effected and advanced to the BC_1F_1 generation is presented Table 3.8.

3.3.12 Testing of recurrent parent purity with SSR markers

DNA was extracted from each of the recurrent parent plants and genotyping of each recurrent parental plants was done with four SSR markers to test their purity. The plants found homozygous at all loci were used in the backcrossing program. The details of numbers of plants genotyped and numbers of pure plants selected and used for pollination are presented in Table 3.9.

3.3.13 Genotyping BC_1F_1 populations with SSR markers for foreground selection

Ten seeds of each of five BC_1F_1 populations, eight seeds of each of three recurrent parents and one seed of each of four donor parents were sown for each sowing of a total of three sets of staggered sowings to ensure nicking of the flowering period. Sowing of first set was done during the fourth week of August 2004, the second set in

Table 3.8. Details of numbers of plant genotyped, markers used, numbers of selected heterozygous plants and numbers of backcrosses effected and advanced to the BC_1F_1 generation

Name of hybrids	No.of plants genotyped	Details of plants	SSR markers used	LG	No. of heterozygous plants selected	Plants selected	No. of backcrosses effected	Detail of crosses	Crosses advanced to BC_1F_1 generation
28B (288) × RIL 189 (312)	05	SP 541-545	*Xtxp*75, *Xtxp*37 *Xtxp*41, XISP10258	A G J	02	SP 541, SP 542	02	SP 541 × SP 405 SP 542 × SP 406	SP 541 ×SP 405 SP 542 ×SP 406
KR 192 (304) × IS 18551 (267)	10	SP 511-520	*Xtxp*75, *Xtxp*37 *Xtxp*41, *Xisp*10258	A G J	10	SP 511, SP 512, SP 513, Sp 514, SP 515, SP 516, SP 517, SP 518, SP 519, SP 520	10	SP 511 × SP 477 SP 512 × SP 479 SP 513 × SP 476 SP 514 × SP 480 SP 515 × SP 480 SP 516 × SP 484 SP 517 × SP 482 SP 518 × SP 482 SP 519 × SP 484 SP 520 × SP 485	SP 511 × SP 477 SP 513 × SP 476 SP 518 × SP 482
20B (186) × RIL 252 (318)	10	SP 521-530	*Xtxp*75, *Xtxp*37 *Xtxp*41 *Xisp*10258	A G J	2	SP 525, SP 529	01	SP 525 × SP 431	SP 525 × SP 431
20B (179) × RIL 153 (248)	10	SP 531- 540	*Xtxp*75, *Xtxp*37 *Xtxp*41 *Xisp*10258	A G J	8	SP 531, SP 532, SP 533, SP 534, SP 535, SP 536, SP 538, SP 540	08	SP 531 × SP 452 SP 532 × SP 451 SP 533 × SP 447 SP 534 × SP 450 SP 535 × SP 448 SP 536 × SP 456 SP 538 × SP 449 SP 540 × SP 453	SP 534 × SP 450 SP 535 × SP 448 SP 540 × SP 453
KR 192 (300) × RIL 252 (319)	09	SP 561-570	*Xtxp*75, *Xtxp*37 *Xtxp*41 *Xisp*10258	A G J	08	SP 561, SP 563, SP 565, SP 566, SP 567, SP 568, SP 569, SP 570	08	SP 561 × SP 465 SP 563 × SP 465 SP 565 × SP 462 SP 566 × SP 466 SP 567 × SP 463 SP 568 × SP 465 SP 569 × SP 464 SP 570 × SP 464	SP 561 × SP 465 SP 568 × SP 465 SP 566 × SP 466
28B (293) × IS 18551 (268)	10	SP 501-510	*Xtxp* 75, *Xtxp* 37, *Xtxp* 41, *Xisp*10258	A G J	0	-	-	-	-
28B (292) × RIL 153 (252)	Not germinated	-	-	-	-	-	-	-	-

Table 3.9. Details of numbers of recurrent parental plants genotyped and number of pure plants selected and used for pollination

Name of recurrent parent	No. of plants genotyped	Details of plants	SSR markers used	LG	No. of homozygous plants selected	Plants used in backcrossing	Details of plants
28B (288)	30	SP 401-430	*Xtxp75*, *Xtxp37*	A	25	02	SP 405, SP 406
			Xtxp41	G			
			XISP10258	J			
20B (186)	15	SP 431-445	*Xtxp75*, *Xtxp37*	A	15	01	SP 431
			Xtxp41	G			
			XISP 10258	J			
20B (179)	15	SP 446-460	*Xtxp75*, *Xtxp37*	A	13	08	SP 447, SP 448,
			Xtxp41	G			SP 449, SP 450,
			XISP10258	J			SP 451, SP 452,
							SP 453, SP 456
KR 192 (300)	15	SP 461-475	*Xtxp75*, *Xtxp37*	A	12	05	SP 462, SP 463,
			Xtxp41	G			SP 464, SP 465,
			XISP10258	J			SP 466,
KR 192 (304)	15	SP 476-490	*Xtxp75*, *Xtxp37*	A	14	07	SP 476, SP 477,
			Xtxp41	G			SP 479, SP 480,
			XISP10258	J			SP 482, SP 484,
							SP 485

September first week, and the third set in the second week of September 2004 at Patancheru.

Individual plant DNA of each BC_1F_1 plant, recurrent parent plant and donor parent plant was extracted from one-week-old seedling tissues. Marker genotyping of individual BC_1F_1 plants, recurrent and donor parent plants was done with eleven SSR markers flanking the targeted shoot fly resistance QTLs (foreground selection) on four linkage groups. A total of 69 heterozygous plants having an appropriate allelic constitution were selected (Table 3.10) before flowering from five BC_1F_1 populations. These selected plants were crossed with their respective recurrent parents (plant × plant cross) to produce BC_2F_1 seed.

Each recurrent parental plant was genotyped with eleven SSR markers and plants conforming to parental type allelic constitution were selected (Table 3.11) and used as pollinators.

Table3.10 Details of genotyping of BC_1F_1 plants with linked marker (foreground selection)

Name of BC_1F_1 population	Number of plant genotyped	Detail of plants	SSR markers used	LG	Heterozygous plants selected	Total selected plants
BC_1F_1 28B (288) × RIL 189 (312) × 28B (288)						
[F_1 (SP 541)] × SP 405	10	SP 610-619	*Xtxp* 75, *Xtxp* 37,	A	SP 612, SP 613, SP 617, SP 619	
			Xtxp 40, *Xixp* 10362, *Xtxp* 312,	E		
			Xixp 10263, *Xgap* 1, *Xtxp* 41	G		
			Xixp 10258, *Xtxp* 65, *Xtxp* 15	J		
[F_1 (SP 541)] × SP 405	5	SP 710-714			SP 710, SP 711	
[F_1 (SP 542)] × SP 406	5	SP 710-714			SP 719	
[F_1 (SP 542)] × SP 406	10	SP 810-819			SP 811, SP 812, SP 814, SP 815, SP 816, SP 817, SP 818	14
BC_1F_1 KR 192(304) × IS 18551 (267) × KR 192(304)						
[F_1 (SP 511)] × SP 477	10	SP 629- 638	*Xtxp* 75, *Xtxp* 37,	A	SP 629, SP 630, SP 633, SP 636	
			Xtxp 40, *Xixp* 10362, *Xtxp* 312,	E		
			Xixp 10263, *Xgap* 1, *Xtxp* 41	G		
			Xixp 10258, *Xtxp* 65, *Xtxp* 15	J		
[F_1 (SP 513)] × SP 476	10	SP 729- 738			SP 729, SP 731, SP 732, SP 736, SP 737	
[F_1 (SP 518) × SP 482	10	SP 829- 838			SP 830, SP 832, SP 833, SP 834, SP 835, SP 836	15
BC_1F_1 20B (186) × RIL 252 (318) × 20B (186)						
[F_1 (SP 525)] × SP 431	10	SP 648- 657	*Xtxp* 75, *Xtxp* 37,	A	SP 649, SP 650, SP 651, SP 655, SP 656, SP 657	
			Xtxp 40, *Xixp* 10362, *Xtxp* 312,	E		
			Xixp 10263, *Xgap* 1, *Xtxp* 41	G		
			Xixp 10258, *Xtxp* 65, *Xtxp* 15	J		
[F_1 (SP 525)] × SP 431	10	SP 748-757			SP 754, SP 757	
[F_1 (SP 525)] × SP 431	10	SP 848- 857			SP 848, SP 849, SP 850, SP 853	12

Name of BC_1F_1 population	Number of plant genotyped	Detail of plants	SSR markers used	LG	Heterozygous plants selected	Total selected plants
Table 3.10 cont---						
BC_1F_1 20B (179) × RIL 153 (248) × 20B (179)						
[F_1 (SP 534)] × SP 450	10	SP 667- 676	*Xtxp* 75, *Xtxp* 37, *Xtxp* 40, *Xixp* 10362, *Xtxp* 312, *Xixp* 10263, *Xgap* 1, *Xtxp* 41 *Xixp* 10258, *Xtxp* 65, *Xtxp* 15	A E G J	SP 668, SP 669, SP 670, SP 672, SP 673 SP 674	
[F_1 (SP 535)] × SP 448	10	SP 767- 776			SP 767, SP 771, SP 772, SP 773, SP 775, SP 776	
[F_1 (SP 540)] × SP 453	10	SP 867- 876			SP 867, SP 868, SP 870, SP 871, SP 873, SP 874, SP 876	19
BC_1F_1 KR 192 (300) × RIL 252 (319) × KR 192 (300)						
[F_1 (SP 561)] × SP 465	10	SP 686- 795	*Xtxp* 75, *Xtxp* 37, *Xtxp* 40, *Xixp* 10362, *Xtxp* 312, *Xixp* 10263, *Xgap* 1, *Xtxp* 41 *Xixp* 10258, *Xtxp* 65, *Xtxp* 15	A E G J	SP 688, SP 692	
[F_1 (SP 568)] × SP 465	10	SP 786-795			Nil	
[F_1(SP 566)] × SP 466	10	SP 886- 895			SP 887, SP 889, SP 890, SP 892, SP 893, SP 894, SP 895	9

Table 3.11 Details of recurrent parental plants genotyped and number of parental type plants selected for (BC_1F_1) backcrossing

Name of recurrent parent	No. of plants genotyped*	Details of plants	Parental type plant used in backcrossing
28B (288)			
SP 405	8	SP 601-608	SP 601, SP 602, SP 604, SP 605, SP 606, SP 607, SP
SP 405	8	SP 701-708	SP 707, SP 708
SP 406	8	SP 801-808	Nil
KR 192			
(304)	8	SP 620- 627	SP 621, SP 622, SP 627
SP 477	8	SP 720-727	SP 723, SP 724, SP 725, SP 726
SP 476	8	SP 820-827	SP 821, SP 822, SP 823, SP 826
SP 482			
20B (186)			
SP 431	8	SP 639- 646	SP 639, SP 640, SP 641, SP 642, SP 643, SP 644
SP 431	8	SP 739-746	SP 740, SP 743, SP 744
SP 431	8	SP 839- 846	SP 840
20 B (179)			
SP 450	8	SP 658-665	SP 658, SP 659, SP 661, SP 664
SP 448	8	SP 758-765	SP 759, SP 762
SP 453	8	SP 858-865	SP 860, SP 861, SP 862, SP 863, SP 865
KR 192			
(300)	8	SP 677- 684	SP 682
SP 465	8	SP 777-784	SP 777, SP 779
SP 465	8	SP 877-884	SP 877, SP 878, SP 880
SP 466			

* Eleven SSR markers used to evaluate purity of recurrent parental plants: *Xtxp*75, *Xtxp*37 (LG: A), *Xtxp*40, *Xtxp*312, *Xisp*10362 (LG: E), *Xgap*1, *Xtxp*141, *Xisp*10263 (LG: G), *Xisp*10258, *Xtxp*65, *Xtxp*15 (LG: J)

Out of 69 heterozygous BC_1F_1plants selected on the basis of foreground molecular data, 8 plants were discarded (Table 3.12) due by infection of maize streak virus at seedling stage.

Table 3.12 Details of discarded BC_1F_1 plants

Name of backcross	No. of plants removed	Details of plant removed
[28B (288) × RIL 189 (312)] × 28B (288)	3	SP 612, SP 613, SP 619
[KR 192 (304) × IS 18551 (267)] × KR 192 (304)	1	SP 636
[20B (186) × RIL 252 (318)] × 20B (186)	1	SP 650
[20B (179) × RIL 153 (248)] × 20B (179)	3	SP 672, SP 771, SP 775
[KR 192 (300) × RIL 252 (319)] × KR 192 (300)	0	-

3.3.14 Parental polymorphism using SSR markers (for background selection)

Initial parental screening with 38 SSR markers was carried out before actual genotyping of selected BC_1F_1 plants. The main objective for screening parental plants with these 38 SSR markers (Table 3.13) was to detect polymorphism among the parents. PCR amplification of each SSR marker was performed in a total volume of 5 ul reaction mixture containing parental genomic DNA, PCR buffer, DNTPs, MgCl2, forward primer labeled with HEX, NED or FAM dye phosporamidities, reverse primers and AmpliTaq gold DNA polymerase. PCR reactions were conducted in 96-well plates in a PE 9700 Perklin-Elimer. DNA thermocycler using a touch down PCR technique. The PCR products were run on an automatic DNA sequencer (i.e. capillary electrophoresis using an ABI 3700). After completion of the sequencer run, presence or absence of allelic fragments was scored using the Genotyper software. When the parents show polymorphism of more than 5 base pairs, the PCR products of the respective backcrossing population plants were separated on 6% non-denaturing PAGE (Poly Acrylamide Gel Electrophoresis) gels. If the polymorphism between the parents was less than 5 base pairs the PCR products were separated by capillary electrophoresis using an ABI Prism 3700 (Perkin-Elimer) DNA sequencer. For this purpose fluorescent labeled primers used. The details regarding fluorescent labeled sorghum SSR primers used in parental polymorphism of the backcrossing populations presented in Table 3.13 and 3.14.

Table 3.13: Parental data of allelic size (bp) of SSR markers used for background selection as determined on the ABI 3700

Sr.No	Marker locus	LG	28B(288)	RIL 189(312)	KR 192(304)	IS 18551(267)	20B(186)	RIL 252(318)	20B(179)	RIL 153(248)	KR 192(300)	RIL 252(319)
1	*Xtxp* 25	B	128	139	**155**	**155**	173	139	173	177	150	138
2	*Xtxp* 296	B	150	170	**163**	**163**	170	166	**170**	**170**	171	166
3	*Xtxp* 298	B	206	197	185	188	209	188	209	197	180	188
4	*Xcup* 63	B	**145**	**145**	**145**	**145**	**103**	**103**	**145**	**145**	**145**	**145**
5	*Xtxp* 283	B	**243**	**243**	**243**	**243**	**243**	**243**	**243**	**243**	**243**	**243**
6	*Xcup* 11	C	163	162	163	170	**172**	**172**	171	163	163	170
7	*Xtxp* 31	C	237	223	209	222	222	219	221	219	209	219
8	*Xcup* 61	C	199	196	196	199	**199**	**199**	199	196	196	199
9	*Xtxp* 228	C	236	246	244	223	224	223	224	246	244	223
10	*Xcup* 32	C	154	153	143	152	154	153	154	152	144	155
11	*Xtxp* 59	C	**206**	**206**	**206**	**206**	**206**	**206**	**206**	**206**	**206**	**206**
12	*Xtxp* 114	C	233	-	217	239	230	233	230	233	**233**	**233**
13	*Xgap* 236	C	179	174	**175**	**175**	**173**	**173**	**173**	**173**	176	172
14	*Xcup* 14	C	211	207	205	211	**211**	**211**	**211**	**211**	204	210
15	*Xgap* 10	D	**250**	**250**	301	250	**250**	**250**	**250**	**250**	295	250
16	*Xcup* 28	D	**164**	**164**	160	164	165	164	165	160	160	164
17	*Xtxp* 21	D	167	169	179	169	173	169	173	179	179	168
18	*Xtxp* 27	D	297	301	-	301	311	301	311	327	-	301
19	*Xgpsb* 50	D	**210**	**210**	246	210	**210**	**210**	**210**	**210**	246	210
20	*Xtxp* 343	D	142	154	**140**	**140**	148	154	-	123	-	155
21	*Xtxp* 289	F	273	288	288	273	**273**	**273**	273	288	288	273
22	*Xgap* 206	F	109	120	123	109	109	118	109	117	123	117
23	*Xcup* 02	F	206	198	192	204	**204**	**204**	**204**	**204**	192	204
24	*Xtxp* 258	F	220	223	223	156	194	182	194	191	222	190
25	*Xtxp* 230	F	169	190	194	-	173	192	173	192	**194**	**194**
26	*Xtxp* 273	H	**222**	**222**	203	190	201	195	216	219	204	195
27	*Xtxp* 47	H	290	280	290	260	**260**	**260**	**260**	**260**	280	260
28	*Xtxp* 105	H	286	286	292	286	**286**	**286**	**286**	**286**	293	286
29	*Xtxp* 321	H	200	210	210	200	210	200	210	200	210	200
30	*Xtxp* 210	H	185	184	201	205	203	205	203	205	201	205
31	*Xtxp* 354	H	162	158	163	154	**155**	**155**	157	156	167	157
32	*Xtxp* 17	I	**186**	**186**	**184**	**184**	**186**	**186**	**186**	**186**	**186**	**186**
33	*Xtxp* 265	I	201	220	203	194	200	195	201	209	220	190
34	*Xtxp* 57	I	245	251	249	241	**241**	**241**	241	251	249	241
35	*Xtxp* 145	I	**237**	**237**	214	240	212	235	212	237	213	235
36	*Xtxp* 317	I	**160**	**160**	161	142	**154**	**154**	153	160	157	153
37	*Xtxp* 6	I	115	79	111	79	94	79	94	115	100	79
38	*Xtxp* 274	I	323	335	329	320	326	320	326	335	329	320

-'= not amlified, Monomorphic parent pairs highlited in bold font

Table3.14 Fluorescent labeled sorghum SSR primers (Applied Biosystems) used for background selection in marker-assisted breeding for shoot fly resistance

SSR locus	Linkage Group	Label F- or R-	Forward (F) primer sequence	Tm	Reverse (R) primer sequence	Tm	SSR motif
Xtxp 25	B	F-Fam	CCATTGAGCTTCTGCTATCTC	57.9	CATTTGTCACCACTAGAACCC	57.9	(CT)12
Xtxp 298	B	F-Fam	GCATGTGTCAGATGATCTGGTGA	60.6	GCTGTTAGCTTCTTCTAATCGTCGGT	63.2	(AGA)23
Xtxp 296	B	F-Hex	CAGAAATAACATATAATGATGGGGTGAA	59.3	ATGCTGTTATGATTTAGAGCCTGTAGAGTT	62.7	(CA)18
Xcup 63	B	F-Ned	GTAAAGGGCAAGGCAACAAG	-	GCCCTACAAAATCTGCAAGC	57.3	(GGATGC)4
Xtxp 283	B	F-Fam	CGCCCGAACTCTTCTTAAATCT	58.4	ATTATGCCCTAACTGCCTTTGA	56.5	(TTC)12
Xcup 11	C	F-Ned	TACCGCCATGTCATCATCAG	-	CGTATCGCAAGCTGTGTTTG	57.3	(GCTA)4
Xtxp 228	C	F-Hex	ACAGGTTGGCGATGTTTCTCT	57.9	TTCTTTTTCGAATTCATTCCTTTT	52.5	(TC)12
Xtxp 31	C	F-Ned	TGCGAGGCTGCCCTACTAG	61	TGGACGTACCTATTGGTGC	56.7	(CT)25
Xcup 32	C	F-Ned	ACTACCACCAGGCACCACTC	-	GTACTTTTTCCCTGCCCTCC	59.4	(AAAAT)4
Xcup 61	C	F-Hex	TTAGCATGTCCACCACAACC	-	AAAGCAACTCGTCTGATCCC	57.3	(CAG)7
Xtxp 114	C	F-Fam	CGTCTTCTACCGCGTCCT	58.2	CATAATCCCACTCAACAATCC	55.9	(AGG)8
Xgap 236	C	F-Fam	GCCAAGAGAAACACAAACAA	-	AGCAATGTATTTAGGCAACACA	-	(AG)20
Xcup 14	C	F-Hex	TACATCACAGCAGGGACAGG	-	CTGGAAAGCCGAGCAGTATG	59.4	(AG)10
Xtxp 59	C	F-Fam	GAAATCCACGATAGGGTAAGG	57.9	GACCCAGAATAGAAGAGAGG	57.3	(GGA)5
Xgap 10	D	F-Ned	GTGCCGCTTTGCTCGCA	-	TGCTATGTTGTTTGCTTGTCCCTTCTC	-	(AG)27
Xtxp 21	D	F-Fam	GAGCTGCCATAGATTTGGTCG	59.8	ACCTCGTCCCACCTTTGTTG	59.4	(AG)18
Xcup 28	D	F-Ned	GGTGTGAGACTGTGAGCAGC	-	TATAGCACGGTTGTTGTGCC	57.3	(TGAG)5
Xtxp 27	D	F-Ned	AACCTTGCCCTATCCACCTC	-	TATGATGAATCAAGGGAGAGG	-	(AG)37
Gpsb 050	D	F-Ned	GGCTTCTTTCCTCTCC	-	GAGTTCTTTTATGTTTTGTGT	-	(CT)10(CA)10
Xtxp 343	D	F-Ned	CGATTGGACATAAGTGTTC	-	TATAAACATCAGCAGAGGTG	-	(AGT)21
Xgap 206	F	F-Ned	ATTCATCATCCTCATCCTCGTAGAA	-	AAAAACCAACCCGACCCACTC	-	(AC)13/(AG)20
Xtxp 289	F	F-Fam	AAGTGGGGTGAAGAGATA	51.4	CTGCCTTTCCGACTC	50.6	(CTT)16+(AGG)6
Xcup 02	F	F-Ned	GACGCAGCTTTGCTCCTATC	-	GTCCAACCAACCCACGTATC	59.4	(GCA)6
Xtxp 258	F	F-Hex	CACCAAGTGTCGCGAACTGAA	-	GCTTAGTGTGAGCGCTGACCAG	-	(AAC)19
Xtxp 230	F	F-Fam	GCTACCGCTGCTGCTCT	57.6	AGGGGGCATCCAAGAAAT	53	(GA)28
Xtxp 321	H	F-Fam	TAACCCAAGCCTGAGCATAAGA	-	CCCATTCACACATGAGACGAG	-	(GT)4+(AT)6+(CT)21
Xtxp 47	H	F-Ned	CAATGGCTTGCACATGTCCTA	57.9	GGTGCGAGCTAGTTAAGTGGG	61.8	(GT)8(GC)5+(GT)6
Xtxp 273	H	F-Fam	GTACCCATTTAAATTGTTTGCAGTAG	-	CAGAGGAGGAGGAAGAGAAGG	-	(TTG)20
Xtxp 105	H	F-Fam	TGGTATGGGACTGGACGG	-	TGTTGACGAAGCAACTCCAAT	-	(TG)5+(CT)6(GTCT)
Xtxp 210	H	F-Fam	CGCTTTTCTGAAAATATTAAGGAC	55.9	GATGAGCGATGGAGGAGAG	56.8	(CT)10
Xtxp 354	H	F-Ned	TGGGCAGGGTATCTAACTGA	57.3	GCCTTTTTCTGAGCCTTGA	54.5	(GA)21+(AAG)3
Xtxp 57	I	F-Hex	GGAACTTTTGACGGGTAGTGC	59.8	CGATCGTGATGTCCCAATC	56.7	(GT)21
Xtxp 145	I	F-Fam	GTTCCTCCTGCCATTACT	53.7	CTTCCGCACATCCAC	-	(AG)22
Xtxp 317	I	F-Hex	CCTCCTTTTCCTCCTCCTCCC	63.7	TCAGAATCCTAGCCACCGTTG	59.8	(CCT)5(CAT)11
Xtxp 265	I	F-Fam	GTCTACAGGCGTGCAAATAAAA	56.5	TTACCATGCTACCCCTAAAAGTGG	61	(GAA)19
Xtxp 274	I	F-Fam	GAAATTACAATGCTACCCCTAAAAGT	-	ACTCTACTCCTTCCGTCCACAT	-	(TTC)19
Xtxp 6	I	F-Fam	ATCGGATCCGTCAGATC	52.8	TCTAGGGAGGTTGCCAC	52.8	(CT)33
Xtxp 17	I	F-Hex	CGGACCAACGACGATTATC	-	ACTCGTCTCACTGCAATACTG	-	(TC)16+(AG)12

3.3.15 Screening of BC_1F_1 selected foreground plants for background selection

A total of 61 BC_1F_1 plants selected through foreground screening from 5 backcross populations were genotyped with 38 SSR marker loci covering the entire genome except the region harboring targeted QTLs (i.e. regions covered in foreground screening). Approximately 2 SSR marker loci were selected to cover the top, middle and bottom portion of the six non-target linkage groups. The main objective of background genotyping was to ascertain recovery of the recurrent parent genome.

Out of 61 BC_1F_1 plants, 12 plants were selected carrying recurrent parental alleles at most of the SSR loci used for background screening along with a few heterozygous loci. Those individuals homozygous for donor parent allele "B" type were rejected as they could only be derived from selfing of their F_1 female parent. The backcross selected 12 individuals advanced for planting the BC_2F_1 generation. A detailed list of background genotyping i.e. number of plants genotyped, number of markers used, plants selected with each targeted QTL, and advanced to the BC_2F_1 generation is presented in Table 3.15.

3.3.16 Genotyping of BC_2F_1 population with SSR marker for fore ground selection

3.3.16.1 Planting of BC_2F_1 population

Numbers of plants to be sown from each of five BC_2F_1 populations to recover a given number of plants possessing the target QTLs were estimated following Sedcole (1977). The sowing of five BC_2F_1 populations and four-donor parents (Table 3.16) was done in two sets. Staggered sowing was employed to ensure nicking of flowering period. The first set was sown in the first week of April 2005 and the second was sown in the 2nd week of April 2005. Sowing of the recurrent parent done in four sets (Table 3.17), with the first set sown in the last week of March, the second set sown in the first week of April, the third set was sown in the second week of April, and the fourth set was sown in the third week of April 2005. One seed was sown in each pot, with pots filled with a mixture of sand, vertisol, and FYM (1:1:1, V: V: V).

Table 3.15. Details of background screening of BC_1F_1 selected plants and advanced to BC_2F_1 generation

Name of cross	No.of BC_1F_1 plants genotyped	No. of markers used	Plants selected	Targeted QTL linkage groups	Backcross plants advanced to BC_2F_1 generation
BC_1F_1 [28B (288) × RIL 189(312)] × 28B (288)	11	38	SP 719	A+J	[BC_1F_1 (SP 719)] × SP 606
			SP 814	A	[BC_1F_1 (SP 814)] × SP 607
			SP 818	E+G+J	[BC_1F_1 (SP 818)] × SP 608
BC_1F_1 [KR 192 (304) × IS 18551 (267)] × KR 192 (304)	14	38	SP 629	A+E	[BC_1F_1 (SP 629)] × SP 621
			SP 830	A+G	[BC_1F_1 (SP 830)] × SP 823
BC_1F_1 [20B (186) × RIL 252 (318)] × 20B (186)	11	38	SP 757	E	[BC_1F_1 (SP 757)] × SP 740
BC_1F_1 [20B (179) × RIL 153 (248)] × 20B (179)	16	38	SP 669	A+G	[BC_1F_1 (SP 669)] × SP 661
			SP 773	A+G+J	[BC_1F_1 (SP 773)] × SP 759
			SP 871	A	[BC_1F_1 (SP 871)] × SP 865
			SP 874	E+J	[BC_1F_1 (SP 874)] × SP 860
BC_1F_1 [KR 192 (300) × RIL 252 (319)] × KR 192 (300)	9	38	SP 889	A+J	[BC_1F_1 (SP 889)] × SP 877
			SP 895	G	[BC_1F_1 (SP 895)] × SP 878

Table 3.16 Details of number of seed planted crosswise and set wise in BC_2F_1 foreground selection

Name of the cross	No. of seed sown		Total no. of plants
	Set I	Set II	
BC2F1 28B (288) × RIL 189 (312) × 28B (288)			
(BC1F1 (719)) × SP 606	07	07	14
(BC1F1 (814)) × SP 607	07	07	14
(BC1F1 (818)) × SP 608	14	14	28
BC2F1 KR 192 (304) × IS 18551 (267) × KR 192 (304)			
(BC1F1 (629)) × SP 621	07	07	14
(BC1F1 (830)) × SP 823	14	14	28
BC2F1 20B (186) × RIL 252 (318) × 20B (186)			
(BC1F1 (757)) × SP 740	07	07	14
BC2F1 20B (179) × RIL 153 (248) × 20B (179)			
(BC1F1 (669)) × SP 661	07	07	14
(BC1F1 (773)) × SP 759	14	14	28
(BC1F1 (871)) × SP 865	07	07	14
(BC1F1 (874)) × SP 860	07	07	14
BC2F1 KR 192 (300) × RIL 252 (319) × KR 192 (300)			
(BC1F1 (889)) × SP 877	14	14	28
(BC1F1 (895)) × SP 878	07	07	14
Donor parents			
IS 18551 (267) SP 828	02	02	04
RIL 252 (318) SP 847	02	02	04
RIL 252 (319) SP 885	02	02	04
RIL 153 (248) SP 866	02	02	04
RIL 189 (312) SP 809	02	02	04

The sowing of three recurrent parents (Table 3.17) was done in four sets to ensure the sufficient pollen availability during the peak crossing period.

Table 3.17 Details of set-wise sowings of recurrent parents

Name/plant no. of recurrent parent	No. of seeds sown				Total
	Set I	Set II	Set III	Set IV	
28B (288)					
SP 606	15	15	-	-	30
SP 607	-	-	15	-	15
SP 608	-	-	-	15	15
KR 192 (304)					
SP 823	12	12	-	-	24
SP 621	-	-	12	12	24
20B (186)					
SP 740	04	04	04	04	16
20B (179)					
SP 661	-	20	-	-	20
SP 759	-	-	20	-	20
SP 865	-	-	-	20	20
SP 860	20	-	-	-	20
KR 192 (300)					
SP 877	-	-	12	12	24
SP 878	12	12	-	-	24

3.3.17 Genotyping of the BC_2F_1 populations with SSR markers for foreground selection

DNA of individual plants of each BC_2F_1 population was extracted from one week old leaf tissue. In case of recurrent parents leaf tissue from one representative plant from each set was used for DNA extraction, and DNA of four plants was pooled in one well. The same procedure was applied for donor parent plant DNA extraction. Genotyping of 224 BC_2F_1 plants was accomplished with 11 SSR marker loci linked to targeted shoot fly resistance QTLs in four linkage groups (A, E, G and J). Around 100 heterozygous BC_2F_1 plants having appropriate allelic constitution were selected before

flowering and crossed with their respective recurrent parents (plant to plant crosses) to produce BC_3F_1 seeds. Details of numbers of plants genotyped, markers used and numbers of heterozygous plants selected for each of the targeted QTLs are presented in Table 3.18.

For recurrent parent plant populations, self-seed of selected genotyped plants used in backcrossing the F_1 plants and selected for their purity were sown for backcrossing to develop the BC_2F_1 populations. The details of recurrent parent seed planted and plants used in backcrossing to develop the BC_2F_1 generation are listed in Table 3.19.

Table 3.18. Detail of foreground BC_2F_1 populations numbers of plants genotyped, and list of selected heterozygous plants

Name of cross	No. of plants geno-typed	Details of plants genotyped	SSR markers used	No. of heterozygous plants selected	Details of selected plants
BC_2F_1 [28B (288) × RIL189 (312) × 28B (288)] × 28B (288)	56	SP 1001 – 1007	*Xtxp75*, *Xtxp37*,	3	SP 1001, SP 1004, SP 1005
		SP 1008 – 1014	*Xisp10362*, *Xtxp312*,	4	SP 1009, SP 1010, SP 1012, SP 1013
		SP 1015 – 1028	*Xtxp40*, *Xisp10263*,	4	SP 1017, SP 1019, SP 1026, SP 1027
		SP 1201 – 1207	*Xgap01*, *Xtxp* 141,	2	SP 1201, SP 1207
		SP 1208 – 1214	*Xisp10258*, *Xtxp15*	1	SP 1212
		SP 1215 – 1228		8	SP 1215, SP 1216, SP 1218, SP 1219, SP 1221, SP 1224, SP 1226, SP 1227
BC_2F_1 [KR 192 (304) × IS 18551 (267) × KR 192 (304)]× KR 192 (304)	42	SP 1029-1035	*Xtxp75*, *Xtxp37*,	4	SP 1031, SP 1033, SP 1034, SP 1035
		SP 1036-1049	*Xisp10362*, *Xtxp312*,	3	SP 1039, SP 1040, SP 1048
		SP 1229-1235	*Xtxp40*, *XISP10263*,	3	SP 1229, SP 1231, SP 1232,
		SP 1236-1249	*Xgap01*, *Xtxp* 141, *Xisp10258*, *Xtxp15*	4	SP 1239, SP 1244, SP 1245,SP 1246
BC_2F_1 [20B(186) × RIL 252 (318) × 20B (186)] × 20B (186)	14	SP 1050- 1056	*Xtxp75*, *Xtxp37*,	3	SP 1052, SP 1053, SP 1055
		SP 1250-1256	*Xisp10362*, *Xtxp312*, *Xtxp40*, *Xisp10263*, *Xgap01*, *Xtxp* 141, *Xisp10258*, *Xtxp15*	1	SP 1253
BC_2F_1 [20B (179) × RIL 153 (248) × 20B (179)] × 20B (179)	70	SP 1057-1063	*Xtxp75*, *Xtxp37*,	5	SP 1057, SP 1060, SP 1061, SP 1062, SP 1063
		SP 1064-1077	*Xisp10362*, *Xtxp312*, *Xtxp40*, *Xisp10263*, *Xgap01*, *Xtxp* 141,	12	SP 1065, SP 1066, SP 1067, SP 1069, SP 1070, SP 1071, SP 1072, SP 1073, SP 1074, SP 1075, SP 1076, SP 1077
		SP 1078-1084	*Xisp10258*, *Xtxp15*	5	SP 1078, SP 1079, SP 1080, SP 1081, SP 1083,
		SP 1085- 1091		4	SP 1086, SP 1087, SP 1090, SP 1091
		SP 1257-1263		4	SP 1258, SP 1260, SP 1261, SP 1262
		SP 1264-1277		11	SP 1264, SP 1265, SP 1266, SP 1267, SP 1269, SP 1271, SP 1273, SP 1274, SP 1275, SP 1276, SP 1277
		SP 1278-1284		2	SP 1278, SP 1284
		SP 1285-1291		2	SP 1285, SP 1291
BC_2F_1 [KR 192(300) × RIL 252(319) × KR 192(300)] × KR 192 (300)	42	SP 1092-1105	*Xtxp75*, *Xtxp37*,	7	SP 1092, SP 1094, SP 1097, SP 1098, SP 1101,
		SP 1106- 1112	*Xisp10362*, *Xtxp312*,		SP 1103, SP 1105
		SP 1292- 1305	*Xtxp40*, *XISP10263*,	2	SP 1106, SP 1109
		SP 1306-1312	*Xgap01*, *Xtxp* 141,	6	SP 1296, SP 1297, SP 1298, SP 1301, SP 1302,
			Xisp10258, *Xtxp15*	0	SP 1303

Table 3.19 Detail of seed planted of each recurrent parent and plant used in backcrossing to advance BC_2F_1 generation

Name of recurrent parent	No. of seeds sown in 4 sets	Plant nos. and source	No. of plants used as pollinators	Detail of plants used as pollinators
28B (288)	60			
	SP 606	SP 901-915	7	SP 902, SP 903, SP 905, SP 906, SP 907 SP 914, SP 915
	SP 606	SP 1113-1127	5	SP 1115, SP 1120, SP 1122, SP 1124, SP 1125
	SP 607	SP 1313-1327	-	-
	SP 608	SP 1401-1415	2	SP 1401, SP 1403
KR 192 (304)	48			
	SP 823	SP 916-927	4	SP 917, SP 923, SP 925, SP 926
	SP 823	SP 1128-1139	2	SP 1128, SP 1132
	SP 621	SP 1328-1339	5	SP 1330, SP 1333, SP 1336, SP 1337, SP 1338
	SP 621	SP 1416-1427	1	SP 1418
20B (186)	16			
	SP 740	SP 928-931	2	SP 929, SP 933
	SP 740	SP 1140-1143	-	-
	SP 740	SP 1340-1343	-	-
	SP 740	SP 1428-1431	1	SP 1433
20B (179)	80			SP 932, SP 934, SP 935, SP 937, SP 938, SP 939, SP 940,
	SP 860	SP 932-951	16	SP 942, SP 943, SP 945, SP 946, SP 947, SP 948, SP 949, SP 950, SP 951
	SP 661	SP 1144-1163	9	SP 1145, SP 1147, SP 1148, SP 1149, SP 1149, SP 1152, S P 1154, SP 1155, SP 1160
	SP 759	SP 1344-1363	4	SP 1342, SP 1349, SP 1356, SP 1361
	SP 865	SP 1432-1451	3	SP 1432, SP 1434, SP 1444
KR 192 (300)	48			
	SP 878	SP 952-963	3	SP 955, SP 957, SP 962
	SP 878	SP 1164-1175	3	SP 1166, SP 1167, SP 1175
	SP 877	SP 1364-1375	3	SP 1365, SP 1369, SP 1374
	SP 877	SP 1452-1463	-	-

3.3.18 Genotyping BC_2F_1 selected foreground plant for back ground selection

68 BC_2F_1 selected on the basis foreground genotyping from five backcrossing populations will be genotyped with 38 SSR marker loci (Table 3.20) covering the entire genome except the region harboring targeted QTLs. This background screening will be restricted to the loci that were heterozygous in the previous generation. Also genotyping will be done for loci that were not amplified in BC_1F_1 background screening.

Table 3.20 Detail list of selected BC_2F_1 plants for background screening

Backcrossing population	Number of plants selected	Details of plants selected	Targeted QTL
BC_2F_1 [28B × (288) × RIL 189 (312)] × 28B (288) × 28B (288)	18	1009	A
		1010	A
		1012	A
		1013	A
		1017	E
		1019	E+G
		1026	J
		1027	G
		1207	A+J
		1212	A
		1215	G
		1216	E
		1218	E+G
		1219	E
		1221	G
		1224	G
		1226	E
		1227	E+G
BC_2F_1 [KR 192 (304) × IS 18551 (267)] × KR 192 (304)] × KR 192 (304)	12	1031	A+G
		1033	A+G
		1034	G
		1039	A

	1048	G
	1229	A+G
	1231	G
	1232	A+G
	1239	A
	1244	A+G
	1245	A+G
	1246	A+G
BC_2F_1 [20B (186) × RIL 252 (318)] × 04 20B(186) × 20B (186)	1052	E
	1053	E
	1053	E
	1253	E
BC_2F_1 [20B (179) × RIL 153 (248)] × 20B 24 (186) × 20B (186)	1057	A+G+J
	1061	G
	1062	G
	1063	A+G
	1065	A+G
	1067	A+J
	1071	A+J
	1075	J
	1076	A+G+J
	1077	J
	1079	A
	1081	A
	1083	A
	1086	E
	1090	E
	1091	E
	1258	A+J
	1260	A+G
	1264	G
	1265	A+G+J
	1267	J

	1271	A+J
	1277	A+J
	1285	E
BC_2F_1 [KR 192 (300) × RIL 252 (319)] × KR 192 (300) × KR 192 (300)	1092	A
	1094	J
	1101	A
	1103	J
	1105	A
	1106	G
	1109	G
	1296	A
	1298	A+J
	1301	J

RESULTS

CHAPTER IV

RESULTS

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal crop of semi-arid regions of Asia, Africa, the Americas and Australia. Generally the lower yields in Asia and Africa are associated with pest damage. Shoot fly is one of the major pests of cultivated sorghum in Asia and Africa. Adaptation of chemical control method is not economically feasible for most of the resource poor sorghum-growing farmers of Asia and Africa. Therefore utilization of host-plant resistance is the most realistic approach to reduce losses caused by sorghum insect pest. The quantitative nature of resistance to this insect, and large environmental variation in its expression hinders genetic manipulation of shoot fly resistance by conventional plant breeding procedures. Therefore efforts are being made to this end in current Ph. D. research project on ' genetic diversity analysis, QTL mapping and marker assisted selection for shoot fly resistance in sorghum.' The results obtained from these studies are presented below objectivewise.

4.1 Application of SSR markers in diversity analysis of sorghum insect resistant germplasm accessions

Assessment of genetic diversity based on SSR marker genotypes was conducted in sorghum germplasm accessions resistant to sorghum shoot fly, spotted stem borer, and sorghum midge following separation of PCR products by polyacrylamide gel electrophoresis (PAGE) and capillary electrophoresis (ABI). Out of 21 SSR primer pairs used for diversity analysis, that for *Xcup*14 was not used for PAGE and that for *Xcup*32 was not used on the ABI because of their poor amplification. Data obtained from 20 SSR loci for each separation method (19 common loci across the two methods) were used to estimate the genetic diversity of the 91 sorghum genotypes studied.

4.1.1 Polyacrylamide gel electrophoresis (PAGE)

Twenty primer pairs were used for separation of PCR products using DNA samples from 91 sorghum genotypes. The allelic composition of each genotype was determined and scored individually from the amplified products separated on 6% denaturing polyacrylamide gels. Only 11 of 20 SSR primer pairs revealed high levels of polymorphism on silver-stained PAGE gels. A total of 69 alleles were detected by silver staining using these 20 SSR primers. On an average 3.45 fragments were

amplified per SSR locus for the 91 sorghum genotypes studied. Gel image of the most polymorphic SSR markers are presented in (Plate. 4.1).

The polymorphism information content (PIC) value of these 20 SSR markers were calculated from the 91 sorghum genotypes evaluated. These 20 SSR markers revealed high levels of polymorphism: 11 of 20 (i.e., 55%) of the SSR primer pairs used detected high levels of polymorphism with PIC values $\geq$0.5. The PIC value range observed was 0.13 to 0.83. The highest level of polymorphism was found with primer pair Sb6-84 (0.83), which detects locus *Xgap*84, followed by those for *Xtxp*15 (0.82), and *Xtxp*320 (0.77). The lowest polymorphism was found with the primer pair for *Xcup*62 (0.13) (Table 4.1).

4.1.2 Capillary electrophoresis (ABI)

The genotypes studied using separation of PCR products PAGE were also assessed for their polymorphism using automated capillary electrophoresis (ABI 3100 or ABI 3700 DNA sequencing machines). A total of 118 alleles generated by 20 SSR primers were detected using the automated capillary electrophoresis system. On average, 5.1 fragments were amplified per SSR locus. The PIC value of each of the 20 SSR markers was calculated from the ABI-generated data set for the 91 sorghum genotypes. In this data set 13/20 (i.e., 65%) of primer pairs detected high levels of polymorphism with PIC values >0.5. The PIC values observed ranged from 0.21 to 0.81. The highest level of polymorphism was found with the primer pairs for *Xtxp*320 (0.81) and *Xtxp*15 (0.81) followed by primer pair Sb6-84 (0.79), and the lowest polymorphism (Table 4.1) was found with the primer pair for *Xcup*60 (0.21). Thus the most polymorphic group of sorghum SSR markers did not show substantial changes across the two PCR product separation and visualization systems (silver-stained gels from PAGE and electronically captured fluorescence measurements during capillary electrophoresis).

4.1.3 PAGE dendrogram

The dendogram for genetic similarity between genotypes based on the PAGE-generated data set showed clustering for geographical origins, sorghum races, raw germplasm vs. elite/improved breeding lines (including stay-green recurrent parents), and specific traits such as insect resistance. The accessions studied were broadly grouped into clusters representing four of the five sorghum races [*durra*, *caudatum* (elite lines derived from zera-zera landraces), *bicolor*, and *guinea*] according to their molecular diversity. The correlation coefficient between the cophenetic matrix

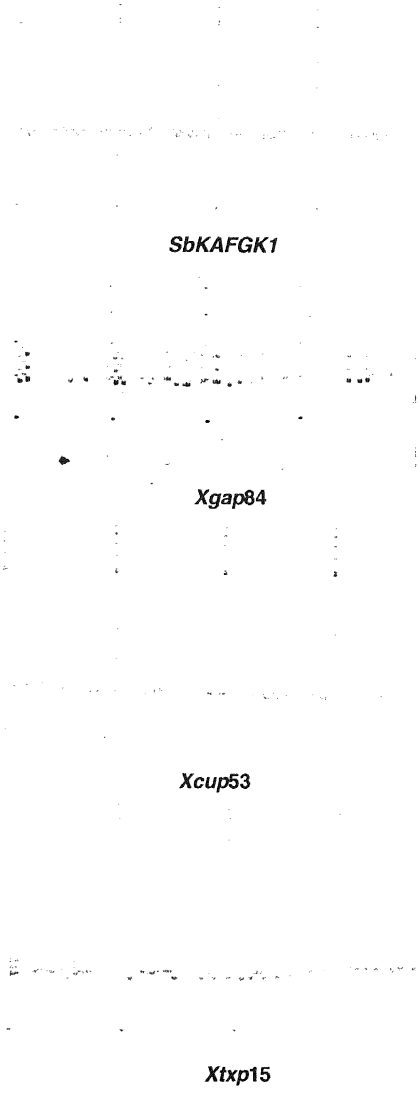


Plate 4.1: Silver-stained gels showing some of the more polymorphic SSR markers used in diversity study.

Table 4.1: Multiplex primer sets used for amplification of SSRs: allele size, No of alleles and Polymorphic information content (%) obtained in diversity study.

			PAGE			ABI		
SSR locus	Expected allele size (bp)	Expected Number of alleles	Observed allele size (bp)	Observed number of alleles	PIC*)	Observed allele size (bp)	Observed number of alleles	PIC
Xcup62	200	2	320/295/190	3	0.13	186/189	2	0.49
Xcup32	170	3	175/170/160	3	0.54	---	---	---
Xcup28	180	4	175/170/160	3	0.53	164/159/151	3	0.56
Xtxp40	160	2	148/145/140	3	0.33	136/134/131/128	4	0.35
Xcup61	210	2	230/205/200	3	0.52	195/198	2	0.44
Xcup02	200/350	5	200/195/190	3	0.64	200/187/156/182/179/176	6	0.71
Xtxp15	240	3	225/222/220/218/210/180	6	0.82	225/126/224/222/220/218/216/214/212/210/208/198	12	0.81
SbKAFGK1	260	3	280/275/270/260/255	5	0.73	295/292/266/257/254	5	0.72
Xcup60	180	2	160/155/150	3	0.60	162/158/153/150	4	0.21
Xcup06	210	2	210/200	2	0.41	201/205	2	0.43
Xtxp114	250	2	215/210	2	0.49	239/233/230/227	4	0.57
Xcup53	200	4	215/212/210/205/200	5	0.63	199/196/195/194/190/186	6	0.61
Xcup63	160	2	160/155/150	3	0.24	144/138/132	3	0.25
Xcup69	240	3	240/230	2	0.49	250/241/239/237	4	0.53

Table 4.1 Cont.---

*Xcup*37	240	3	220/215/180	3	0.14	210/208/205/204/201	5	0.35
*Xcup*11	185	3	185/180	2	0.48	122/121/168/163/162	5	0.53
*Xtxp*320 (PhyB)	300	4	320/295/290/285/280	5	0.77	292/288/286/283/280/278/277/274/271/269/268/266/265/262/255	15	0.81
*Xgap*84	200	5	205/200/195/193/190/188	6	0.83	220/218/216/214/212/210/209/206/204/200/198/196/194/192/190/188/186/184/182/180/178	21	0.79
Xcup 7	200/280	5	290/280/270/190	4	0.55	271/268/267/259/256/253/193/190	8	0.55
*Xcup*52	260	3	280/270/260	3	0.49	250/249/242	3	0.62
*Xcup*14	200	6	---	---	---	210/208/206/204	4	0.65
Total number of alleles				69			118	

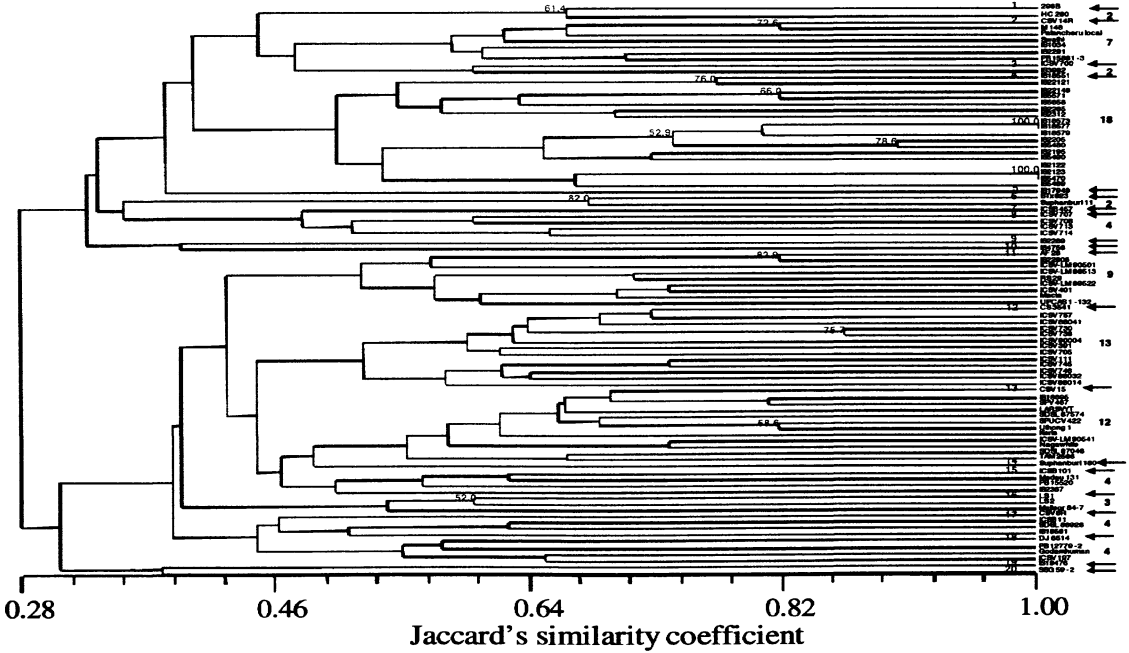
computed from the dendrogram and the original similarity matrix was r = 0.74 (t = 16.7, P=1). The results suggested good fit of the tree generated from the rough data. The coefficient of similarity values ranged from 0.28 to 1.00 (Fig. 4.1).

Ninetyone sorghum genotypes could be separated into two super clusters at the 30% level of similarity. The first super cluster consisted largely of *durra* landrace genotypes resistant to sorghum shoot fly and/or spotted stem borer. The second super cluster consisted largely of genotypes having resistance to sorghum midge and elite zera-zera derivatives being used as recurrent parents in a marker-assisted backcross programme for the stay-green trait. These 91 sorghum genotypes diverged into 20 clusters at approximately the 50% level of similarity. Among these 20 clusters, the largest was cluster 4 (18 genotypes); followed by cluster 12 (13 genotypes); cluster 13 (12 genotypes); and, cluster 11(9 genotypes). However, some of the clusters (e.g., clusters 5, 7, 9, 10, 14, 19 and 20) accommodated only single genotype (IS 17948, ICSB 457, IS 2269, IS 4756, Suphanburi 160, IS 19476, and SSG 59-2, respectively). Clusters 1, 3, and 6 accommodated only two genotypes each.

Cluster 1 had two genotypes 296B and HC 260. This cluster was well supported with an operational bootstrap value of 61.4%. Elite hybrid maintainer line 296B is susceptible insect pests but is a potent combiner for high grain yield. It has been used extensively in hybrid development programs in India. This elite line is used as a susceptible parent in shoot fly resistance QTL mapping studies reported else where in this thesis.

Cluster 4 included 18 genotypes, most of which exhibits resistance to sorghum shoot fly and/or spotted stem borer. All sorghum genotypes in the cluster originated from the *durra* race. This group contains genotype IS 18551, which has been used as the resistant parent in development of two ICRISAT sorghum mapping populations targeting shoot fly resistance. Most of the genotypes found in this cluster possess late maturity and medium grain yield potential but have relatively high degree if insect resistance. IS 22121, IS 2265, IS 2312, IS 2195, and IS 2123 have been identified as sources of resistance against both sorghum shoot fly and spotted stem borer. Most of the genotype pairs in this cluster exhibited operational bootstrap values greater than 50%, which provides confidence about their clustering. In particular, genotype pairs involving the group of IS 2122, IS 2123, and IS 5470, and the group of IS 18573, and IS 18577 actually exhibited 100% operational bootstrap values, indicating that they are perhaps identical as they could not be distinguished based on silver-stained PAGE

Figure 4.1. Dendrogram generated from data for 91 sorghum accessions using SSR genotype data revealed by silver-stained PAGE of PCR products from 20 polymorphic loci distributed across 9 of 10 sorghum linkage groups.



gel banding patterns for PCR products of 20 SSR primer pairs previously demonstrated to detect polymorphism in cultivated sorghum

Cluster 6 includes two genotypes, BTx623 and Suphanburi 11. The operational bootstrap value 82% for these two genotypes strongly supports this cluster. Both genotypes are susceptible to sorghum shoot fly and BTx623 was used as susceptible parent of the first ICRISAT sorghum RIL population developed for QTL mapping for shoot fly resistance.

Cluster 8 consists of four genotypes representing an intermediate population developed from crosses of *durra* and *caudatum* materials. These are all elite breeding lines and known for their combination shoot fly resistance with better agronomic performance. The genotypes from this group could be used as a source for the development of a mapping population for grain yield and shoot fly resistance, but it appears that a single representative of this group would be suffice, at least initially.

Cluster 11 includes nine genotypes: a sub-cluster of two germplasm lines exhibiting high levels of midge resistance and a loose sub-cluster of the remaining seven agronomically elite genotypes (i.e., diverse recurrent parents in ICRISAT's backcross programme for marker-assisted introgression of stay-green QTLs). Either AF 28 or IS 22806 could be used as the resistant parent of a new mapping population if we choose to map midge resistance, but since these two entries cluster closely (operational boot strap value of 82.9%) based on their molecular marker genotypes, it is likely that the bases of their midge resistance(s) are similar. The remainder of the genotypes in this cluster are elite breeding lines and released varieties from several different countries, and all are largely be based on crosses of zera-zera derivatives.

The specially of cluster 12 is that except for converted zera-zera line CS 3541 (CSV 4), all of its constituent genotypes were bred at ICRISAT-Patancheru in a program to combine insect resistance (to sorghum shoot fly or sorghum midge) with superior agronomic performance and excellent grain quality. These ICRISAT genotypes were developed from crosses involving converted zera-zera landraces.

Twelve genotypes are found in cluster 13 is another elite group of materials, consisting largely of recurrent parents for the stay-green backcrossing programme. Most of these genotypes are agronomically elite caudatum-type breeding lines or improved cultivars adapted to tropical sorghum production zones of Latin America, Africa or Asia. Some of them also exhibits resistance to sorghum midge.

LS 1, LS 2 and Malisor 84-7 form a separate cluster (cluster 16) of improved genotypes, with a moderate operational bootstrap value of 52%. The first two genotypes originated from the People's Republic of China and the third was developed from guinea -caudatum materials in ICRISAT's sorghum breeding programme in Mali. All these lines are potential recurrent parents for the stay-green marker-assisted backcrossing programme, but any one can be used as they are similar to each other.

Cluster 18 contains four genotypes having various degrees of eliteness, midge resistance, and shoot fly resistance. Single genotype clusters 19 and 20 appear to represent the grassy *bicolor* race of sorghum.

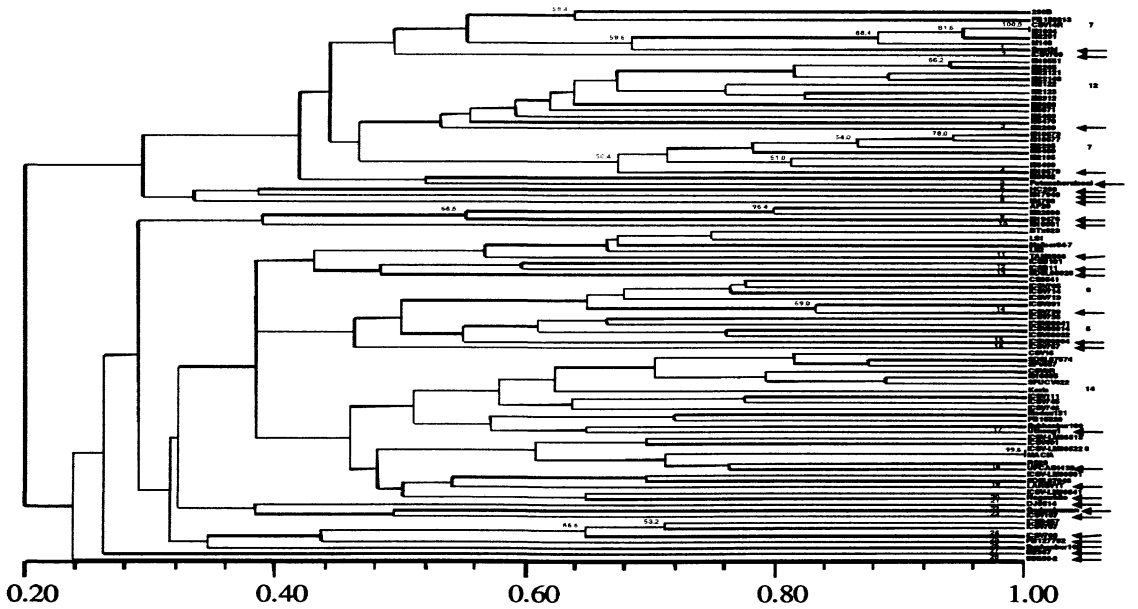
4.1.4 ABI dendrogram

The UPGMA method was used for generating a dendrogram as we did for the PAGE-generated marker data set. The results suggested a good fit for generation of the tree.For the SSR data set generated using the ABI sequencing machines, the coefficients of similarity ranged from 0.21 to 1.00 .

Marker alleles detected in the ABI-generated data set grouped 91 sorghum genotypes into 28 clusters at the 50% level of similarity. When compared to the dendrogram from the PAGE-generated data set, the number of clusters detected with the ABI-generated data was comparatively higher (Fig. 4.2). This may be due to the greater sensitivity of the automated sequencer, which allows it to detect SSR alleles differing by smaller numbers of repeat units so that it can effectively detect higher levels of polymorphism.

According to the operational bootstrap values obtained, only a few clusters were supported at the 50% level. Two pairs of test entries (CSV 14R and IS 1034, and, Macia and ICSV-LM 89522) appeared to be almost identical in their genetic make-up based on the ABI-generated marker data sets be, as they had 100% levels of similarity. These two tight clusters were each supported by strong operational bootstrap values (100%). It is possible that one of the genotypes in such a pair might have been developed as an improved line from the other genotype, or the two of them shared genetic material at loci that were assessed in this study due to one or more common ancestors. However, if it is not the case, it will be necessary to reconfirm the lack of marker polymorphism between such pairs of lines, starting with extraction of DNA from seedlings from selfed true-to-type plants of each line in order to

Figure 4.2. Dendrogram generated from data for 91 sorghum accessions using SSR genotype data revealed by capillary electrophoresis of PCR products from 20 polymorphic loci distributed across 9 of 10 sorghum linkage groups.



completely eliminate the possibility of a mix-up in samples having identical marker genotype data sets.

The largest cluster according to the ABI-generated SSR marker data set was cluster 17, which consisted of 14 genotypes. Many of the genotypes in this cluster show midge fly resistance and/or are agronomically elite lines selected as potential recurrent parents for the ICRISAT marker-assisted backcrossing programme for the stay-green component of terminal drought tolerance. All these lines are agronomically elite caudatum-type breeding lines and released varieties.

Another 12 genotypes were grouped together to form cluster 3. Genotypes in this cluster originated from the durra race and possess moderate levels of resistance to sorghum shoot fly and/or spotted stem borer. Seven additional durra genotypes were grouped in adjacent cluster 4. These genotypes also have sorghum shoot fly and/or spotted stem borer resistance. Actually these two clusters based on the ABI-generated data set (3 and 4) formed in a single cluster in the PAGE-generated data set (4). The use of single representative from ABI-generated cluster 3 and another from ABI-generated cluster 4, as parents in mapping populations targeting shoot fly and/or spotted stem borer resistance, would seem to be a reasonable starting point. As the resistant parent (IS 18551) of both currently available shoot fly resistant mapping populations falls in ABI-generated cluster 3, any future shoot fly resistance mapping population should have its resistant parent from ABI-generated cluster 4. Several genotype pairs (i.e., IS 18551 and IS 2265 in cluster 3, and all possible combinations involving IS 18573, IS 18577, IS 2205, IS 2195, and IS 5490 in cluster 4) exhibited operational bootstrap values greater than 50%, indicating good fit of the genotypes in these clusters.

Cluster 14 contained six improved genotypes. Most of these have sorghum shoot fly resistance and some of them have sorghum midge resistance, all in agronomically superior zera-zera-derived genetic backgrounds similar to that of insect pest susceptible CS 3541. Cluster 15, which included five genotypes could be designated as a cluster of agronomically superior midge resistant breeding lines. Nearly all genotypes from clusters 14 and 15 were developed at ICRISAT-Patancheru from crosses designed to introgressed insect resistance into elite zera-zera backgrounds having superior agronomic characteristics and excellent grain quality. A single selected genotype from these two clusters could be used for development of a mapping population targeting for grain yield, grain quality, and insect resistance.

Compared to the clustering patterns obtained from the PAGE-generated data set, many genotypes formed single-genotype clusters at the 50% level of similarity when the ABI-generated marker data set was used. These genotypes included ICSV 700, HC 260, IS 17948, IS 4756, IS 18581, SDSL 88928, ICSV 757, DJ 6514, Godahuman, ICSV 197, PB 12779-2, Suphanbur 11, IS 2367, and SSG 59-2. Among these single-genotype clusters, many of them originated from different countries; e.g., Suphanbur came from Thailand, Godamhuman originated from Sudan, and IS 18581 and IS 26367 are Nigerian breeding lines.

By and large, most of the clusters that appeared from the PAGE-generated SSR marker data set were separated further and their positions relative to other clusters changed moderately in the dendrogram based upon the ABI-generated SSR marker data set. This is expected as the ABI should give a more accurate picture than PAGE because of its superior ability to detect small polymorphisms between the genotypes. For example except for a very few large clusters of related breeding products or insect resistance germplasm accession, all clusters detected based on the PAGE-generated marker data sets were separated into distinct sub-groups by the ABI-generated marker data set. If we look at around the 40% level of similarity, both the PAGE- and ABI-generated data sets detect 12 clusters. But positions of the genotypes within these clusters were slightly modified by the superior sensitivity of the PCR product separation on the ABI machines. At the same time if we look the clustering pattern at around the 70% level of similarity, both of the systems classified the accessions into a larger number of clusters, which indicates that the 91 studied genotypes were well diverged in their genetic make-up. Finally, according to both the ABI- and PAGE-generated data sets there are very few genotypes forming single-genotype clusters (e.g., genotype SSG 59-2) at even the 30% level of similarity, revealing their distant relatedness to the remainder of the cultivated sorghums sampled in this study, and their distinctness was well supported by very low operational bootstrap values (0.6%).

All of the genotypes that were well distinguished from each other at the 50% level of similarity had divergent geographical origins. Though materials of dissimilar geographic origins have sometimes fallen in different clusters, most materials of diverse origins intermingled with each other within clusters irrespective of their origin. For example breeding lines IS 18573, IS 18577 and IS 18579 from Nigeria fall in cluster 4 (for both PAGE- and ABI-generated dendograms). But the other

genotypes in this group were predominantly of Indian origin. Another breeding line from Nigeria, IS 18581, was loosely associated with several unrelated genotypes in cluster 15 of the PAGE dendrogram, but was found in single-genotype cluster 10 of the ABI dendrogram. Modest shifts of this type, where poor ability of PAGE to discriminate among similar alleles was overcome by the superior sensitivity of the ABI sequencers, were commonly found in our dendrograms.

Many reasons may be suggested for unexpected associations of genotypes thought to be of distinctly different geographic origin. Most of the genotypes are cultivated. One well-known possible reason for similarities is due to widespread exchange of genotypes across regions; especially of materials having superior performance for traits (e.g., yield potential and grain quality) that may be of common interest across regions. In addition, lines are introduced from other countries for specific purposes like development of male-sterile lines, or as sources of insect and disease resistance. Though they are thought to have come from one state, their origins could be quite different so that though they are now derived from distinct geographic regions, originally they appear to have been derived from a common gene pool and/or to share common origins in the distant past.

4.2 Phenotyping RILs from cross 296B × IS 18551 for components of resistance to sorghum shoot fly

An experimental study was carried out to characterize the recombinant inbred lines (RILs) developed from cross 296B (susceptible) × IS 18551 (resistant) in order to improve understanding of the genetic makeup of shoot fly resistant components in sorghum. The experiments were conducted under two environments viz. late *kharif* 2002 (E_1) and *rabi* 2004/2005 (E_2) at Patancheru. The observations were recorded on different components of resistance to shoot fly and other traits. The entry mean performance of shoot fly resistance and other traits for individual RIL and its parents, evaluated under late *kharif* and *rabi* seasons, are presented in Appendix Iv and V respectively. The results combined from phenotypic and combined phenotypic and genotypic data are presented under suitable headings.

4.2.1 Estimates of phenotypic and genotypic variation

4.2.1.1 Mean performance of parents

The mean performance of the two parents revealed significant phenotypic differences (Table 4.2) for the shoot fly resistance and other agronomic traits, except for time to 50% flowering (days) in E_1; and overall recovery score, aphid damage score, and

Table 4.2 Means and standard errors for two parents of cross 296B (susceptible) × IS 18551 (resistant) for different components of resistance to shoot fly and other traits in two screening environments at Patancheru during 2002 – 2004

Character	E1 (*Kharif*, Patancheru)		SE (±)	E2 (*Rabi*, Patancheru)		SE (±)
	P1 (296B)	P2 (IS 18551)		P1 (296B)	P2 (IS 18551)	
Glossiness intensity (scale)	5.0**	1.0**	0.26	5.0**	1.1**	0.35
Seedling vigor I (scale)	-	-	-	4.3**	3.0**	0.42
Pigmentation (scale)	-	-	-	2.9**	1.1**	0.38
Oviposition I (%)	68.7**	45.0**	5.71	55.2**	15.5**	7.40
Oviposition II (%)	95.5**	79.0**	3.42	81.2**	33.1**	6.61
Deadhearts I (%)	75.7**	51.5**	3.73	60.0**	17.0**	7.59
Deadhearts II (%)	96.0**	68.5**	2.45	77.4**	25.8**	6.60
Seedling height I (cm)	-	-	-	7.9**	11.6**	0.85
Trichome density (upper surface) (no./microscopic field)	0.0**	152.2**	6.70	0.0**	189.7**	7.97
Trichome density (lower surface) (no./microscopic field)	0.0**	73.0**	3.86	0.0**	83.1**	4.91
Time to 50% flowering (days)	89.4	87.8	2.0	82.0**	75.0**	1.84
Plant height (cm)	110.5**	244.0**	8.94	105.0**	161.5**	7.73
Overall recovery score (scale)	8.1**	2.9**	0.53	4.0	3.4	0.62
Aphid score (scale)	8.0**	5.0**	0.53	5.3	4.2	0.42
Midge score (scale)	-	-	-	6.3**	8.6**	0.46
Agronomic score (scale)	-	-	-	1.2**	3.8**	0.30
Grain yield (g/plot)	194**	1074**	100	393	539	110

* significant at 5% and ** significant at 1% level

Glossiness (1 - 5 scale): 1 = high intensity of glossiness, 5 = non-glossy,
Seedling vigor (1 - 5 scale): 1 = high vigor,5 = low vigor, Midge score: 1= less than 10% Midge damaged spikelet, 9= more than 81% midge damaged spikelet, Aphid density/injury% :1 =10% leaf injury and 9= more than 80% leaf injury, Pigmentation : 1=plumule or leaf sheath with dark pink pigment (non-tan type), 5= plumule or leaf sheath with green color (tan type), Agronomic score 1= Good productive potential and good adaptation, 5 = poor productive potential and poor adaptation - data not recorded

grain yield in E_2. The parental performance under different environments varied for all the traits, except for glossiness (E_1, E_2), recovery resistance (E_2), and aphid damage (E_2).

The resistant parent IS 18551 (P_2) showed maximum leaf glossiness (score 1.0 and 1.1 in E_1 and E_2, respectively), moderate seedling vigour 1 (3.0 in E_2), high trichome density [189.7 in E_2, and 152.2 inE_1) on upper surface of leaf blade; and 83.1 in E_2 and 73.0 in E_1 no./ microscopic field on lower surface of leaf blade, and high seedling height (11.6 cm in E_2). For time to 50% flowering, there were no significant differences among the parents in E_1, while in E_2, significant differences were observed between the parents. The resistant parent IS 18551 was tall [244.0 cm (E_1) and 161.5 (E_2) cm], and had better shoot fly resistance recovery score [2.9 in E_1 and 3.4 in E_2], and lower aphid damage score [4.2 and 5.0 in E_2 and E_1, respectively] than its susceptible counterpart 296B. The resistant parent also recorded high midge damage (8.6 in E_2) and showed poor agronomic desirability (3.8 in E_2). Oviposition incidence (%) and deadheart incidence (%) were significantly lower in P_2 than P_1, in both screening environments. However, the range of phenotypic values for these traits varied significantly in the two screening environments. The phenotypic values for oviposition for IS 18551 were lower in environment E_2 [15.5% and 33.1% plants with eggs at 14 and 21 DAE, respectively] than in E_1 [45.0% plants with eggs at 14 DAE, and 79.0% plants with eggs at 21 DAE]. Similarly the deadheart formation in IS 18551 was lower in environment E_2 (17% deadhearts at 21DAE, and 25.8% deadhearts at 28 DAE) than environment E_1 (51.5% and 68.5% deadhearts at 21 and 28 DAE, respectively). IS 18551 also showed high pigmentation (1.1), i.e. dark pink coloration at the seedling stage (non-tan type).

The susceptible parent, 296B (P_1) was non-glossy, with low trichome density on both leaf surfaces in both screening environments. However, 296B showed poor seedling vigor (4.3 in E_2), and least seedling height (7.9 in E_2). 296B had significantly higher oviposition (68.7% and 95.5% plants with eggs at 14 and 21 DAE, respectively) in environment E_1 than in E_2 (55.2% and 81.2% plants with eggs at 14 and 21 DAE, respectively). Similarly, the deadheart incidence in 296B was significantly higher in E_1 (75.7% and 96.1% deadhearts at 21 and 28 DAE, respectively) than in E_2 (60.0% and 77.4% deadhearts at 21 and 28 DAE, respectively). This parent also showed a high pigmentation score (2.9, tan type) in E_2. The two parental lines flowered almost at same time (89 days and 88 days for 296B

and IS 18551, respectively) in E_1, but 296B flowered late (82 days) than the parent IS 18551 (75 days) in E_2. Parent 296B was shorter (110 cm and 105 cm in E_1 and E_2, respectively) than IS 18551. 296B showed poor recovery (8.1 and 4.0 in E_1 and E_2, respectively) and high aphid damage (8.0 and 5.3 in E_1 and E_2, respectively) than IS 18551 in both screening environments.

4.2.1.2 Mean and ranges of RILs

The mean and ranges of RIL population for different shoot fly resistance components and agronomic traits (Table 4.3) varied between the environments, except for the glossiness (score 3.6 and 3.5 in E_1 and E_2, respectively). The mean value for trichome density on the upper surface of leaf blade was greater in E_2 (87.3 per microscopic field) than in E_1 (80.6 per microscopic field). Similarly, the mean values for trichome density on the lower leaf surface were higher in E_2 (43.7 per microscopic field) than in E_1 (32.0 per microscopic field). The RIL mean values were lower for oviposition incidence (35.0% and 57.8% plants with eggs at 14 and 21 DAE, respectively) and deadhearts incidence (35.0% and 50.6% plants with deadhearts at21 and 28 DAE, respectively) in E_2 than in E_1 (61.2% and 89.7% plants with eggs at 14 and 21 DAE; and 73.3% and 87.7% plants with deadhearts at21 and 28 DAE, respectively). The observed ranges of RIL means were larger for oviposition incidence and deadhearts incidence in E_2 than in E_1. For other agronomic traits, RIL means for time to 50% flowering and plant height (73 days and 166 cm, respectively) were lower in E_2 than in E_1 (83 days and 195 cm, respectively). RIL means for overall recovery score (4.2) and aphid damage score (4.9) in E_2 were lower than in E_1 (5.7 and 6.4, respectively). The mean performance of the RILs for grain yield was better in E_1 (519.1 g plot^{-1}) than in E_2 (479.0 g plot^{-1}). The mean performance of the RILs for midge damage was high (7.2) and agronomic score was fairly good (2.8) in E_2.

4.2.1.3 Analysis of variance

The analysis of variance for different shoot fly resistance components (Table 4.4) showed that variances due to genotypes (RILs) were significant for all the traits studied based on performances in individual screening environments, as well as averages across the two screening environments. For shoot fly resistance component traits such as glossiness score, oviposition incidence, deadhearts incidence, and trichome density on the lower surface of the leaf blade, genotypic variances were greater in environment E_2 than in environment E_1. However, for trichome density on the upper surface of the leaf blade, time to 50% flowering, plant height, overall

Table 4.3 Mean, standard errors and range of phenotypic values for RILs derived from the cross 296B (susceptible) × IS 18551 (resistant) for different components of resistance to shoot fly and other traits in two screening environments at Patancheru during 2002 - 2004

Character	E1 (*Kharif*, Patancheru)				E2 (*Rabi*, Patancheru)			
	RIL mean	SE(±)	Mid parent value	Range	RIL mean	SE(±)	Mid parent value	Range
Glossiness intensity (scale)	3.6	0.28	3.0	1.0-5.0	3.5	0.40	3.05	1.3-5.0
Seedling vigor I (scale)	-	-		-	3.3	0.43	3.65	1.0-5.0
Pigmentation (scale)	-	-		-	1.9	0.40	2.0	1.0-5.0
Oviposition I (%)	61.2	5.75	56.9	41.0-81.0	35.0	7.35	35.3	0.0-72.6
Oviposition II (%)	89.7	3.42	87.3	75.1-99.2	57.8	6.62	57.1	16.7-90.7
Deadhearts I (%)	73.3	3.69	63.6	53.0-85.0	35.1	7.58	38.5	5.6-76.0
Deadhearts II (%)	87.7	2.55	82.3	69.0-97.0	50.6	6.62	51.6	25.9-83.6
Seedling height I (cm)	-	-		-	9.6	0.83	9.8	4.0-13.7
Trichome density (upper surface) (no./microscopic field)	80.6	6.7	76.1	0.0-213.0	87.3	8.0	94.9	0.0-240.0
Trichome density (lower surface) (no./microscopic field)	32.0	3.80	36.5	0.0-92.7	43.7	4.95	41.6	0.0-137.7
Time to 50% flowering (days)	83.4	2.09	88.6	67.0-97.0	72.8	1.82	78.5	61.0-84.0
Overall recovery score (scale)	5.7	0.54	5.5	2.0-9.0	4.2	0.62	3.7	2.0-9.0
Aphid score (scale)	6.4	0.53	6.5	4.0-9.0	4.9	0.42	4.8	3.3-7.3
Midge score (scale)	-	-		-	7.2	0.47	7.4	4.0-9.0
Agronomic score (scale)	-	-		-	2.8	0.30	2.5	1.0-4.3
Plant height (cm)	194.6	9.81	177.3	118-284	165.9	7.78	133.2	100-218
Grain yield (g/plot)	519.1	99.5	634	87-1120	479.0	108.8	439.0	56-1018

Glossiness (1 - 5 scale): 1 = high intensity of glossiness, 5 = non-glossy,
Seedling vigor (1 - 5 scale): 1 = high vigor,5 = low vigor, Midge score: 1= less than 10% Midge damaged spikelet, 9= more than 81% midge damaged spikelet, Aphid density/injury% :1 =10% leaf injury and 9= more than 80% leaf injury, Pigmentation : 1=plumule or leaf sheath with dark pink pigment (non-tan type), 5= plumule or leaf sheath with green color (tan type), Agronomic score 1= Good productive potential and good adaptation, 5 = poor productive potential and poor adaptation - data not recorded

4.4 Genotypic varances, G X E interaction and respective standard errors for components of resistance to shoot fly and other traits in sorghum RIL mapping population derived from cross 296B X IS18551 under individual and across season screening environment

Character	E1 (late *kharif*)		E2 (*rabi*)		E1E2 (across seasons)			
	$\sigma 2g$	SE ±	$\sigma 2$ g	SE ±	$\sigma 2$ g	SE ±	$\sigma 2$ g x e	SE ±
Glossiness intensity	3.08**	0.27	3.21**	0.35	4.6**	0.21	1.7**	0.34
Seeedling vigor I	-	-	2.54**	0.42	-	-	-	-
Pigmentation	-	-	2.91**	0.37	-	-	-	-
Oviposition I (%)	166.2**	5.70	493.5**	7.40	363.2**	4.50	299.8**	7.20
Oviposition II (%)	75.15**	3.40	432.3**	6.60	255.8**	3.40	250.7**	5.20
Deadhearts I (%)	144.58**	3.70	856.2**	7.60	330.2**	3.80	274.1**	5.90
Deadhearts II (%)	99.37**	2.50	410.8**	6.60	266.8**	3.10	242.6**	4.80
Seedling height I (cm)	-	-	5.89**	0.82	-	-	-	-
Trichome density (upper surface) (no./microscopic field)	11309.1**	4.80	8685.9**	8.10	13317.7**	4.60	6571.2**	7.10
Trichome density (lower surface) (no./microscopic field)	2064.5**	2.90	2615.7**	4.97	3092.1**	2.70	1583.8**	4.10
Time to 50% flowering (days)	129.1**	2.10	68.3**	1.81	141.4**	1.50	56.9**	2.30
Overall recovery score(scale)	4.9**	0.54	3.99**	0.62	5.53**	0.40	3.41**	0.62
Aphid score	4.0**	0.53	1.9**	0.42	4.17**	0.35	1.7**	0.55
midge score	-	-	6.36**	0.65	-	-	-	-
Agronomic score	-	-	1.1**	0.30	-	-	-	-
Plant height (cm)	4362.2**	9.10	1263.4**	6.90	4927.5**	6.60	793.6**	10.40
Grain yield (g/plot)	160726.0**	99.00	7563.0**	190.0	152599**	73.70	83873**	115.30

- data not recorded

* significant at $p=0.05$

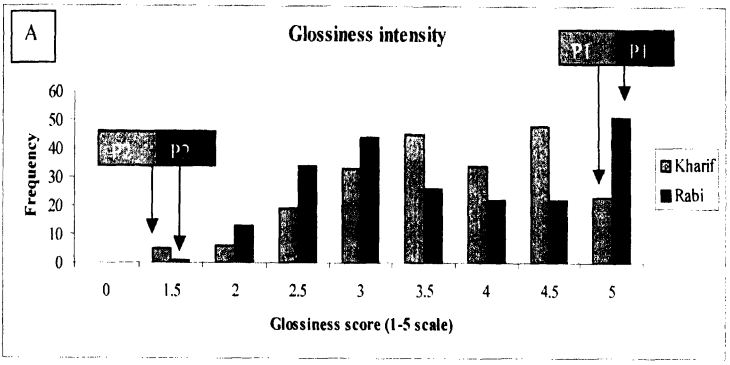
** significant at $p=0.01$

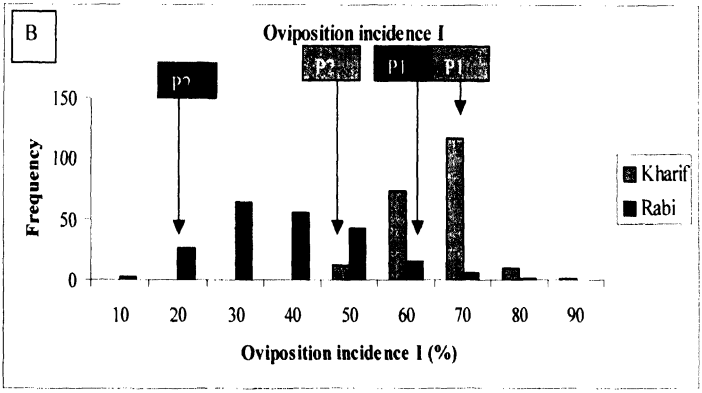
recovery score, aphid damage score, and grain yield, the genotypic variances were greater in environment E_1 than in environment E_2. The across-season analysis revealed that variances due to genotypes (G) and G × E interaction were significant for all the traits observed. The genetic variance values were more for resistance components such as glossiness, trichome density (both upper and lower surfaces of seedling leaf blades), time to 50% flowering, aphid damage score, and plant height more than double the G × E interaction variance. For other traits such as Oviposition, deadhearts, overall recovery resistance score, and grain yield, the genotypic variances were more than the variance due to G × E interaction.

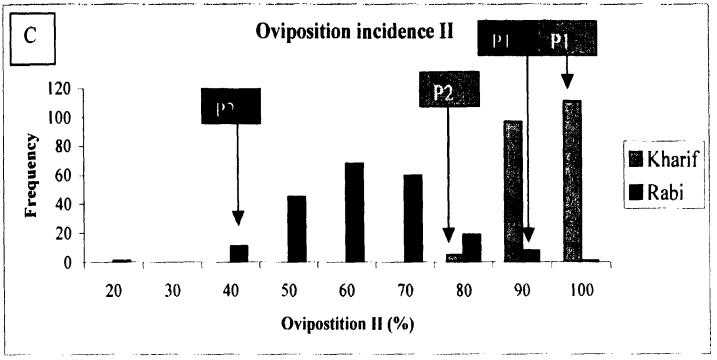
4.2.1.4 Frequency distribution

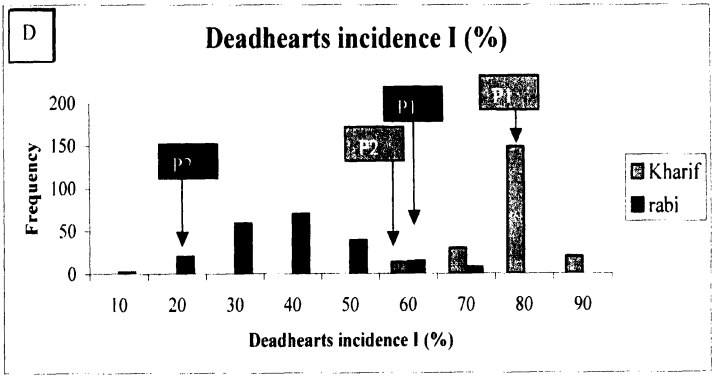
The variation for shoot fly resistance and other traits was represented graphically using frequency distributions of entry means in the two screening environments. The measurements were grouped into equally spaced classes on the X-axis, and the frequency of individuals falling in each class was plotted on the Y-axis. The resulting histograms showed normal curves (Fig 4.3 A-Q). In general, frequency distributions for most of the traits under study approximated a normal curve in the rabi screening environment. But in case of the 2002 kharif environment, the frequency distributions for a few resistance traits were skewed. The values for means and ranges of these characters varied, and the peaks were seen at different points for each of the traits in the two testing environments. In the 2004 rabi environment, the distribution curves were normal, except for glossiness, trichome density (both on upper and lower surfaces of seedling leaf blades), seedling vigor I, pigmentation score, and midge damage score. In case of the 2002 kharif environment, the frequency curves were normal, except leaf glossiness, oviposition II, deadhearts II, trichome density (both on upper and lower surfaces of seedling leaf blades), plant height, and aphid damage. For the trait leaf glossiness, although the character varied continuously, it showed a kind of bimodal distribution, which was evident in each of the screening environments. For trichome densities on the upper and lower surfaces of the leaf, the histograms drawn showed discontinuous distributions, i.e., bimodal distributions that were skewed towards trichomelessness in both the kharif and the rabi environments. For oviposition II and deadhearts II, the histograms showed discontinuous distribution, which were skewed towards shoot fly preference for egg laying and high deadheart incidence in the 2002 kharif screening environment. The histograms for plant height and aphid damage also showed discontinuous distribution, which were skewed

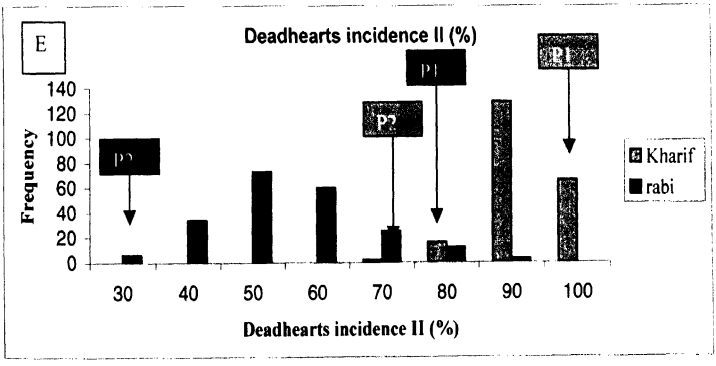
Figure 4.3 (A – Q). Frequency distribution of 213 RILs derived from cross 296B × IS 18551 for components of shoot fly resistance and few agronomic traits in two screening environments, viz., late *kharif* (E1) and *rabi* (E2) at Patancheru. On the X-axis groups of concerned trait are plotted and on the Y-axis frequencies of each group (i.e. the number of RILs in each group) are plotted.

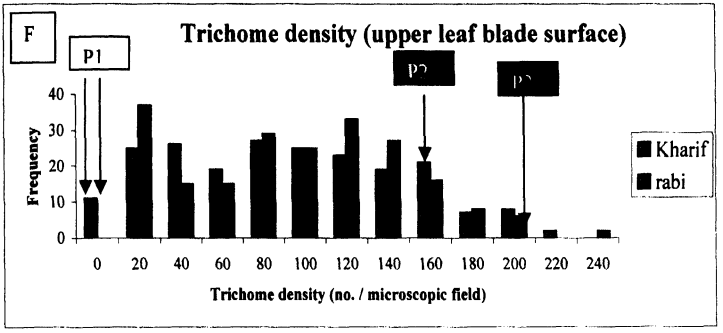


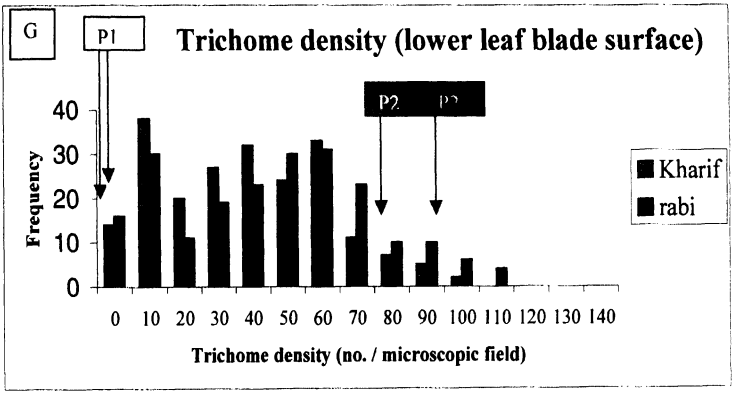


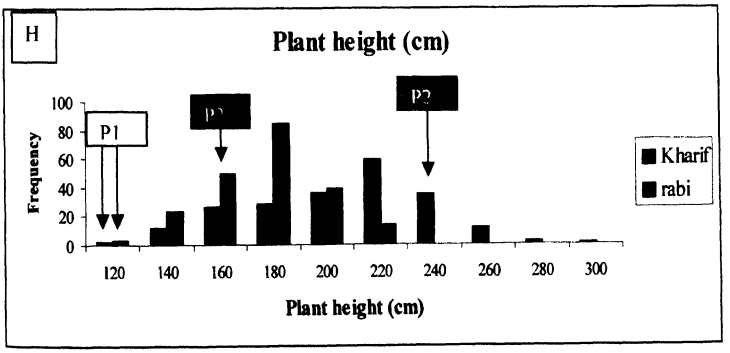

C
Oviposition incidence II
P1
P1
P2
P3
120
100
80
60
40
20
0
Frequency
Kharif
Rabi
20
30
40
50
60
70
80
90
100
Ovipostition II (%)

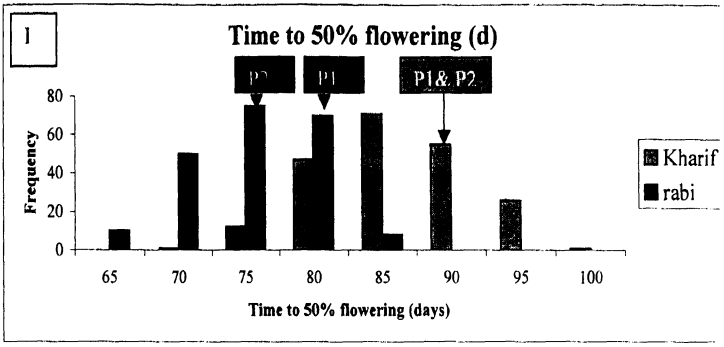

D
Deadhearts incidence I (%)
P1
P1
P2
P3
200
150
100
50
0
Frequency
Kharif
rabi
10
20
30
40
50
60
70
80
90
Deadhearts incidence I (%)

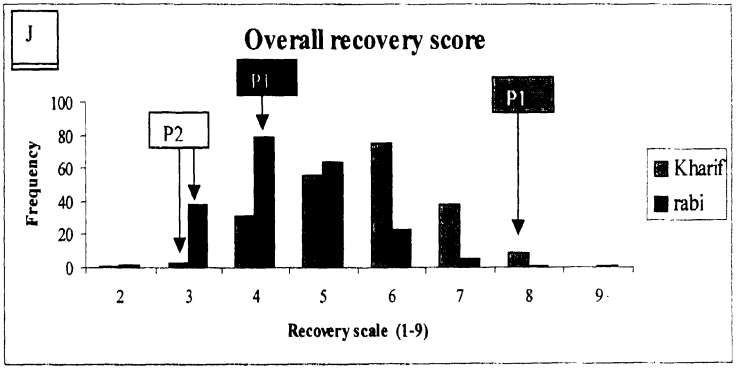

E
Deadhearts incidence II (%)
P1
P1
P2
P3
140
120
100
80
60
40
20
0
Frequency
Kharif
rabi
30
40
50
60
70
80
90
100
Deadhearts incidence II (%)

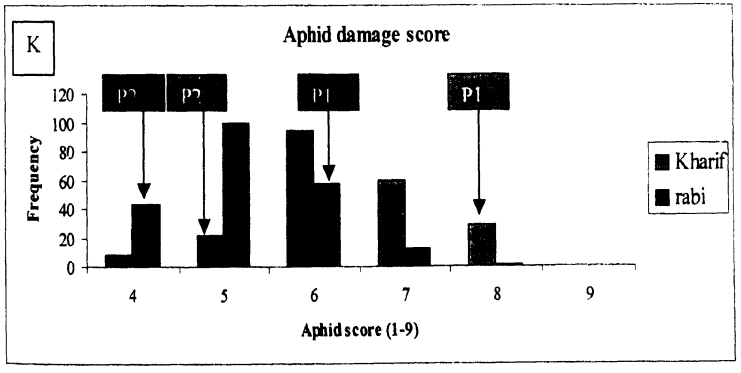

F
Trichome density (upper leaf blade surface)
P1
Frequency
40
30
20
10
0
0 20 40 60 80 100 120 140 160 180 200 220 240
Trichome density (no. / microscopic field)
Kharif
rabi

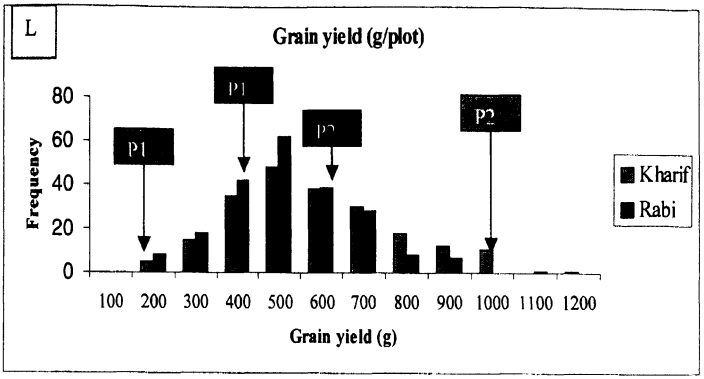

G
P1
Trichome density (lower leaf blade surface)
Frequency
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
Trichome density (no. / microscopic field)
Kharif
rabi

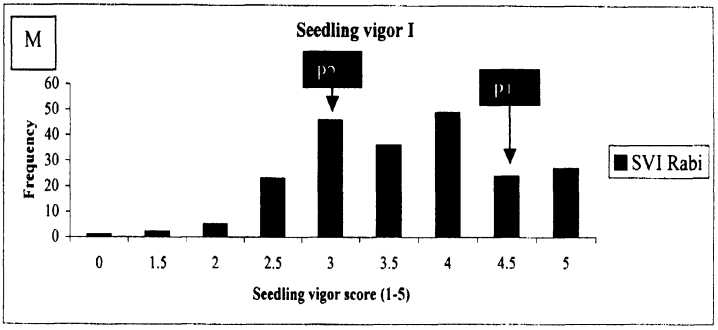

H
Plant height (cm)
P1
Frequency
100
80
60
40
20
0
120 140 160 180 200 220 240 260 280 300
Plant height (cm)
Kharif
rabi

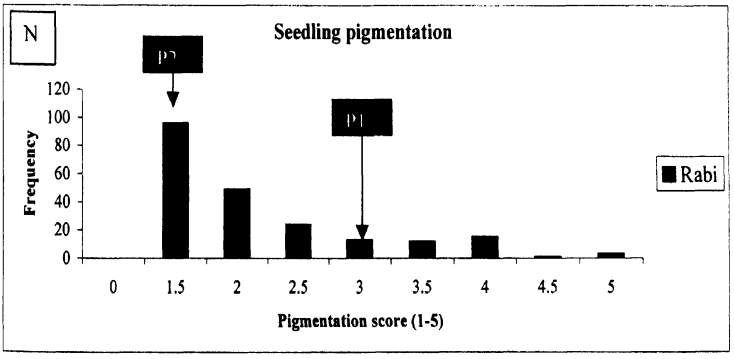

I
Time to 50% flowering (d)
P1& P2
Frequency
80
60
40
20
0
65
70
75
80
85
90
95
100
Time to 50% flowering (days)
Kharif
rabi

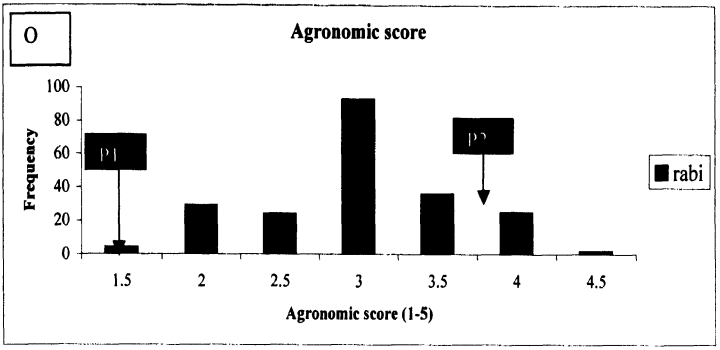

J
Overall recovery score
P1
P2
P1
Frequency
100
80
60
40
20
0
2
3
4
5
6
7
8
9
Recovery scale (1-9)
Kharif
rabi

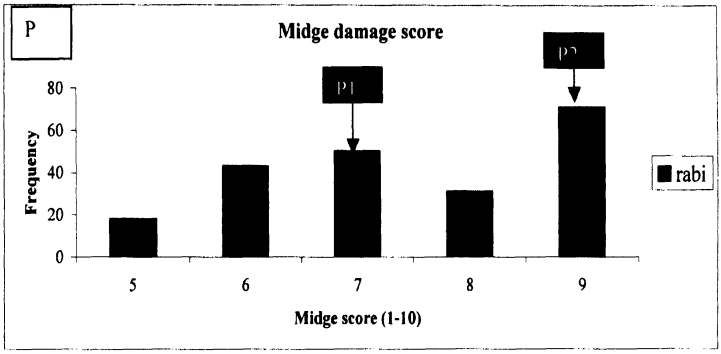

K
Aphid damage score
P1
P1
Frequency
120
100
80
60
40
20
0
4
5
6
7
8
9
Aphid score (1-9)
Kharif
rabi

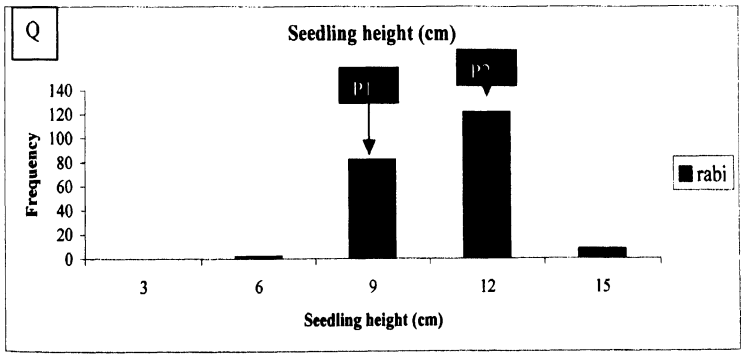

L
Grain yield (g/plot)
P1
P1
P2
P2
Frequency
80
60
40
20
0
100 200 300 400 500 600 700 800 900 1000 1100 1200
Grain yield (g)
Kharif
Rabi


M
Seedling vigor I
P2
P1
Frequency
60
50
40
30
20
10
0
0 1.5 2 2.5 3 3.5 4 4.5 5
Seedling vigor score (1-5)
SVI Rabi


N
Seedling pigmentation
P2
P1
Frequency
120
100
80
60
40
20
0
0 1.5 2 2.5 3 3.5 4 4.5 5
Pigmentation score (1-5)
Rabi


O
Agronomic score
100
80
60
40
20
0
Frequency
P1
P2
rabi
1.5
2
2.5
3
3.5
4
4.5
Agronomic score (1-5)


P
Midge damage score
80
60
40
20
0
Frequency
P1
P2
rabi
5
6
7
8
9
Midge score (1-10)


Q
Seedling height (cm)
140
120
100
80
60
40
20
0
Frequency
P1
P2
rabi
3
6
9
12
15
Seedling height (cm)

towards greater plant height and lower aphid damage score in the 2002 kharif screening environment. For seedling vigor I, pigmentation score, and midge damage, the histograms showed discontinuous distributions in the 2004 *rabi* screening environment. Seedling vigor I showed a bimodal distribution, while pigmentation was skewed towards dark-pink pigment (non-tan foliage), while midge damage score was skewed towards susceptibility.

4 .2.1.5 Transgressive segregation

The RILs lying outside the parental limits were identified based on trial entry means across the two screening environments. The RIL population mean and individual parent means were subjected to T-test to assess the significance of differences between the means (Table 4.5). The analysis revealed that RIL means differed significantly from both the parents for shoot fly resistance traits such as glossiness, oviposition incidence, deadhearts incidence, trichome density (both on upper and lower surfaces of seedling leaf blades), plant height, overall recovery score, aphid damage and grain yield. For oviposition at 21 DAE, the RIL mean did not differ significantly from that of resistant parent IS 18551. Transgressive segregants with phenotypic values outside the parental limits were observed for most of the traits, except for leaf glossiness (both parents), deadhearts II (296B), trichome density (both on upper and lower surfaces of seedling leaf blades) (296B), plant height (296B), and grain yield (IS 18551). For oviposition I and II, deadhearts I and II, overall recovery score, aphid damage score, and grain yield, the RIL population mean was less than the mid-parental value. In contrast to glossiness, the trichome density (both on upper and lower surfaces of seedling leaf blades) and plant height, RIL population mean value were greater than the mid-parental value. The proportion of RIL outside the parental limits were greater for those outside the low-scoring parents, and lower for outside high-scoring parents.

4.2.1.5.1 Glossiness

The deviation of RIL mean from mid-parental value was positive, but no transgressive segregant RIL were observed with phenotypic values outside the high- and low-scoring parent.

4.2.1.5.2 Oviposition incidence (%)

The RIL population mean deviated from mid-parental value towards that of the resistant parent. Favourable transgressive segregants were observed at 14 and 21 DAE. However, the proportion of RIL lying outside the mean of the low-scoring parent (IS 18551) was comparatively higher for oviposition II (at 21 DAE) than for oviposition I (14 DAE).

Table 4.5 Means of parents, the RIL population, their difference and proportion of RILs with values outside the parental limits based on pooled means over two screening environments

Character	P1 296B	P2 IS 18551	Midparenta value	RIL population mean	Test of significance of means		Proportions outside the parental limits	
					P1/RIL	P2/RIL	P1	P2
Glossiness intensity	5.0	1.1	3.0	3.6	**	**	0.000	0.000
Oviposition I (%)	66.3	40.9	53.6	49.9	**	**	0.015	0.092
Oviposition II (%)	93.0	72.6	82.8	76.1	**	ns	0.003	0.281
Deadhearts I (%)	72.9	46.8	59.9	56.9	**	**	0.015	0.054
Deadhearts II (%)	92.6	62.6	77.6	71.8	**	**	0.000	0.057
Trichome density (upper surface) (no./microscopic field)	0.0	157.4	78.7	83.6	**	**	0.000	0.042
Trichome density (lower surface) (no./microscopic field)	0.0	74.4	37.2	37.7	**	**	0.000	0.039
Plant height (cm)	109.5	232.3	170.9	182.3	**	**	0.000	0.015
Overall ecovery score (scale)	7.4	2.9	5.2	5.1	**	**	0.019	0.007
Aphid damage score (scale)	7.6	4.8	6.2	5.8	**	**	0.003	0.096
Grain yield (g/plot)	228.7	999.6	614.2	502.1	**	**	0.027	0.000

ns : nonsignificant * significant at $p = 0.05$ ** significant at $p = 0.01$

4.2.1.5.3 Deadhearts incidence (%)

The RIL population mean deviated from mid-parental values for this trait. Transgressive segregants were observed at both the observation stages. No transgressive segregant RIL was observed with phenotypic values outside the high-scoring susceptible parent 296B for deadhearts II. However, the proportion of RIL lying out-side the low-scoring resistant parent, IS 18551 was comparatively higher for deadhearts II (at 28 DAE) than for deadhearts I (at 21 DAE).

4.2.1.5.4 Trichome density

Mean trichome density in the RIL population was on par with the mid-parental value for trichome density on the lower surface of leaf blades; however, favourable transgressive segregants were observed for this trait, which were relatively high in frequency with values outside the high-scoring parent IS 18551 (RIL nos. 39, 50, 80, 110, 117, 129, 140, 208, 242, and 258). For the trichome density on the upper surface of leaf blades, the mean of the RIL population deviated positively from the mid-parental value and the proportion of favourable transgressive segregants was even higher than that observed for trichome density on the lower surface of leaf blades. Transgressive segregants with phenotypic values lying outside the high-scoring IS 18551 included RIL nos. 33, 42, 50, 80, 117, 129, 174, 208, 241, 242, and 258 for trichome density on the upper surface of the leaf.

4.2.1.5.5 Plant height

The two parents differed significantly for plant height. The mean value of the RIL was higher than the mid-parental value. Transgressive segregants were observed exhibiting greater plant height than taller parent IS 18551, although their proportion was low.

4.2.1.5.6 Overall recovery score

Mean recovery resistance score of RIL population was at par with the mid-parental value for this trait. A low proportion of transgressive segregants were observed, with a few RILs outside the low scoring parental values. The proportion of transgressive RILs was higher for individuals with values lying outside the high-scoring susceptible parent 296B.

4.2.1.5.7 Aphid damage score

The RIL population mean deviated from the mid-parental value for this trait. Transgressive segregants were observed outside limits of both the high and low-scoring parents; however, the proportion of RILs lying outside the low-scoring (more resistant) parent IS 18551 was comparatively higher.

4.2.1.5.8 Grain yield

The two parents of the RIL population differed significantly for grain yield under conditions of moderate to severe shoot fly pressure. The mean value of the RIL population was lower than the mid-parental value. A low proportion of transgressive segregants were observed for this trait. No favourable transgressive segregate were observed. All observed transgressive segregates for this trait had grain yield values lying outside the lower-scoring shoot fly susceptible parent 296B.

4.2.1.6 Inheritance of resistance

4.2.1.6.1 Broad-sense heritability

Estimates of broad-sense (operational) heritability (entry mean basis) for shoot fly resistance components and other traits at maturity were obtained from the data collected in individual environments *viz.* E_1 (kharif 2002) and E_2 (rabi 2004). Heritability estimates were also obtained based on average performance over these two environments (Table 4.6).

4.2.1.6.1.1 Glossiness

Heritability estimates were consistently high for leaf glossiness (h^2>0.85) in the two screening environments, but were moderate (h^2>0.64) across environments.

4.2.1.6.1.2 Oviposition incidence (%)

Operational heritability was low to moderate, but consistent for oviposition in the two individual screening environments, and also at different observation intervals. In kharif 2002, the operational heritability estimates for oviposition incidence (%) were low at both observational stages (h^2 = 0.20 at 14 DAE, and h^2 = 0.38 at 21 DAE), while in rabi 2004, operational heritabilities were higher (h^2 = 0.67 and h^2= 0.70 at 14 and 21 DAE, respectively). However, operational heritability across seasons for both stages was low (h^2= 0.17 and 0.02), indicating a significant influence of the screening environment, and/or genotype × environment interaction.

4.2.1.6.1.3 Deadhearts incidence (%)

Operational heritability estimates for deadhearts were low to moderate in the two screening environments. In kharif 2002, the heritability estimates were moderate, but consistent at both the observation stages (h^2 = 0.62 and 0.74 for deadhearts at 21 and 28 DAE, respectively); while in *rabi* 2004, the estimates were moderate (h^2 = 0.63 and 0.68 for deadhearts at 21 and 28 DAE, respectively). However, the operational heritability estimates across seasons were quite low (0.17and 0.09 for deadhearts at 21 and 28 DAE, respectively), indicating significant influence of the screening environment, and genotype × environment interaction.

Table 4.6 Operational heritability estimates (broad-sense; entry mean basis) for components of resistance to shoot fly and other traits in sorghum RIL population derived from cross 296B (susceptible) × IS 18551 (resistant) evaluated under two screening environments and across environments, at Patancheru

Character	E1 (*Kharif*, Patancheru)	E2 (*Rabi*, Patancheru)	E1E2 (across season)
Glossiness intensity (scale)	0.90	0.88	0.64
Seedling vigor I (scale)	-	0.79	-
Pigmentation (scale)	-	0.85	-
Oviposition I (%)	0.20	0.67	0.17
Oviposition II (%)	0.38	0.70	0.02
Deadhearts I (%)	0.62	0.63	0.17
Deadhearts II (%)	0.74	0.68	0.09
Seedling height I (cm)	-	0.63	-
Trichome density upper leaf surface (no./microscopic field	0.98	0.98	0.51
Trichome density lower leaf surface (no./microscopic field)	0.97	0.97	0.49
Time to 50% flowering (days)	0.87	0.85	0.60
Overall recovery score (scale)	0.76	0.71	0.38
Aphid damage score (scale)	0.71	0.72	0.59
Midge damage score (scale)	-	0.90	-
Agronomic score (scale)	-	0.75	-
Plant height (cm)	0.91	0.86	0.84
Grain yield (g/plot)	0.75	0.53	0.45

Glossiness (1 - 5 scale): 1 = high intensity of glossiness, 5 = non-glossy,

- Seedling vigor (1 - 5 scale): 1 = high vigor,5 = low vigor, Midge score: 1= less than 10% Midge damaged spikelet, 9= more than 81% midge damaged spikelet, Aphid density/injury% :1 =10% leaf injury and 9= more than 80% leaf injury, Pigmentation : 1=plumule or leaf sheath with dark pink pigment (non-tan type), 5= plumule or leaf sheath with green color (tan type), Agronomic score 1= Good productive potential and good adaptation, 5 = poor productive potential and poor adaptation -= data not recorded

4.2.1.6.1.4 Seedling vigor and leaf sheath pigmentation

The operational heritability estimates for these traits were high in the rabi 2004. (h^2 = 0.79 and 0.85 for seedling vigor, and pigmentation score, respectively.

4.2.1.6.1.5 Seedling height

Seedling height showed moderate operational heritability in 2004 *rabi* season (h^2 = 0.63)..

4.2.1.6.1.6 Trichome density

Trichome density showed high operational heritability estimates (h^2 >0.95) in both the environments for upper and lower leaf blade surfaces. However, the combined season analyses, lower estimates of operational heritability were seen for both upper (h^2 = 0.51) and lower (h^2 = 0.49) leaf surfaces, indicating the genotype × environment interaction.

4.2.1.6.1.7 Time to flowering

In both screening environments, time to flowering showed high and consistent operational heritability (h^2 = 0.87 and 0.85 in the 2002 *kharif* and 2004 *rabi*, respectively). Across seasons, this trait showed moderate heritability (h^2 = 0.60), probably as a result of differences in photoperiod sensitivity of the parental lines contributing to genotype × environment interaction.

4.2.1.6.1.8 Overall recovery score

Moderate operational heritabilities were recorded for overall recovery score in individual seasons (h^2= 0.76 and 0.71 for 2002 *kharif* and 2004 *rabi* seasons, respectively). However, low operational heritability estimates were obtained in across-season analysis (h^2 = 0.38).

4.2.1.6.1.9 Aphid damage score

Moderate operational heritabilities were recorded for aphid damage score in individual seasons (h^2 = 0.71 and 0.72 for *kharif* 2002 and *rabi* 2004 seasons, respectively), and a moderate operational heritability estimate was obtained in the across-season analysis (h^2 = 0.59).

4.2.1.6.1.10 Midge damage score and agronomic score

High and moderate operational heritabilities estimates were recorded in the 2004 *rabi* for midge damage score (h^2 = 0.90) and for agronomic deasirability (h^2 = 0.75), respectively.

4.2.1.6.1.11 Plant height

Plant height showed high operational heritability estimates (h^2> 0.80) in both screening environments, as well as in the combined analysis across environments (h^2 = 0.84).

4.2.1.6.1.12 Grain yield

Moderate heritability was observed for grain yield under conditions of moderate to severe shoot fly pressure in individual seasons (h^2 = 0.75 and 0.53 for kharif 2002 and rabi 2004, respectively), and a moderate to low operational heritability estimate was obtained in the across-season analysis (h^2=0.45).

4.3 QTL mapping

For QTL mapping and Q × E interaction analysis, the linkage map constructed using the population of 213 RILs derived from cross 296B × IS 18551 was used. The software package Plab QTL was used to analyze the data by composite interval mapping (CIM) procedures. The CIM method was implemented using a LOD value of 2.5 as the threshold for QTL significance. The genetic model chosen was additive × additive interaction for this $F_{3:8}$ recombinant population. The software calculates additive effects and estimates the portion of phenotypic variation explained by each individual QTL. The results from CIM analysis for identification of QTLs with significant effects for shoot fly resistance components are described below for two individual phenotypic environments and across these phenotypic environments. Among shoot fly resistance and grain yield or component traits studied, QTLs were identified for all traits in both the screening environments except in case of oviposition I and deadhearts I in the kharif 2002 environment.

4.3.1 QTL analysis in single environment

The phenotypic data from two phenotyping environments and genotypic data for 213 RILs were subjected to QTL analysis. The results of the two single-environment analyse are presented in Table 4.7 and Figure 4.4. The results of this QTL analysis for shoot fly resistance components are described below.

4.3.1.1 Glossiness (score)

Based on the CIM analysis of phenotypic data from the two Patancheru screening environments (kharif 2002 and rabi 2004), three QTLs were detected using data from the kharif environment and two QTLs using data from the rabi environment, accounting for 51.1% and 28.7% of the observed phenotypic variances in these environments, respectively. Of the QTLs detected, one mapped on LG 'J' in both

Table 4.7 Characteristics of QTLs associated with putative components of resistance to shoot fly (in two screening environments, *kharif* and *rabi*) based on Composite Interval Mapping (PLABQTL, LOD>2.5) using 213 RILs derived from cross 296B (susceptible) × IS 18551 (resistant)

Environment/trait	Linkage group	Position	Marker interval	Support interval (cM)	Peak LOD[1]	R^2%	Effect[4] (additive)
Glossiness intensity							
Kharif, Patancheru (E_1)	E	24	*Xtxp*40-*Xtxp*159	0-40	3.55	7.6	-0.264
	H	86	*XSbAGD*02-*Xtxp*294	84-94	3.14	6.6	-0.199
	J	28	*Xisp*215-*Xisp*258	22-54	17.99	36.9	-0.601
	Sum: 3 QTLs					51.1	
	Final simultaneous fit				LOD = 20.08	Adj. R^2 = 33.5%	
						Adj.genotypic var. ex.[3] = 48.5%	
Rabi, Patancheru (E2)	G	136	*Xcup*67 - *X cup*73	102-158	3.10	7.3	-0.332
	J	32	*Xisp*215 - *Xisp*258	20-62	9.35	21.4	-0.465
	Sum: 2 QTLs					28.7	
	Final simultaneous fit				LOD = 10.34	Adj. R^2 = 18.6%	
						Adj.genotypic var. ex. = 25.1%	
Seedling vigor I (scale)	A	72	*Xtxp*75 - *Xtxp*37	52-106	2.51	5.3	-0.203
Rabi , Patancheru (E2)	B	258	*Xtxp*01- *Xtxp*348	250-272	5.27	10.8	-0.300
	D	280	*Xisp*335 - *Xisp*343	264-300	3.86	8.1	0.280
	Sum: 3 QTLs					24.2	
	Final simultaneous fit				LOD = 7.53	Adj. R^2 = 12.6%	
						Adj.genotypic var. ex. = 22.4%	
Kharif, Patancheru (E1)	**Phenotypic observation not recorded**						
Oviposition I (%)							
Kharif, Patancheru (E1)	**QTLs not found**						
Rabi, Patancheru (E2)	F	2	*Xtxp*10 - *Xisp*318	0-14	3.30	7.2	2.448
	Sum: 1 QTL					7.2	
	Final simultaneous fit				LOD = 1.72	Adj. R^2 = 2.8%	
						Adj.genotypic var. ex. = 6.4%	

Table 4.7 cont.---

Environment/trait	Linkage group	Position	Marker interval	Support interval (cM)	Peak LOD	R^2%	Effect (additive)
Oviposition II (%)							
Kharif, Patancheru (E1)	E	22	*Xtxp*40 - *Xtxp*159	0-42	2.86	6.1	-0.558
	J	26	*Xisp*215 - *Xisp*258	8-64	3.25	8.0	-0.623
	Sum: 2 QTLs					14.1	
	Final simultaneous fit				LOD = 3.99	Adj. R^2 = 6.5%	
						Adj.genotypic var. ex. = 46.7%	
Rabi, Patancheru (E2)	F	10	*Xtxp*10- *Xisp*318	0-32	3.06	6.7	2.623
	G	104	*Xgap*01 - *Xcup*67	78-126	3.40	7.3	-3.751
	Sum: 2 QTLs					14	
	Final simultaneous fit				LOD = 5.06	Adj. R^2 = 8.7%	
						Adj.genotypic var. ex. = 19.0%	
Deadhearts I (%)							
Kharif, Patancheru (E1)	**QTLs not found**						
Rabi, Patancheru (E2)	F	2	*Xtxp*10 - *Xisp*318	0-14	3.90	8.4	2.475
	Sum: 1 QTL					8.4	
	Final simultaneous fit				LOD = 2.31	Adj. R2 = 4.5%	
						Adj.genotypic var. ex. = 10.0%	
Deadhearts II (%)							
Kharif, Patancheru (E1)	F	12	*Xtxp*10 - *Xisp*318	4-14	3.02	6.6	-1.437
	Sum: 1 QTL					6.6	
	Final simultaneous fit				LOD = 0.55	Adj. R^2 = 0.2%	
						Adj.genotypic var. ex. = 0.6%	
Rabi, Patancheru (E2)	F	24	*Xisp*318 - *Xtxp*230	12-38	3.14	7	2.518
	G	104	*Xgap*01 - *Xcup*67	88-122	5.29	11.2	-4.395
	Sum: 2 QTLs					18.2	
	Final simultaneous fit				LOD = 5.98	Adj. R^2 = 10.5%	
						Adj.genotypic var. ex. = 23.9%	

Table 4.7 cont.---

Environment/trait	Linkage group	Position	Marker interval	Support interval (cM)	Peak LOD[1]	R^2%	Effect[4] (additive)
Seedling height I (cm)							
Rabi, Patancheru (E2)	B	264	*Xtxp*348 - *Xtxp*207	254-272	3.63	7.6	0.258
	I	62	*Xtxp*17 - *Xisp*347	60-66	2.88	6.7	0.203
	Sum: 2 QTLs					14.3	
	Final simultaneous fit				LOD = 4.13	Adj. R^2 = 6.8%	
						Adj.genotypic var. ex. = 18.3%	
Kharif, Patancheru (E1)	**Phenotypic observation not recroded**						
Trichome density (upper leaf blade surface) (no./microscopic field)							
Kharif, Patancheru (E1)	G	112	*Xgap*01 - *Xcup*67	104-122	15.53	29	48.798
	Sum: 1 QTLs					29	
	Final simultaneous fit				LOD = 13.35	Adj. R^2 = 24.4%	
						Adj.genotypic var. ex. = 25.4%	
Rabi, Patancheru (E2)	G	124	*Xgap*01 - *Xcup*67	110-126	7.31	15	22.367
	Sum: 1 QTL					15	
	Final simultaneous fit				LOD = 8.39	Adj. R^2 = 15.8%	
						Adj.genotypic var. ex. = 16.8%	
Trichome density (lower leaf balde surface) (no./microscopic field)							
Kharif, Patanchru (E1)	G	118	*Xgap*01 - *Xcup*67	108-136	15	28.4	16.839
	Sum: 1 QTL					28.4	
	Final simultaneous fit				LOD = 15.2	Adj. R^2 = 27.3%	
						Adj.genotypic var. ex. = 29.1%	
Rabi, Patancheru (E2)	C	26	*Xtxp*69 - *Xtxp*34	22-32	2.92	6.2	-7.482
	G	112	*Xgap*01 - *Xcup*67	106-126	7.27	14.9	13.127
	Sum: 2 QTL					21.1	
	Final simultaneous fit				LOD = 9.67	Adj. R^2 = 17.3%	
						Adj.genotypic var. ex. = 18.8%	

Table 4.7 cont.---

Environment/trait	Linkage group	Position	Marker interval	Support interval (cM)	Peak LOD	R^2%	Effect (additive)
Plant height (cm)							
Kharif, Patancheru (E1)	I	2	*Xtxp*145 - *Xcup*36	0-4	7.24	16.7	13.592
	Sum: 1 QTL					16.7	
	Final simultaneous fit				LOD = 4.64	Adj. R^2 = 8.%	
						Adj.genotypic var. ex. = 11.9%	
Rabi, Patancheru (E2)	I	4	*Xcup*36 - *Xtxp*317	2-6	6.71	14.5	7.819
	Sum: 1 QTL					14.5	
	Final simultaneous fit				LOD = 5.32	Adj. R^2 = 10.0%	
						Adj.genotypic var. ex. = 15.2%	
Time to 50 % flowering (days)							
Kharif, Patancheru (E1)	A	68	*Xtxp*75 - *Xtxp*37	52-84	4.04	8.4	-1.915
	I	32	*Xisp*264 - *Xcup*12	22-40	3.62	7.5	1.28
	Sum: 2 QTLs					15.9	
	Final simultaneous fit				LOD = 6.70	Adj. R^2 = 11.8%	
						Adj.genotypic var. ex. = 19.1%	
Rabi, Patancheru (E2)							
	A	68	*Xtxp*75 - *Xtxp*37	56-82	4.90	10.2	-1.928
	Sum: 1 QTLs					10.2	
	Final simultaneous fit				LOD = 3.56	Adj. R^2 = 6.5%	
						Adj.genotypic var. ex. = 9.9%	
Overall recovery score							
Kharif Patancheru (E1)	B	136	*Xtxp*50 - *Xtxp*04	90-158	3.57	7.5	-0.306
	C	32	*Xtxp*69 - *Xtxp*34	24-42	2.50	5.3	0.238
	E	38	*Xtxp*40 - *Xtxp*159	26-54	6.18	12.8	-0.435
	G	110	*Xgap*01 - *Xcup*67	90-126	3.18	6.8	-0.311
	Sum: 4 QTLs					32.4	
	Final simultaneous fit				LOD = 10.44	Adj. R^2 = 17.1%	
						Adj.genotypic var. ex. = 38.0%	
Rabi Patancheru (E2)	**QTLS not found**						

Table 4.7 cont.---

Environment/trait	Linkage group	Position	Marker interval	Support interval (cM)	Peak LOD	R^2%	Effect (additive)
Aphid damage score							
Kharif, Patancheru (E1)	E	34	*Xtxp*40 - *Xtxp*159	22-46	6.94	14.3	-0.392
	J	150	*Xtxp*15 - *Xtxp*283	140-160	7.70	17.3	-0.349
	Sum: 2 QTLs					31.5	
	Final simultaneous fit				LOD = 12.73	Adj. R^2 = 22.7%	
						Adj.genotypic var. ex. = 59.7%	
Rabi, Patancheru (E2)	J	144	*Xtxp*15 - *Xtxp*283	132-156	4.47	10.4	-0.229
	Sum: 1 QTL					10.4	
	Final simultaneous fit				LOD = 3.26	Adj. R^2 = 6.0%	
						Adj.genotypic var. ex. = 13.2%	
Environment/trait	**Linkage group**	**Position**	**Marker interval**	**Support interval (cM)**	**Peak LOD**	**R^2%**	**Effect (additive)**
Pigmentation score							
Kharif, Patancheru (E1)	**Phenotypic observations not recorded**						
Rabi, Patancheru (E2)	A	52	*Xtxp*75 - *Xtxp*37	30-66	3.51	7.13	-0.225
	I	28	*Xisp*264 - *Xcup*12	20 -40	5.91	12.00	-0.309
	Sum: 2 QTLs					19.13	
	Final simultaneous fit				LOD = 9.43	Adj. R^2 = 16.9%	
						Adj.genotypic var. ex. = 25.6%	
Midge damage score							
Kharif, Patancheru (E1)	**Phenotypic observations not recorded**						
Rabi, Patancheru (E2)	C	100	*Xtxp*114 - *Xtxp*218	92-126	2.63	5.5	0.310
	I	2	*Xtxp*145 - *Xcup*36	0-4	6.69	15.5	0.575
	Sum: 2 QTLs					21	
	Final simultaneous fit				LOD = 5.86	Adj. R^2 = 10.2%	
						Adj.genotypic var. ex. = 13.4%	

Table 4.7 cont.---

Environment/trait	Linkage group	Position	Marker interval	Support interval (cM)	Peak LOD	R^2%	Effect (additive)
Agronomic score							
Kharif, Patancheru (E1)	**Phenotypic observations not recorded**						
Rabi, Patancheru (E2)	A	76	*Xtxp*75 - *Xtxp*37	58-94	2.57	5.5	0.135
	Sum: 1 QTL					5.5	
	Final simultaneous fit				LOD = 2.79	Adj. R^2 = 5.0%	
						Adj.genotypic var. ex. = 9.9%	
Grain yield (g/plot)							
Kharif, Patancheru (E1)	E	56	*Xtxp*40 - *Xtxp*159	34-62	3.40	7.4	39.6
	G	114	*Xgap*01 - *Xcup*67	100 -126	5.74	12.1	73.4
	I	66	*Xtxp*17 - *Xisp*347	60-66	2.81	6.7	41.1
	Sum: 3 QTL					26.2	
	Final simultaneous fit				LOD = 6.96	Adj. R^2 = 11.5%	
						Adj.genotypic var. ex. = 26.0%	
Rabi, Patancheru (E2)	C	98	*Xtxp*114 - *Xtxp*218	90-130	3.08	6.4	-21.792
	Sum: 1 QTL					6.4	
	Final simultaneous fit				LOD = 2.55	Adj. R^2 = 4.55%	
						Adj.genotypic var. ex. = 16.6%	

Glossiness (1 - 5 scale) : 1 = high intensity of glossiness, 5 = non-glossy

Seedling vigor I (1 - 5 scale) : 1 = high vigor, 5 = low vigor

[1] : Log 10 likelihood

[2] : Percentage of adjusted phenotypic variance explained

[3] : Percentage of adjusted genotypic variance explained

[4] : + sign indicates that the homozygous IS 18551 allele genotype has a numerically greater value for the trait than does the homozygous 296B allele genotype; while - sign indicates that the homozygous 296B allele genotype has a numerically greater

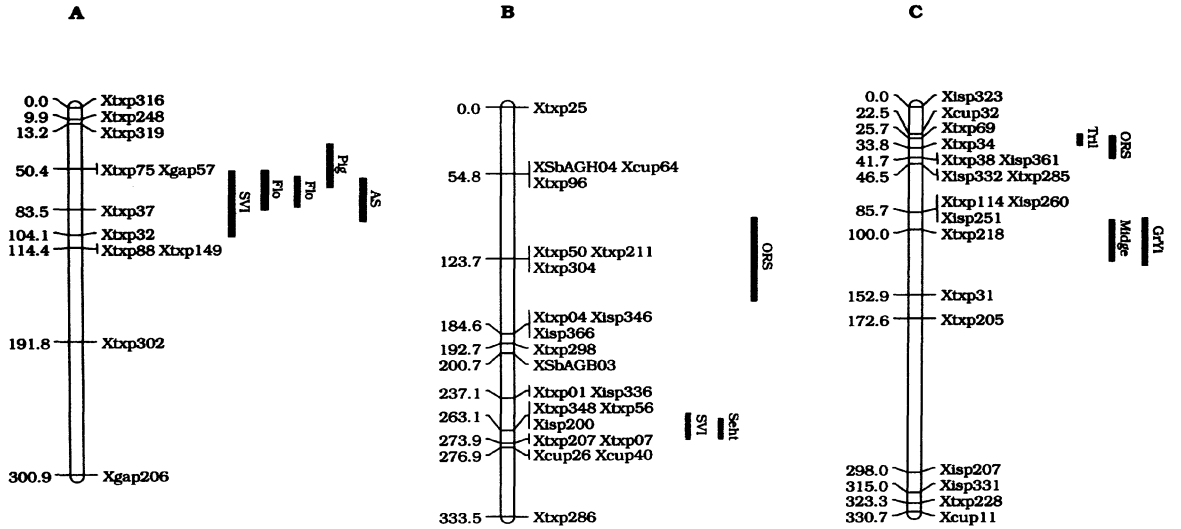
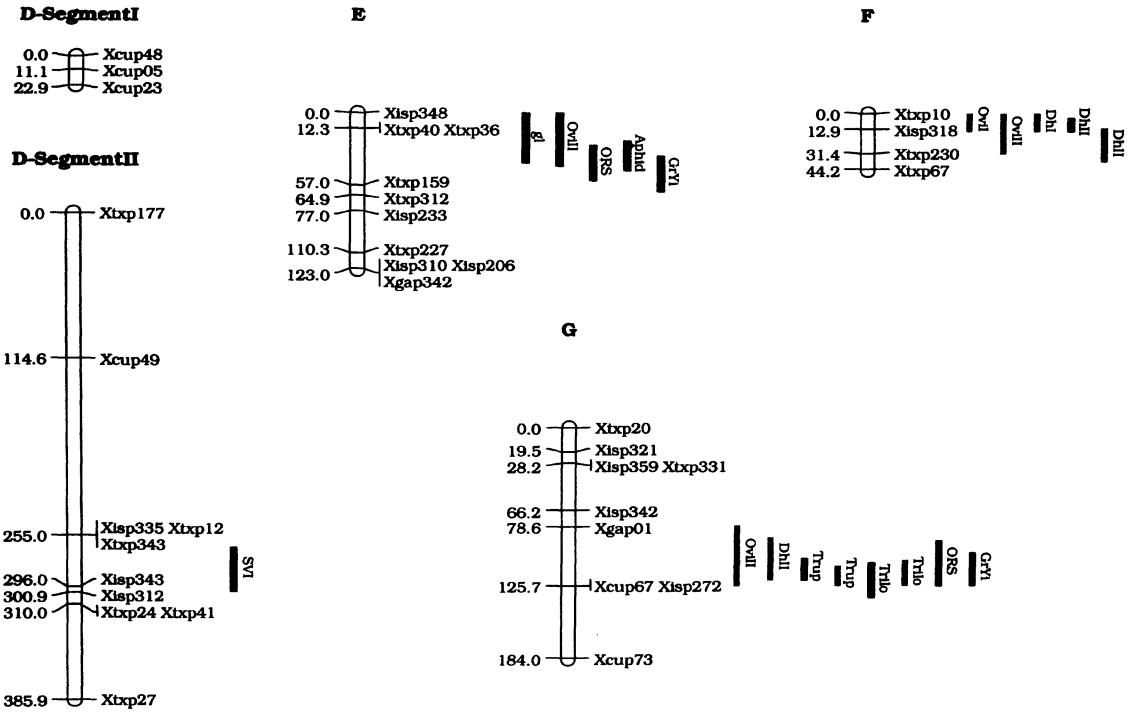
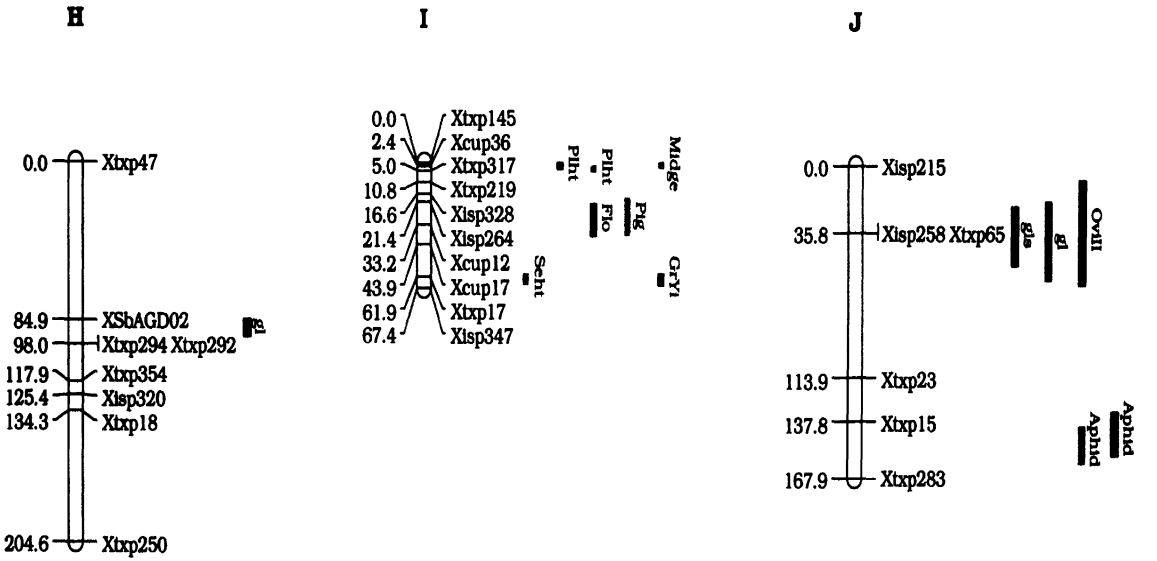


Figure 4.4. QTL positions of shoot fly resistance component traits for 213 recombinant inbred lines derived from cross 296B × IS 18551, detected in individual screening environments, late *kharif* 2002 (indicated by dark blue color) and *rabi* 2004/05 (indicated by pink color), at Patancheru.


D-SegmentI
0.0 Xcup48
11.1 Xcup05
22.9 Xcup23
D-SegmentII
0.0 Xtxp177
114.6 Xcup49
255.0 Xisp335 Xtxp12
Xtxp343
SV1
296.0 Xisp343
300.9 Xisp312
310.0 Xtxp24 Xtxp41
385.9 Xtxp27
E
0.0 Xisp348
12.3 Xtxp40 Xtxp36
57.0 Xtxp159
64.9 Xtxp312
77.0 Xisp233
110.3 Xtxp227
123.0 Xisp310 Xisp206
Xgap342
gl
OvdI
ORS
Aphid
GrY1
F
0.0 Xtxp10
12.9 Xisp318
31.4 Xtxp230
44.2 Xtxp67
OvdI
OvdI
DhI
DhII
DhII
G
0.0 Xtxp20
19.5 Xisp321
28.2 Xisp359 Xtxp331
66.2 Xisp342
78.6 Xgap01
125.7 Xcup67 Xisp272
184.0 Xcup73
OvdI
DhI
Trup
Trup
Trlo
Trlo
ORS
GrY1


H
0.0
Xtxp47
84.9
XSbAGD02
98.0
Xtxp294 Xtxp292
117.9
Xtxp354
125.4
Xisp320
134.3
Xtxp18
204.6
Xtxp250
gl
I
0.0
Xtxp145
2.4
Xcup36
5.0
Xtxp317
10.8
Xtxp219
16.6
Xisp328
21.4
Xisp264
33.2
Xcup12
43.9
Xcup17
61.9
Xtxp17
67.4
Xisp347
Plht
Plht
Plht
Flo
Plg
Midge
Sebt
GrY1
J
0.0
Xisp215
35.8
Xisp258 Xtxp65
113.9
Xtxp23
137.8
Xtxp15
167.9
Xtxp283
gls
gl
Ovtll
Aphid
Aphid

screening environments. The QTL on LG 'J' is a major QTL explaining 36.9% (kharif) and 21.4% (rabi) of the observed phenotypic variance for glossiness intensity. The final simultaneous fits revealed peak LOD values of 20.08 (kharif) and 10.34 (rabi), explaining 33.5% and 18.6% of the adjusted phenotypic variances, respectively. Parent IS 18551 contributed glossiness alleles for all of the detected QTLs, with additive effects ranging from –0.199 to –0.601 (negative sign indicated that greater degree of glossiness is from parent IS 18551).

4.3.1.2 Seedling vigor I (score)

For seedling vigor I, phenotypic observations were not recorded in the late kharif (E_1) environment, while in the rabi (E_2) environment the trait was measured and phenotypic data from E_2 detected three QTLs for seedling vigor score. Theses putative QTLs were mapped on LGs 'A', 'B' and 'D', and together explain 24.2% of the observed phenotypic variance. The final simultaneous fit of these three QTLs using data from the E_2 environment revealed a peak a LOD value 7.53 and 12.6% of the adjusted phenotypic variance was explained by these three QTLs. Out of these three detected QTLs for seedling vigor I, two QTLs (one each on LG 'A' and 'B'), exhibited favorable additive genetic effects contributed by IS 18551 alleles (-0.203 for the QTL on LG A and –0.300 for the QTL on LG B), while the QTL mapped on LG 'D' exhibited favorable additive genetic effects from the 296B allele (0.280). The QTL mapped on LG 'B' appears to be the most important QTL, as it explained 10.8% of observed phenotypic variance for seedling vigor I.

4.3.1.3 Oviposition incidence I

For oviposition I, no QTL was detected in the late *kharif* (E_1) screening environment. The most probable reason for this could be the very little difference between the RIL parents and hence very limited variation for oviposition incidence I in the RIL population due to high shoot fly pressure in late *kharif* screening environment. In the *rabi* screening (E_2) environment, one QTL was detected on LG 'F' explaining 7.2% of the observed phenotypic variance. The final simultaneous fit analysis revealed that only 2.8% of the adjusted phenotypic variance for oviposition I was explained by this single QTL for the which peak LOD value was just 1.72. It was observed that this single putative QTL exhibited favourable additive effects contributed by the 296B alleles (susceptible parent).

4.3.1.4 Oviposition incidence II

For oviposition II, the QTL analysis detected two QTLs in each of the two screening environments. For late *kharif* (E_1) the two QTLs detected were mapped on LG 'E' and LG 'J' and together explained 14.1% of the observed phenotypic variance for this trait. Based on the phenotypic values collected in screening environment E_2 (*rabi*), two QTLs were mapped, one each on LG 'F' and LG 'G', and together these explained 14.0% of the observed phenotypic variance. The final simultaneous fit analysis revealed that only 6.5% of the adjusted phenotypic variance was explained by two QTLs for which peak LOD value was 3.99 in E_1 environment. While in E_2, final simultaneous fit of the two QTLs detected explained 8.7% of the adjusted phenotypic variance, for which a peak LOD value of 5.06 was observed. The favourable additive genetic effects for the QTLs detected in E_1 and E_2 were mostly contributed by the IS 18551 (resistant parent) alleles. The exception was one QTL mapped on LG 'F' in E_2 for which favourable additive effects were contributed by alleles from 296B.

4.3.1.5 Deadhearts incidence I

For deadhearts I, no QTL was found using phenotypic data collected in the late *kharif* (E_1) screening environment. However, from data collected in the *rabi* (E_2) environment, one QTL was detected on LG 'F', accounting 8.4% of the observed phenotypic variance. The final simultaneous fit analysis revealed that only 4.5% of the adjusted phenotypic variance was explained by this single QTL for which the peak LOD value was 2.31. It was observed that favourable additive genetic effects for this QTL on LG 'F' were contributed by alleles from susceptible parent 296B.

4.3.1.6 Deadhearts incidence II

QTL analysis for this trait revealed one QTL and two QTLs that were detected in screening environments E_1 (late *kharif* 2002) and E_2 (*rabi* 2004), respectively. One of these QTLs was mapped on LG 'F' in both screening seasons and accounted for 6.6% and 7.0% of the observed phenotypic variances in E_1 and E_2, respectively. The total observed phenotypic variance explained by the two detected QTLs in E_2 was 18.2%. The second QTL detected for deadhearts II in E_2 was mapped on LG 'G' and accounted for 11.2% of observed phenotypic variance. This is the most important QTL for this trait detected in the *rabi* 2004 screening environment. The final simultaneous fit analysis for this trait in this environment revealed that only 10.5% of the adjusted phenotypic variance was explained by these two QTLs, for which peak

LOD value was 5.98. Favourable additive genetic effects were contributed by IS 18551 (resistance parent) alleles at the QTL on LG 'F' in the late *kharif* screening environment and for the QTL mapped on LG 'G' in the *rabi* screening environment. In contrast, for the QTL mapped on LG 'F' in the *rabi* screening environment, allele from susceptible parent 296B contributed the favourable additive genetic effects.

4..3.1.7 Seedling height I (cm)

Seedling height I was not recorded in the *kharif* screening environment (E_1). For the *rabi* season screen (E_2) two QTLs for seedling height I were mapped, one each on LG 'B' and LG 'I'. Together these QTLs explained about 14.3% of the observed phenotypic variance. Final simultaneous fit analysis revealed that only 6.8% of the adjusted phenotypic variance was explained by these two QTLs, with a peak LOD score of 4.13. The favourable additive genetic effects for these two QTLs for seedling height I were contributed by IS 18551 (resistant parent) alleles.

4.3.1.8 Trichome density (upper leaf blade surface) (no./microscopic field)

For trichome density on the upper surface of the seedling leaf blade, QTL analysis detected one QTL each in both the *kharif* 2002 (E_1) and *rabi* 2004 (E_2) screening environments. Interestingly, the QTL detected was mapped at the same position on LG 'G' in both screening environments. The detected QTL explained 24.4% of the adjusted phenotypic variance with a peak LOD score of 13.35 in E_1 and 15.8% of the adjusted phenotypic variance with a peak LOD score of 8.39 in E_2. The allele for high trichome density on the upper surface of the seedling leaf blade was inherited from resistant parent (IS 18551) for the QTL detected in both of these screening environments. This major QTL detected on LG 'G' mapped to marker interval *Xgap*01-*Xcup*67.

4.3.1.9 Trichome density (lower leaf blade surface) (no./microscopic field)

For trichome density on the lower surface of the seedling leaf blade, one QTL in the *kharif* 2002 screening (E_1) and two QTLs in the *rabi* 2004 screening (E_2) were detected. One QTL was mapped at same position on LG 'G' in both screening environments and an additional QTL was mapped on LG 'C' in E_2. The detected QTL on LG 'G' explained 27.3% of the adjusted phenotypic variance in E_1 and had a peak LOD score of 15.2. While in E_2 the two QTLs detected on LG 'G'and LG C explained 17.3% of the adjusted phenotypic variance and had a peak LOD score 9.7. For the QTL mapped on LG G governing trichome density on the lower surface of the leaf blade, IS 18551 contributed the favourable alleles. However, the favourable additive

genetic effect for the QTL mapped on LG 'C' was contributed by alleles from susceptible parent 296B. Based on analysis of pooled means across E_1 and E_2, one major QTL governing trichome density on the lower surface of the leaf blades mapped LG 'G' between markers *Xgap*01 and *Xcup*67.

4.3.1.10 Plant height (cm)

One QTL for plant height was detected in each of the screening environment. Interestingly, the QTLs detected were mapped on to common position on LG 'I' in these environments. The QTL detected in E_1explained 8.7% of the adjusted phenotypic variance and had a peak LOD score of 4.64. In E_2, the QTL detected explained 10.0% of the adjusted phenotypic variance and had a peak LOD score of 5.32. The additive genetic effects for increased plant height were contributed by alleles from taller parent IS 18551 in both screening environments. The QTL detected in both environments mapped very near to marker locus *Xcup*36.

4.3.1.11 Time to 50% flowering (d)

Two QTLs for time to 50% flowering were detected in the kharif 2002 screening environment (E_1) and one QTL was detected in the shorter day length rabi 2004 screening environment (E_2). One QTL mapped on LG 'A' was mapped in the same position in both E_1 and E_2 and the other QTL mapped on LG 'I' in longer day length screening environment (E_1). The QTLs detected on LG A, explained 11.8% of the adjusted phenotypic variance for flowering time in E_1 with a peak LOD value of 6.7 and explained 6.5% of the adjusted phenotypic variance for this trait in E_2 with a peak LOD value of 3.56. The favourable additive genetic effects (early flowering) were contributed by IS 18551 alleles for the QTL detected on LG 'A' in both screening environments, while for the QTL detected in E_1 on LG 'I', 296B allele contributed favourable additive genetic effects.

4.3.1.12 Overall recovery score

For overall recovery score, the analysis detected four QTLs expressed in the kharif 2002 screening environment (E_1) but no QTL was found for this trait in rabi 2004 (E_2) screening environment. In E_1 one QTL was mapped on each of LG 'B', LG 'C', LG 'E' and LG 'G'. These four QTLs explained 32.4% of the observed phenotypic variance. Final simultaneous analysis revealed that, only 17.1% of the adjusted phenotypic variance was explained by these four QTLs, which together managed a peak LOD value of 10.44. The favourable additive genetic effects for the QTLs detected on LG 'B', LG 'E' and LG 'G' were contributed by IS 18551 alleles, while for the QTL detected on LG 'C' the favourable effects were contributed by alleles from susceptible parent 296B.

4.3.1.13 Ahid damage score

For aphid damage score, two QTLs were detected based on phenotypic evaluation in the kharif 2002 screening environment and one QTL was detected based on screening in E_2. One of the QTLs detected mapped to same position of LG 'J' for both screening environments and one QTL mapped to LG 'E' based on screening in E_1. The two QTLs together explained 31.5% of the observed phenotypic variance for this trait in E_1. Final simultaneous analysis revealed that 22.7% of adjusted phenotypic variance was explained by these two QTLs, which had a combined peak LOD score of 12.7 for E_1. The single QTL detected in E_2 explained 10.4% of observed phenotypic variance. Final simultaneous fit analysis revealed that only 6.0% of the adjusted phenotypic variance was explained by this single QTL, with a peak LOD score of 3.26. Favourable additive genetic effects for aphid low incidence was contributed by alleles from shoot fly resistant parent IS 18551 in both screening environments. A major QTL for aphid resistance was mapped on LG 'J' in the marker interval *Xtxp*15-*Xtxp*283.

4.3.1.14 Pigmentation score

Pigmentation was not recorded in the 2002 *kharif* screening environment (E_1) From the 2004 *rabi* screening environment data two QTLs for foliage color were detected, one each on LG 'A' and LG 'I', which explained 7.1% and 12.0% of the observed phenotypic variance, respectively. For both of these QTLs positive additive genetic effects that is darker foliage color were contributed by alleles from IS 18551. The final simultaneous fit analysis revealed that only 16.9% of the adjusted phenotypic variance for pigmentation score was explained by these two QTLs, which had a combined peak LOD value of 9.43 that was significantly better than the best single QTL model for this trait.

4.3.1.15 Midge damage score

The midge damage score was not recorded in the 2002 *kharif* season screening environment (E_1). For the 2004 *rabi* screening environment (E_2), two QTLs were detected, one each on LG 'C' and LG 'I', explaining 5.5% and 15.5% of the observed phenotypic variance, respectively. The simultaneous fit analysis revealed that only 10.2% of the adjusted phenotypic variance was explained by these two QTLs, which had a combined peak LOD score of 5.86. The favourable additive genetic effects for low midge damage score were contributed by 296B alleles.

4.3.1.16 Agronomic score

Agronomic score of the RILs and their parents was not recorded in the 2002 kharif screening environment (E_1). For the 2004 *rabi* screening environment (E_2) one QTL was detected on LG 'A' explaining 5.5% of the observed phenotypic variance for agronomic score. The final simultaneous fit analysis revealed that only 5.0% of the phenotypic variances was explained by this single QTL, with a peak LOD score of 2.8. The additive effects for desirable agronomic score were contributed by 296B alleles in this environment.

4.3.1.17 Grain yield (g/plot)

For grain yield under moderate to severe shoot fly pressure, the QTL analysis detected three QTLs in E_1 and one QTL in E_2. In 2002 *kharif* screening environment E_1, one QTL was mapped on each of LG 'E', LG 'G' and LG 'I'. These three QTLs together explained 26.2% of the observed phenotypic variance for grain yield under severe shoot fly pressure, while in the 2004 *rabi* screening environment (E_2) one QTL was mapped on LG 'C', which explained 6.4% of observed phenotypic variance for this trait. Final simultaneous fit analysis for the data from E_1 revealed that a total of 11.5% of the adjusted variance could be explained by the three detected QTLs, which together had a peak LOD value of 6.96. Favourable additive genetic effects were contributed by the IS 18551 parental alleles in the kharif screening environment, while 296B alleles contributed favourable effects in the *rabi* screening environment.

4.3.2 QTL analysis across the two screening environments

In order to determine chromosomal regions that are important for the expression of the traits under different environmental conditions and also to detect the Q × E interaction effects, QTL analysis was done based on pooled means of the phenotypic values averaged over the two screening environments. The results are presented in Table 4.8 and Figure 4.5.

4.3.2.1 Glossiness score

For glossiness two QTLs were detected in the across-environments analysis, together explaining about 41.6% of the total observed phenotypic variance in pooled entry means. These QTLs were mapped on LG 'G' and LG 'J'. The QTL mapped on LG 'J' was a major QTL explaining about 33.6% of total phenotypic variance with a LOD peak value 15.92. For both of the QTLs exhibited non-significant Q × E interaction. Both of the QTLs the favourable additive genetic effects were contributed by IS 18551 alleles. After adjustment of the phenotypic variance for Q × E interaction and A × A epistatic interaction, the two QTL explained 31.1% of the phenotypic variance with a combined peak LOD value of 18.0.

Table 4.8 Characteristics of QTLs associated with putative components of resistance to shoot fly (based on across-season averages) based on Composite Interval Mapping (PLABQTL, LOD>2.5) using 213 RILs derived from cross 296B (susceptible) × IS 18551 (resistant)

Environment/trait	Linkage group	Position	Marker interval	Support interval (cM)	Peak LOD[1]	R^2 (%)	Effect[4] (additive)	Q × E interaction
Glossiness score	G	108	*Xgap* 01-*Xcup* 67	88-146	3.76	8.0	-0.291	NS
	J	30	*Xisp* 215-*Xisp* 258	22-52	15.92	33.6	-0.495	NS
	Sum: 2 QTLs					41.6		
	Final simultaneous fit				LOD = 18.01	Adj. R^2 = 31.1%		
						Adj.genotypic var. ex.[3] = 48.6%		
Oviposition I (%)	C	84	*Xisp* 332-*Xtxp* 114	66-94	4.42	9.2	-1.248	NS
	F	0	*Xtxp* 10-*Xisp* 318	0-12	2.68	5.9	0.849	**
	Sum: 2 QTLs					15.1		
	Final simultaneous fit				LOD = 3.89	Adj. R^2 = 6.4%		
						Adj.genotypic var. ex. = 37.4%		
Oviposition II (%)	C	44	*Xtxp* 38-*Xtxp* 332	34-48	2.80	6.0	-0.972	NS
	F	24	*Xisp* 318-*Xtxp* 230	12-36	3.05	6.8	1.250	*
	G	96	*Xgap* 01-*Xcup* 67	72-112	4.76	10.1	-2.109	*
	J	56	*Xisp* 215-*Xtxp* 23	22-80	3.46	7.6	-1.855	NS
	Sum: 4 QTLs					30.5		
	Final simultaneous fit				LOD = 9.26	Adj. $R^{\wedge 2}$ = 15.1%		
						Adj.genotypic var. ex. = 75.6%		

Table 4.8 cont.—

Trait	Linkage group	Position	Marker interval	Support interval (cM)	Peak LOD[1]	R^2%	Effect[4] (additive)	Q X E interaction
Deadhearts I (%)	**QTL not found**							
Deadhearts II (%)	F	32	*Xtxp* 230-*Xtxp* 67	22-38	3.59	11.5	1.229	*
	G	98	*Xgap* 01-*Xcup* 67	76-118	3.65	7.8	-2.447	*
	Sum: 2 QTLs					19.3		
	Final simultaneous fit				LOD = 6.01	Adj. R^2 = 10.6%		
						Adj.genotypic var. ex. = 11.7%		
Trichome density (upper leaf blade surface) (no./microscopic field)								
	G	116	*Xgap* 01 - *Xcup* 67	106-126	16.38	30.05	36.915	NS
	Sum: 1 QTL					30.05		
	Final simultaneous fit				LOD = 16.7	Adj. R^2 = 29.6%		
						Adj.genotypic var. ex. = 50.3%		
Trichome density (lower leaf blade surface) (no./microscopic field)								
	F	44	*Xtxp* 230-*Xtxp* 67	34-44	2.89	9.3	4.896	NS
	G	120	*Xgap* 01-*Xcup* 67	110-126	14.38	27.4	15.448	NS
	Sum: 2 QTLs					36.7		
	Final simultaneous fit				LOD = 18.79	Adj. R^2 = 32.4%		
						Adj.genotypic var. ex. = 63.4%		
Plant height (cm)	I	4	*Xcup* 36-*Xtxp* 317	2-6	7.38	15.6	9.373	NS
	Sum: 1 QTL					15.6		
	Final simultaneous fit				LOD = 5.21	Adj. R^2 = 9.8%		

Table 4.8 cont.—

Environment/trait	Linkage group	Position	Marker interval	Support interval (cM)	Peak LOD[1]	R^2 (%)	Effect[4] (additive)	Q × E interaction
						Adj.genotypic var. ex. = 11.7%		
Time to 50% flowering (d)	A	70	*Xtxp* 75-*Xtxp* 37	62-80	9.15	18.2	-2.021	NS
	E	64	*Xtxp* 159-*Xtxp* 312	60-72	3.68	7.8	0.887	NS
	Sum 2 QTLs					26.0		
	Final simultaneous fit				LOD= -8.86	Adj. R^2 = 15.9%		
						Adj.genotypic var. ex. = 32.3%		
Overall recovery score	E	8	*Xtxp* 348-*Xtxp* 40	0-14	2.91	6.2	-0.154	NS
	J	124	*Xtxp* 23-*Xtxp* 15	112-138	2.82	6.3	-0.187	NS
	Sum 2 QTLs					12.5		
	Final simultaneous fit				LOD= 4.95	Adj. R^2 = 8.5%		
						Adj.genotypic var. ex. = 14.1%		
Aphid damage score	E	40	*Xtxp* 40-*Xtxp* 159	18-64	2.58	5.5	-0.191	**
	J	148	*Xtxp* 15-*Xtxp* 283	138-156	9.26	20.4	-0.312	NS
	Sum 2 QTLs					25.9		
	Final simultaneous fit				LOD= 10.35	Adj. R^2 = 18.6%		
						Adj.genotypic var. ex. = 48.9%		
Grain yield (g/plot)	A	74	*Xtxp* 75-*Xtxp* 37	56-94	2.50	5.4	30.067	NS
	G	122	*Xgap* 01-*Xcup* 67	106-134	5.26	11.2	33.001	NS
	I	66	*Xtxp* 17-*Xisp* 347	60-66	3.64	8.6	22.063	NS
	Sum 3 QTLs					25.2		
	Final simultaneous fit				LOD= 7.38	Adj. R^2 = 12.3%		
						Adj.genotypic var. ex. = 27.3%		

Glossiness (1-5 scale) : 1 = high intensity of glossiness, 5 = non-glossy

[1] : Log 10 likelihood

[2] : Percentage of adjusted phenotypic variance explained [3] : Percentage of adjusted genotypic variance explained

[4] : + sign indicates that the homozygous IS 18551 allele genotype has a numerically greater value for the trait than does the homozygous 296B allele genotype; while - sign indicates that the homozygous 296B allele genotype has a numerically greater value

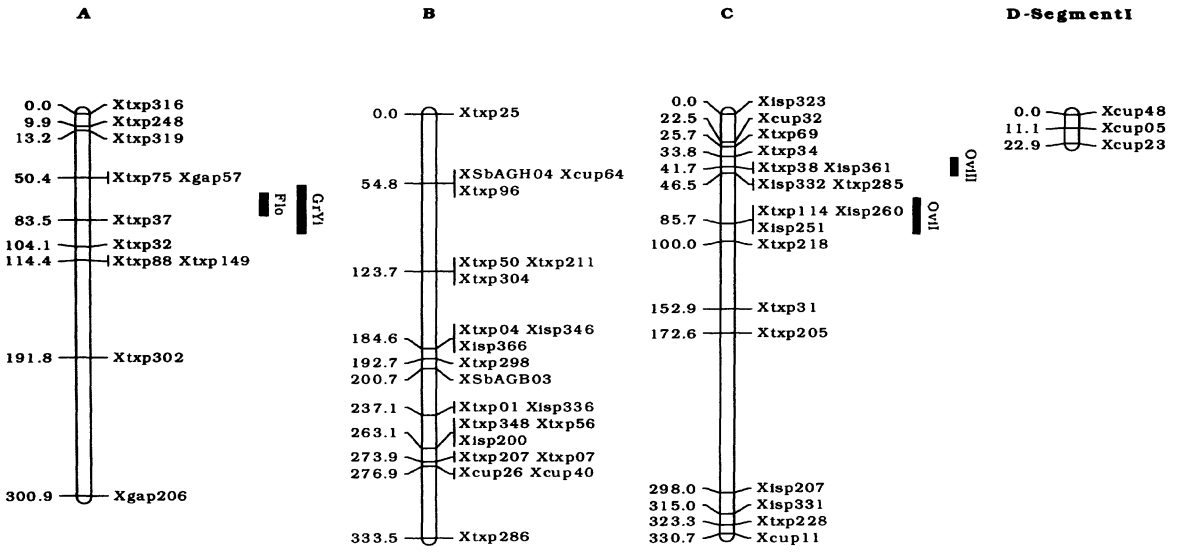


Fig 4.5: QTL positions of shoot fly resistance component traits mapped in 213 recombinant inbred lines derived from cross 296B × IS 18551, based on analysis across two screening environments at Patancheru during 2002-2004.

Fig. 4.5 cont.--

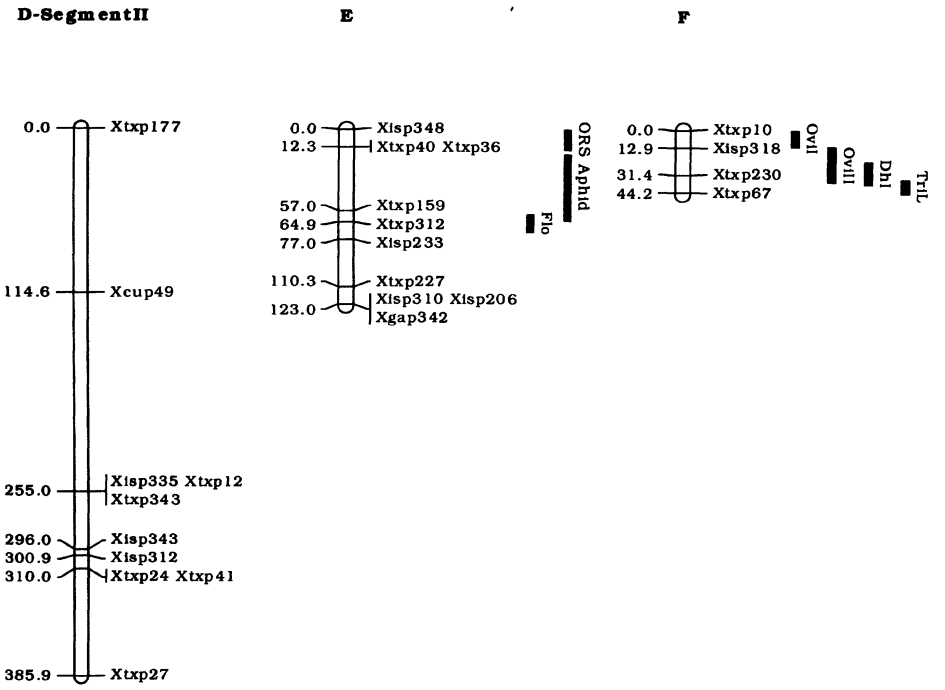
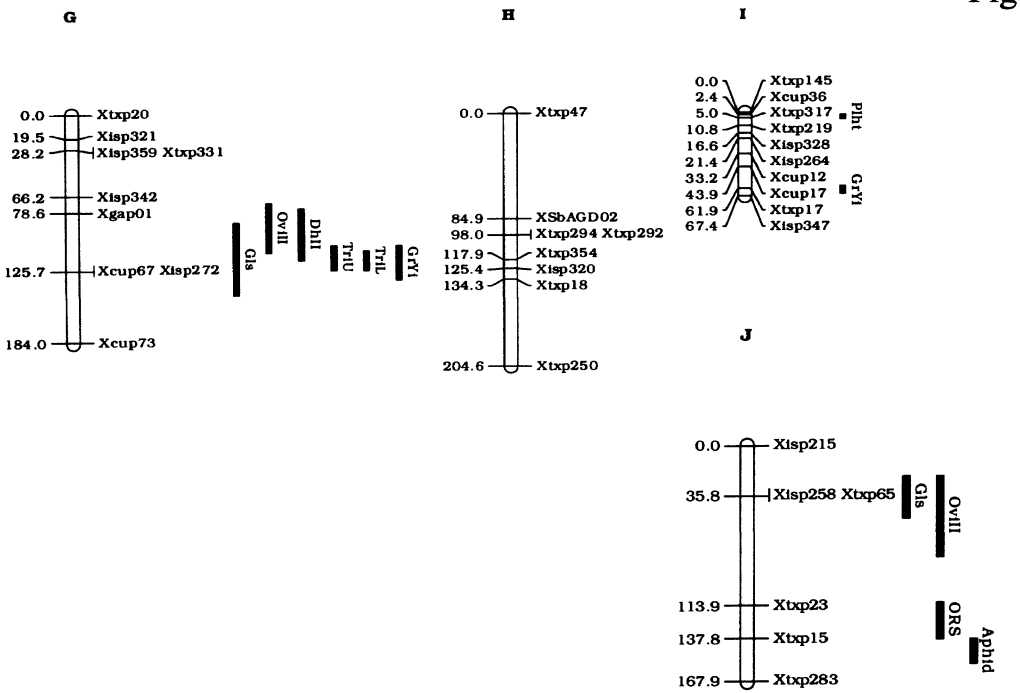

D-SegmentII
0.0 Xtxp177
114.6 Xcup49
255.0 Xisp335 Xtxp12 Xtxp343
296.0 Xisp343
300.9 Xisp312
310.0 Xtxp24 Xtxp41
385.9 Xtxp27
E
0.0 Xisp348
12.3 Xtxp40 Xtxp36
57.0 Xtxp159
64.9 Xtxp312
77.0 Xisp233
110.3 Xtxp227
123.0 Xisp310 Xisp206 Xgap342
ORS Aphid
Flo
F
0.0 Xtxp10
12.9 Xisp318
31.4 Xtxp230
44.2 Xtxp67
Ovl1
Ovl1
Dhl
TriL

Fig. 4.5 cont--


G
0.0 Xtxp20
19.5 Xisp321
28.2 Xisp359 Xtxp331
66.2 Xisp342
78.6 Xgap01
125.7 Xcup67 Xisp272
184.0 Xcup73
Gis
OvIII
DhII
LHU
THL
GyV1
H
0.0 Xtxp47
84.9 XSbAGD02
98.0 Xtxp294 Xtxp292
117.9 Xtxp354
125.4 Xisp320
134.3 Xtxp18
204.6 Xtxp250
I
0.0 Xtxp145
2.4 Xcup36
5.0 Xtxp317
10.8 Xtxp219
16.6 Xisp328
21.4 Xisp264
33.2 Xcup12
43.9 Xcup17
61.9 Xtxp17
67.4 Xisp347
PhIt
GyV1
J
0.0 Xisp215
35.8 Xisp258 Xtxp65
113.9 Xtxp23
137.8 Xtxp15
167.9 Xtxp283
Gis
OvIII
ORS
Aphid

4.3.2.2 Oviposition incidence I

For oviposition I two QTLs were detected in the across-environments analysis, together explaining about 15.1% of the total phenotypic variance for pooled entry means. These QTLs were mapped on LG 'C' and LG 'F'. The QTL for oviposition I mapped on LG 'F' exhibited significant Q × E interaction, while the other QTL mapped on LG 'C' exhibited non-significant Q × E interaction. Favourable additive genetic effects were contributed by alleles from resistant parent IS 18551 for the QTL mapped on LG 'C'. For the QTL mapped on LG 'F', favourable additive genetic effects were contributed by alleles from susceptible parent 296B. Final simultaneous fit analysis revealed that two QTLs together explained only 6.4% of adjusted phenotypic variance in pooled RIL means for oviposition I with a peak LOD value of 3.8.

4.3.2.3 Oviposition incidence II

For oviposition II four QTLs were detected by the across-environments analysis, explaining together about 30.5% of the observed phenotypic variance. These four QTLs were mapped, one each, on LG 'C', LG 'F', LG 'G', and LG 'J'. The QTLs mapped on LG 'F' and LG 'G' explained about 6.8% and 10.1% of observed phenotypic variance, respectively; and these two QTLs exhibited significant Q × E interaction. While QTLs mapped on LG 'C' and LG 'J' exhibited non-significant Q × E interaction and explained 6.0% and 7.6%, respectively of observed phenotypic variance. Final simultaneous fit analysis revealed that only 15.1% of adjusted phenotypic variance could be explained by these four QTLs together with a peak LOD value 9.26. The favourable additive genetic effects were contributed by IS 18551 alleles for the QTLs mapped on LG 'G', LG 'J', and LG 'C', while for the QTL mapped on LG 'F' alleles from susceptible parent 296B contributed favourable additive genetic effects.

4.3.2.4 Deadhearts incidence II

For deadhearts I no QTL was found across analysis. Failure to detect the significant QTLs for deadhearts I using the pooled mean across E_1, E_2 the most probable reason for this could be the very little differences between the RIL parents and hence very limited variation for deadhearts I in the RIL population due to high shoot fly pressure in late kharif screening environment. In the *rabi* screening initially the deadhearts per cent was low due to low population of shoot fly and differences was limited.

QTL analysis using the phenotypic mean values for the individual RIL progenies, averaged across two screening environments, detected two QTLs for deadhearts incidence at the second observed stage and these mapped on LG 'F' and LG 'G'. Final simultaneous fit of these two QTLs together explained 10.6% of the adjusted phenotypic variance for this trait with a peak LOD value of 6.01. They both exhibited significant Q × E interaction. For the QTL on LG 'G', favourable additive genetic effects were contributed by alleles from resistant parent IS 18551. However, for the QTL on LG 'F', alleles from susceptible parent 296B contributed the favourable genetic effects.

4.3.2.5 Trichome density (upper leaf blade surface) (no./microscopic field)

Across environment analysis found one major QTL for trichome density of the upper surface of the leaf blade. This QTL mapped on LG 'G' and explained about 30.1% of the observed phenotypic variance for this trait with a peak LOD value of 16.38. The Q × E interaction for this trait was non-significant and 29.6% of the adjusted phenotypic variance for pooled RIL entry means was explained by this major QTL. The alleles for increased trichome density on the upper leaf blade surface a putative shoot fly resistance component were inherited from resistant parent IS 18551.

4.3.2.6 Trichome density (lower leaf blade surface) (no./microscopic field)

Two QTLs were detected for this trait in the across-season analysis. The QTL mapped on LG 'G' was a major QTL explaining about 27.5% of the observed phenotypic variance with a peak LOD value of 14.4. The second QTL mapped on LG 'F' explaining 9.3% of observed phenotypic variance. Both of QTLs exhibited non-significant Q × E interaction. These two QTLs together explained 32.4% of the adjusted phenotypic variance for pooled RIL means of this trait. Favourable additive genetic effects (increased trichome density) for both QTLs were contributed by IS 18551 alleles.

4.3.2.7 Plant height (cm)

One QTL was detected for plant height in the across-season analysis. This QTL mapped on LG 'I' and explained about 15.6% of observed phenotypic variance with a peak LOD value of 7.38. This QTL exhibited non-significant Q × E interaction. This single QTL explained only 9.8% of adjusted phenotypic variance in pooled RIL means of this trait in the final simultaneous fit analysis with had peak lod values 5.21. Favourable additive genetic effects for increased height were contributed by alleles from shoot fly resistant parent IS 18551.

4.3.2.8 Time to 50% flowering (d)

Two QTLs were detected for flowering time in the across-season analysis. These QTLs were mapped on LG 'A' and LG 'E'; and together explained 26.0% of observed phenotypic variance for pooled mean flowering time. One major QTL for this trait on LG 'A' explained about 18.2% of observed phenotypic variance with a peak LOD value of 9.15. Both QTLs exhibited non-significant Q × E interaction. Final simultaneous fit analysis revealed that only 15.9% of adjusted phenotypic variance in pooled RIL means for this trait could be explained by these two QTLs together. The QTL on LG 'A' had favourable additive genetic effects for early flowering contributed by IS 18551 alleles, while the QTL for this trait on LG 'E' had favourable additive effects contributed by 296B alleles.

4.3.2.9Overall shoot fly recovery score

Two QTLs were detected for this trait in the across-season analysis. These two QTLs were mapped on LG 'E' and LG 'J'. In the final simultaneous fit analysis these two QTLs together explained only 8.5% of the adjusted phenotypic variance for pooled RIL means for this trait with a peak LOD value of 4.95. Both these QTLs exhibited non-significant Q × E interaction for this trait. Favourable additive genetic effects (better overall recovery) were contributed by alleles from resistant parent IS 18551.

4.3.2.10 Aphid damage score

Two QTLs were detected in the across-seasons analysis for this trait. These mapped on LG 'E' and LG 'J'. Final simultaneous fit analysis of the QTLs together explained only 18.6% of adjusted phenotypic variance in pooled RIL means for this trait with a peak LOD value of 10.35. The QTL mapped on LG 'J' for this trait was a major one, explaining 20.4% of observed phenotypic variance with a peak LOD value 9.26. The QTL mapped on LG 'E' exhibited significant Q × E interaction, while the QTL mapped on LG 'J' showed non-significant Q × E interaction. The favourable additive genetic effects for both QTLs were contributed by IS 18551 alleles.

4.3.2.11 Grain yield (g/plot)

Three QTLs were detected for this trait under conditions of moderate to severe shoot fly pressure in the across-season analysis. These mapped on LG 'A', LG 'G', and LG 'I'. These three QTLs together explained about 12.3% of adjusted phenotypic variance with a peak LOD value of 7.38. All three QTLs exhibited non-significant Q × E interaction. The favourable additive genetic effects for these three QTLs were contributed by alleles from resistant parent IS 18551.

4.4 Marker- assisted selection for shoot fly resistance traits in sorghum

A backcross breeding program is aimed at gene introgression from a donor line into the genomic background of a recipient line. The potential utilization of molecular markers in such programs has received considerable attention in the recent past. Markers can be used to assess the presence of the introgressed gene (foreground selection) when direct phenotypic evaluation is not possible or too expensive or only possible late in development. Markers can also be used to accelerate the return to the recipient parent genotype at other loci (background selection). The use of molecular markers for background selection in backcross program has been tested experimentally and proved to be very efficient. In the present study, the target for introgression of a QTL (Quantitative Trait Locus), that is a gene or gene block whose position is not known with certainty, but only estimated. In fact introgressing the favourable allele of a QTL by recurrent backcrossing can be a powerful mean to improve the economic value of elite lines provided the expression of the QTL is not reduced in the recipient genomic background.

4.4.1 Marker-assisted breeding for shoot fly resistance and component traits

Conventional breeding for shoot fly resistance and its component traits is often an extremely slow and laborious process and because of significant genotype × environment interactions, the results tend to be location specific. The application of DNA markers and QTL mapping technology is expected to facilitate breeding for complex traits such as shoot fly resistance. After mapping QTL(s) for shoot fly resistance and its component traits in a donor parent, markers linked to the QTL(s) be employed to transfer these QTL(s) from that donor (resistant parent) to a agronomically elite but more susceptible parent (recurrent parent). This process is referred to as foreground selection in a marker-Assisted Breeding (MAB) program. In addition, selection for recurrent parent alleles at marker(s) unlinked to the QTLs can be used during the MAB program to hasten recovery of recurrent parent genotypes in genomic regions that are not involved with the target QTL(s) (background selection).

Sajjanar and Folkertsama et al. (2005 unpublished) evaluated that 252 RILs of a BTx623 × IS 18551 derived mapping population for shoot fly resistant component traits in three environments. Same set of RILs was genotyped using 109 SSR marker loci and QTL analysis was performed with the aim of identifying the genomic regions associated with shoot fly resistance. QTL analysis using Plab QTL Version 1.1 revealed the presence of 28 QTLs detected at least in two of the three

screening environments. Closely linked markers were identified for four QTLs for deadhearts incidence. In the present study efforts are being made to transfer these four deadhearts QTLs, by marker-aided selection, into three elite hybrids parental lines developed at SRS, MAU, Parbhani. The markers associated with shoot fly resistance traits are listed below.

Table 4.9a Target genomic regions, linked SSR markers and associated shoot fly resistance QTLs for marker-assisted selection

Linkage group	Associated SSR markers	QTLs co-localized with genomic regions
A=SBI-01	*Xtxp*75, *Xtxp*37	Deadhearts I, Oviposition I
E=SBI-07	*Xisp*10362, *Xtxp*40, *Xtxp*312	Deadhearts I, Oviposition I
G=SBI-10	*Xisp10*263, *Xgap*01, *Xtxp*141	Glossiness, Trichome density of upper and lower leaf blade surfaces, Seedling vigor II, Oviposition I and II, and Deadhearts I and II
J=SBI-05	*Xisp10*258, *Xtxp*65, *Xtxp*15	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II

4.4.2 Checking the DNA concentration

After isolating the DNA samples of parental plants, all effected crosses ($F_{1\ hybrids}$) and their backcross populations were loaded in to 1.2% agrose gels along with standard for testing the DNA quantity and quality. If the bands were clear, this indicated good DNA quality. DNA concentrations were also assessed with a Spectrafluorplus spectrophotometer using the green fluorescent dye Picogreen™. Likewise DNA quality and quantity was assessed for all generations and dilutions were made accordingly to produce working solutions for each sample having a DNA concentration of 2.5 ng/µl.

PCR was done with selected SSR primers for both foreground and background selection for all backcross generations. After each PCR reaction was completed, PCR products were electrophoretically separated on 6% non-denaturing PAGE gels and they were scored for parental band after silver staining. Parental and backcrossing population samples had PCR products for some primer pairs separated on an automatic DNA sequencer (ABI 3700) and amplified products were then scored using the Genotyper software.

4.4.3 Parental genotyping with SSR markers

Twelve SSR marker loci linked to targeted shoot fly resistance trait of QTLs were used for genotyping recurrent and donor parent plants to detect polymorphism between the three recurrent and four donor parents. The results of parent polymorphism screenig, (Table 4.9b) showed that the flanking markers *Xtxp*75-*Xtxp*37 (LG A), *Xtxp*312 (LG E), *Xtxp*141 (LG G), and *Xisp*10258 (LG J) exhibited allele size differences greater than 5 bp between all twelve (3 recurrent x 4 donor parents) cross combinations.

The remaining flanking SSR markers viz. [*Xtxp*40 (LG E), *Xgap*01 (LG G), *Xtxp*65 (LG J), and *Xtxp*15 (LG J)] exhibited allele size differences less than 5 bp between recurrent and donor parent pairs, except that *Xtxp*40 (LG E) was monomorphic between all three recurrent parents (28B, 20B, KR 192 and the RIL 252 donor parent, and for marker *Xgap*01 (LG G) recurrent parent 20B was monomorphic with all four donor parents. Marker locus *Xixp*10362 (LG E) exhibited allele size difference more than 5 bp between all recurrent and donor parent pairs except that KR 192 was monomorphic with donors RIL 189, IS 18551 and RIL 153;. marker *Xisp*10263 (LG G) was monomorphic with recurrent parent 20B and donor parents IS 18551 and RIL 252,but polymorphic with donor parent RIL 153,similarly for this marker KR 192 was monomorphic with RIL 153 but polymorphic with donor parent IS 18551 and RIL 252. Marker *Xtxp*94 (LG J) was monomorphic for all three recurrent parents with all four donor parents.

After detecting polymorphism between recurrent and donor parents, homozygous parental-type plants of the two parents were selected at seedling stage for subsequent crosses (plant to plant crosses). Finally we succeeded to develop 7 F_1 hybrids, which are listed below:

28B(288) × RIL 189(312)

KR 192(304) × IS 18551(267)

20B(186) × RIL 252(318)

20B(179) × RIL 153(248)

KR 192(300) × RIL 252(319)

28B(293) × IS 18551(268)

28B(292) × RIL 153(252)

Out of the seven F_1 hybrids listed above, the "hybrid " progeny for cross 28B(293) x IS18551 (268) showed only the recurrent parent alleles (indicating that crossing had

Table 4.9b. Parental polymorphism (allele size, bp) using eleven SSR markers that were used for foreground selection in marker-assisted breeding for shoot fly resistance in this study

SSR Markers	LG	28B	20B	KR 192	RIL 189	IS 18551	RIL 252	RIL153
Xtxp75	A	165	167	169	150	150	150	150
Xtxp37	A	177	184	188	167	167	167	167
Xisp10362	E	370	350	**360**	**360**	**360**	365	**360**
Xtxp40	E	**135**	**135**	**135**	138	138	**135**	138
Xtxp312	E	165	170	230	185	180	195	180
Xisp10263	G	-	**320**	**340**	-	**320**	**320**	**340**
Xgap01	G	252	**254**	252	254	254	254	254
Xtxp141	G	156	150	150	162	156	156	156
Xisp10258	J	190	190	195	185	185	185	185
Xtxp65	J	130	130	130	132	132	132	132
Xtxp94	J	**211**	**211**	**211**	**211**	**211**	**211**	**211**
Xtxp15	J	225	223	224	223	221	221	221

failed and the progeny were in fact selfed of recurrent parent 28B(293)) based on molecular data scored with SSR markers. Similarly hybrid progeny of cross 28B(292) x RIL 153(252) fail to germinate. Thus, only five crosses (F_1 hybrid) were advanced for generating backcross populations.

4.4.4 Screening of F_1 and BC_1F_1 populations based on recurrent parent 28B with SSR markers

4.4.4.1 Testing hybridity with SSR markers

Five plants putatively produced from cross 28B (288) × RIL 189 were genotyped at seedling stage using four SSR loci linked with targeted QTLs (Table 4.10a). Two heterozygous F1 hybrid plants were selected (plate4.2) and crossed with recurrent parent 28B to produce BC_1F_1 seed.

4.4.4.2 Genotyping BC_1F_1 population [28B(288) × RIL 189(312)] × 28B(288), recurrent parent and donor parent with SSR marker for foreground selection

Thirty plants of this backcross population were screened at the seedling stage at loci detected by eleven SSR primer pairs that targeted shoot fly resistance QTLs (Table 4.10b). Based on scoring SSR molecular data fourteen heterozygous plants having appropriate allelic constitutions were selected (plate 4.3.4.4.4.5.4.6) at seedling stage and crossed with recurrent parent 28B (plant to plant cross) to generate BC_2F_1 population. Details of fourteen-introgressed plant with targeted QTL and its associated shoot fly resistance traits presented below (Table 4.10c).

Table 4.10b: Molecular data of BC1F1 population [28B(288) × RIL 189(312)]× 28B(288), recurrent and donor parent for foreground selection

Plant #	LG	F.Size	601	602	603	604	605	606	607	608	Fsize	609	Fsize
Name of SSR Locus													
Xtxp75	A	170	A	A	-	A	A	A	A	A	155	B	170\155
Xtxp37	A	180	A	A	-	A	A	A	A	A	170	B	180\170
Xtxp40	E	135	A	A	A	A	A	A	A	A	138	B	135\138
Xtxp36	E	MONOMORPHIC											
Xisp 10362	E	370	A	A	A	A	A	A	A	A	360	B	370\360
Xtxp312	E	165	A	A	-	A	A	A	A	A	185	B	165\185
Xtxp217	G	MONOMORPHIC											
Xisp 10263	G		-	-	-	-	-	-	-	-	-	-	-
Xgap1	G	252	A	A	-	A	-	-	A	A	254	B	252\254
Xtxp141	G	160	A	A	A	A	A	A	A	A	165	B	160\165
Xisp 10258	J	190	A	A	A	A	A	A	A	A	185	B	190\185
Xtxp65	J	130	A	A	A	A	-	A	A	A	132	B	130\132
Xtxp225	J	MONOMORPHIC											
Xtxp15	J	225	scorable								223		225\223

BC$_1$F$_1$ progeny [28B(288) × RIL 189(312)] × 28B(288)

Plant #	610	611	612	613	614	615	616	617	618	619	710	711	712	713	714	715	716	717	718	719	810	811	812	813	814	815	816	817	818	819
Xtxp75	B	A	H	H	B	-	H	A	A	H	H	H	H	H	A	H	H	A	A	H	A	A	H	A	H	H	H	H	A	A
Xtxp37	B	B	H	H	H	H	H	A	H	A	H	H	H	H	A	H	H	A	A	H	A	A	H	A	H	H	H	H	A	A
Xtxp40	H	-	A	A	A	H	H	H	H	H	H	H	H	A	A	-	A	A	H	A	A	-	H	A	A	-	A	-	-	A
Xtxp36	MONOMORPHIC										MONOMORPHIC										MONOMORPHIC									
Xisp 10362	H	B	H	A	H	B	H	H	H	H	-	H	H	A	A	A	H	A	A	A	A	H	H	A	A	H	A	H	H	A
Xtxp312	-	B	A	H	A	H	A	H	H	H	H	H	A	B	H	H	A	H	H	H	A	H	H	A	A	A	A	H	H	H
Xtxp217	MONOMORPHIC										MONOMORPHIC										MONOMORPHIC									
Xisp 10263	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xgap1	B	-	H	H	H	-	A	H	B	H	H	-	H	-	H	-	A	A	H	A	A	-	A	H	A	O	A	-	H	H
Xtxp141	H	-	H	H	H	A	A	H	B	H	H	H	B	B	H	H	A	A	H	A	A	H	A	A	A	H	H	A	H	H
Xisp 10258	H	H	H	A	H	A	B	H	A	H	H	H	A	H	B	A	A	A	A	H	H	A	H	H	A	H	H	A	H	H
Xtxp65	H	H	H	H	-	-	H	H	A	H	H	H	A	H	H	A	A	A	A	H	H	A	H	-	A	-	H	A	H	H
Xtxp225	MONOMORPHIC										MONOMORPHIC										MONOMORPHIC									
Xtxp15											H	H	B	H	H	H	H	H	A	H	H	H	H	H	H	-	A	H	H	H

A allele = 28B(288)
B allele = RIL 189(312)
H allele = heterozygote
'-'= not apmlified
o= off type
Selected plants are in bold font

Table 4.10a: Genotypic data of hybrid (F1) 28B(288) x RIL189(312) for foreground selection

	28B(288) × RIL 189(312)				
Plant no.	**541**	**542**	543	544	545
SSR marker	**41**	**42**	43	44	45
Xtxp 75(170/155)	**H**	**H**	A	A	A
Xtxp 37(180/170)	**H**	**H**	A	A	A
Xisp 10258(190/185)	**H**	**H**	A	A	A
Xtxp 141(160/165)	**H**	**NA**	A	A	A

A allele= 28B(288)
H allele= heterozygote 28B(288)/RIL189(312)
NA = not amplified

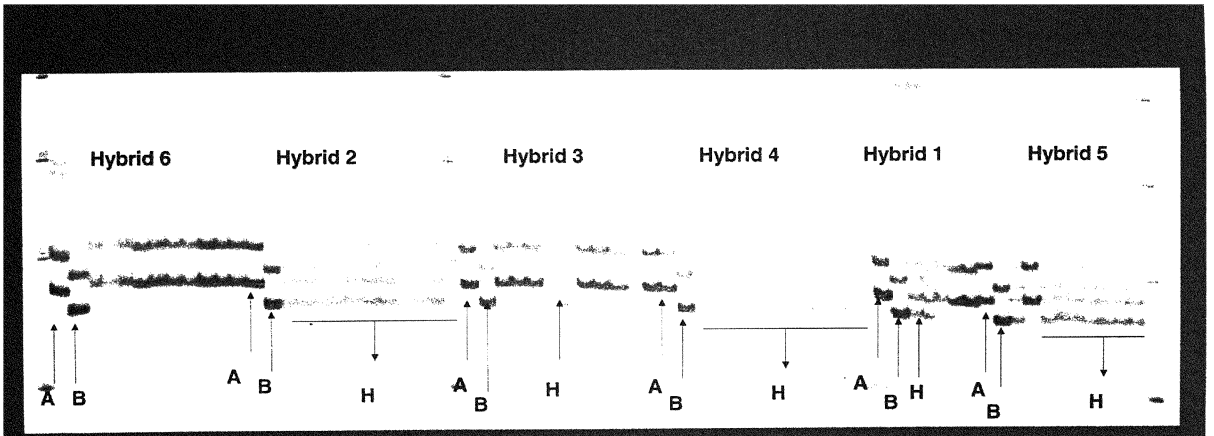


Plate 4.2. Genotyping of five hybrid (F_1) populations (data for hybrid I to V as shown in Tables 4.10a, 4.11a, 4.12a, 4.13a and 4.14a, respectively) to confirm hybridity using SSR locus *Xtxp75*

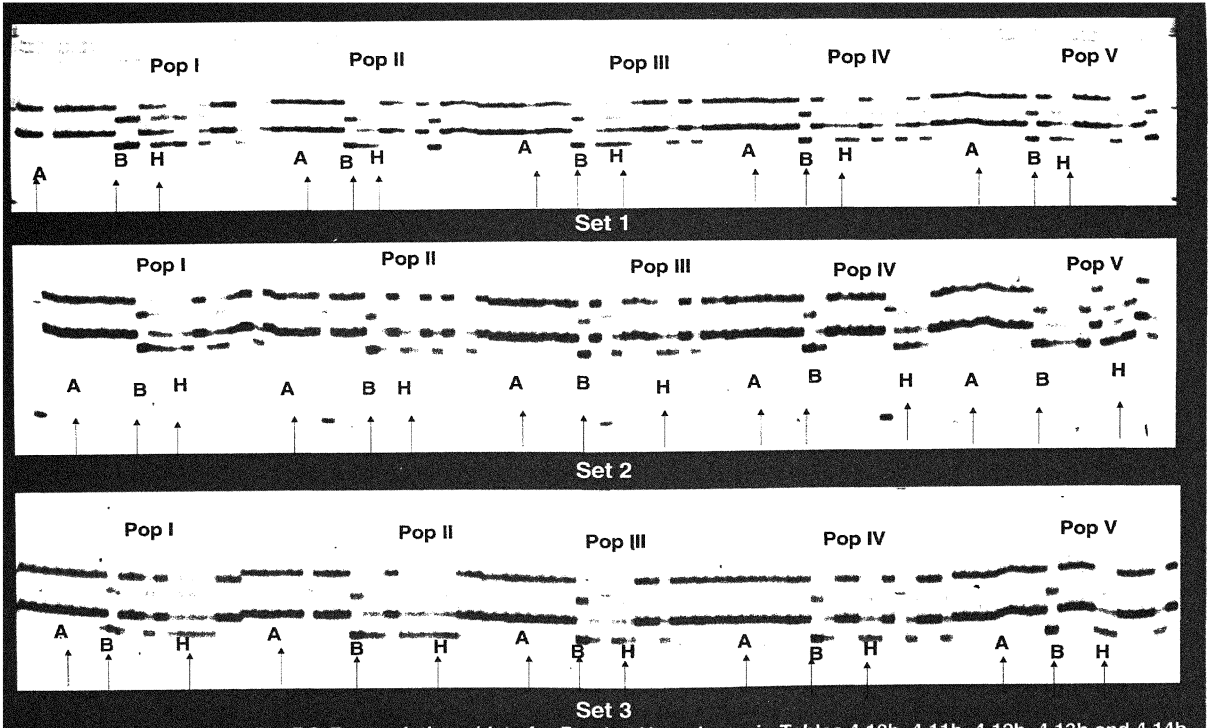
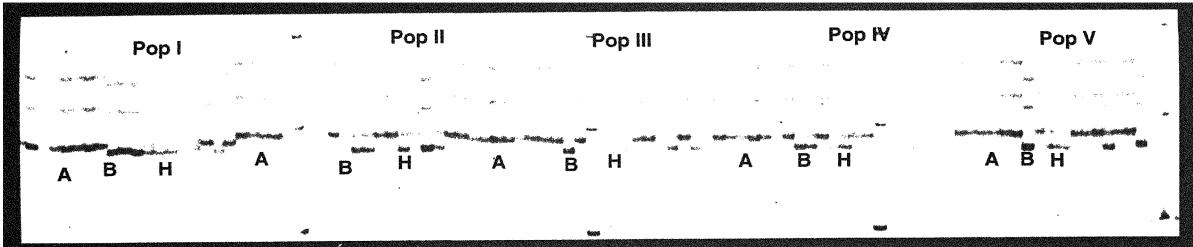
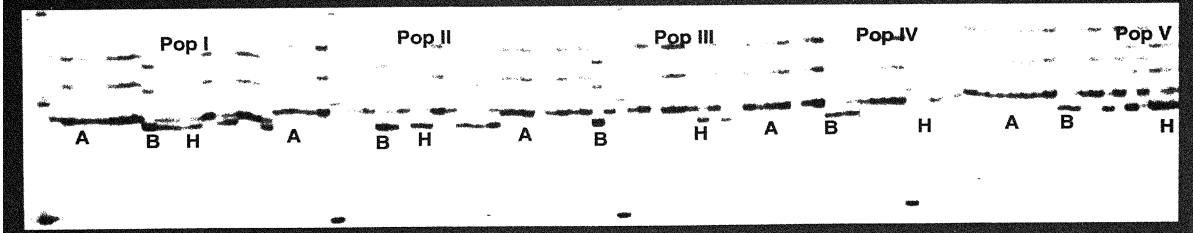


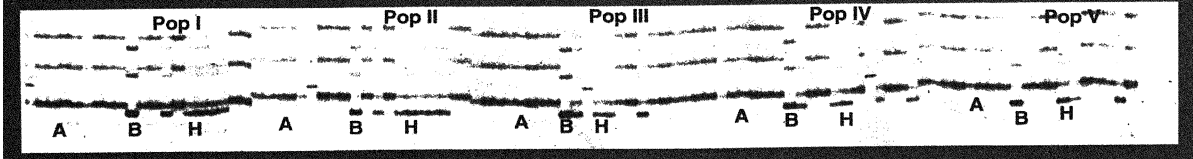
Plate 4.3. Genotyping of five BC$_1$F$_1$ populations (data for Pop I to V as shown in Tables 4.10b, 4.11b, 4.12b, 4.13b and 4.14b, respectively) for foreground screening using SSR locus *Xtxp75* in three different sowing sets (Set 1, Set 2 and Set 3)



Set 1



Set 2



Set 3

Plate 4.4. Genotyping of five BC_1F_1 populations (data for Pop I to V as shown in Tables 4.10b, 4.11b, 4.12b, 4.13b and 4.14b, respectively) for foreground screening using SSR locus *Xtxp*37 in three different sowing sets (Set 1, Set 2 and Set 3)

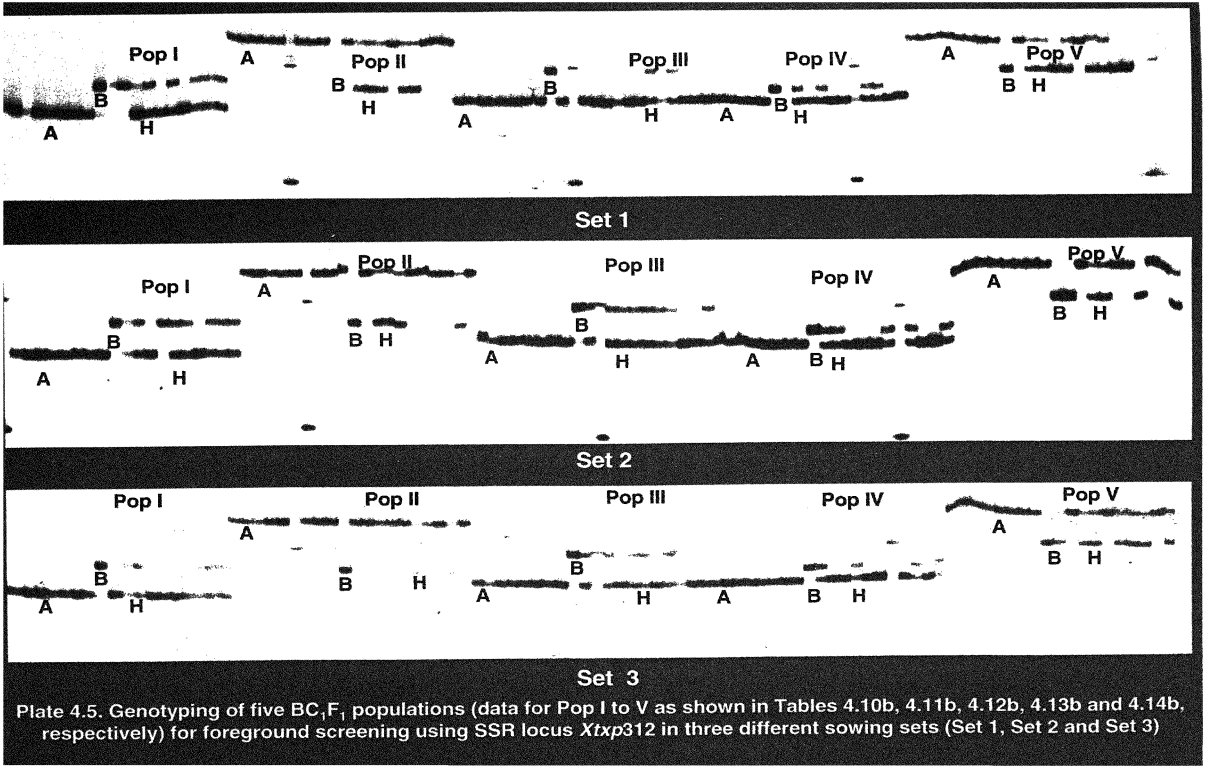


Plate 4.5. Genotyping of five BC_1F_1 populations (data for Pop I to V as shown in Tables 4.10b, 4.11b, 4.12b, 4.13b and 4.14b, respectively) for foreground screening using SSR locus *Xtxp*312 in three different sowing sets (Set 1, Set 2 and Set 3)

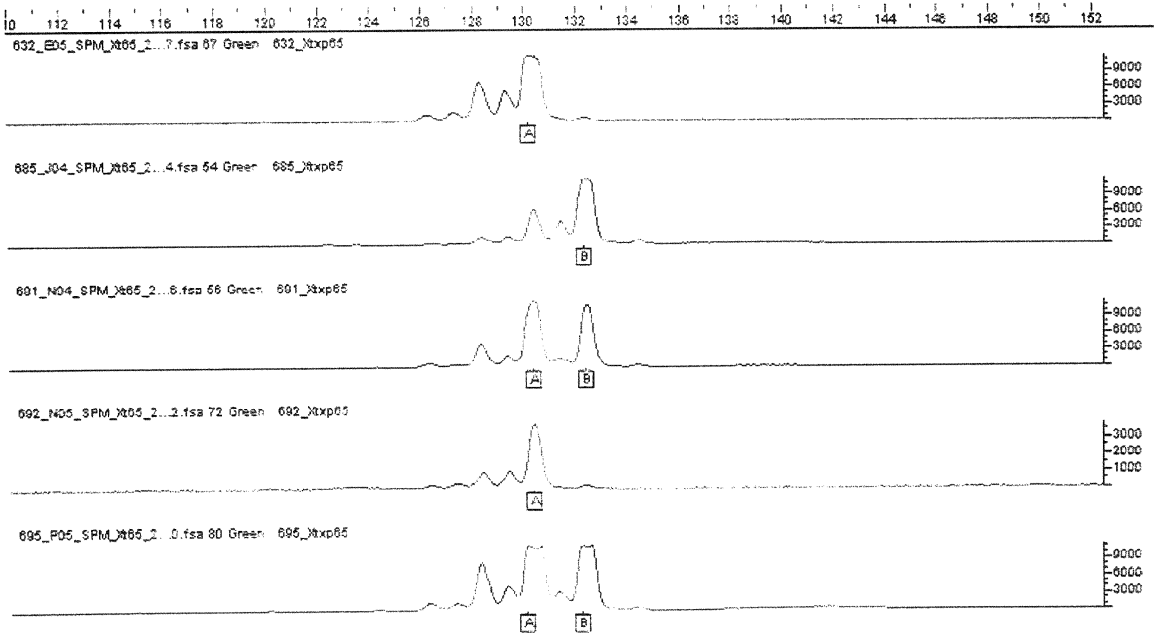


Fig 4.6. Graphical presentation (chromatograph) of BC_1F_1 population screening with SSR marker *Xtxp65* analyzed using the ABI prism 3700 DNA sequencer.

Table 4.10c List of introgressed plant with the targeted QTL and associated characters

No. of heterozygous plant selected	Targeted QTL linkage group	Shoot fly resistance trait association
3	LG A	Deadhearts I and Oviposition I
1	LG E	Deadhearts I and Oviposition I
2	LG A+E	Deadhearts I and Oviposition I
1	LG A+J	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
3	LG E+G+J	Glossiness, Trichome density (upper and lower leaf blade surfaces), Seedling vigor, Oviposition I and II, and Deadhearts I and II
3	LG A+E+J	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
1	LG A+G+J	Glossiness, Trichome density (upper and lower leaf blade surfaces), Seedling vigor, Oviposition I and II, and Deadhearts I and II

4.4.5 Screening of F_1 and BC_1F_1 populations based on recurrent parent KR 192(304) with SSR marker

4.4.5.1 Testing hybridity with SSR markers

Ten plant putatively produced from cross [KR 192(304) × IS 18551(267)] were genotyped using four SSR loci linked with targeted QTLs (Table 4.11a). All ten plants were identified at seedling stage (plate 4.2) as heterozygous and crossed with selfed progeny of recurrent parent KR 192(304) to generate BC_1F_1 seeds

4.4.5.2 Genotyping BC_1F_1 population [KR 192(304) × IS 18551(267)] × KR 192(304), recurrent parent and donor parent with SSR markers for foreground selection

Thirty plants of this backcross population were genotyped at seedling stage with eleven SSR marker loci linked to targeted QTLs for shoot fly resistance traits (Table 4.11b). Fifteen plants having appropriate allelic constitutions were identified and used as females for backcrossing (plate 4.3,4.4,4.5,4.6) with recurrent parent KR 192(304) to generate BC_2F_1 seeds. The details of selected BC_1F_1 QTL introgression

Table 4.11a: Genotypic data of hybrid (F_1) KR192(304) x IS 18551(267) for foreground selection

	KR 192(304) x IS 18851(267)									
Plant no.	511	512	513	514	515	516	517	518	519	520
SSR marker locus										
Xtxp 75(170/155)	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**
Xtxp 37(190/170)	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**
Xisp 10258(195/185)	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**
Xtxp 141	-	-	-	-	-	-	-	-	-	-

H= heterozygote KR192(304)/IS 18551(267)

-'= not amlified

Table 4.11b: Molecular data of BC1F1 population [KR192(304) × IS 18551(267)] × KR192(304), recurrent and donor parent for foreground selection

		Recurrent parent KR 192(304)								DP IS18551(267)			BC₁F₁ progeny [KR192(304) × IS 18551(267)] × KR192(304)																													
Plant	LG	Fsize	620	621	622	623	624	625	626	627	F size	628	F size	629	630	631	632	633	634	635	636	637	638	729	730	731	732	733	734	735	736	737	738	829	830	831	832	833	834	835	836	837	838
Name of SSR locus																																											
Xtxp 75	A	170	-	A	A	A	A	A	A	A	155	B	170\155	H	H	A	A	H	A	B	A	A	A	H	A	H	H	A	H	A	-	H	A	H	H	A	H	H	H	H	H	A	A
Xtxp 37	A	190	A	A	A	A	-	A	-	A	170	B	190/170	H	H	A	A	H	A	B	H	A	A	H	A	H	H	A	A	H	H	H	H	A	H	A	H	H	H	H	H	A	A
Xtxp 40	E	135	A	A	A	A	A	A	A	A	138	B	135\138	A	H	H	H	A	A	H	A	A	A	A	H	H	-	A	H	A	H	-	A	A	A	A	A	A	H	H	A	H	-
Xtxp 36	E	MONOMORPHIC											MONOMORPHIC										MONOMORPHIC										MONOMORPHIC										
Xisp 10362	E	360	MONOMORPHIC								360	-	360\360	MONOMORPHIC																				MONOMORPHIC									
Xtxp 312	E	230	A	A	A	A	A	A	A	A	180	-	230\180	A	H	H	H	A	H	H	A	A	A	A	H	H	H	A	A	A	A	H	A	A	A	A	A	A	H	A	A	H	A
Xtxp 217	G	MONOMORPHIC											MONOMORPHIC										MONOMORPHIC										MONOMORPHIC										
Xisp 10283	G	340	A	A	A	A	A	A	A	A	320	B	340\320	H	H	B	H	A	-	A	H	H	A	-	H	H	H	A	A	A	H	A	A	A	H	H	H	H	A	A	H	A	A
Xgap1	G	252	A	A	A	A	A	A	A	A	254	B	252\254	H	-	H	H	A	H	A	H	-	A	A	-	H	H	A	A	A	H	-	A	A	H	A	A	H	A	H	H	O	-
Xtxp 141	G	155	A	A	A	A	A	A	A	A	160	B	155/160	H	H	H	A	A	H	A	H	A	A	H	A	H	H	A	A	A	H	A	H	A	H	A	A	A	A	A	H	A	A
Xisp 10258	J	195	A	A	A	A	A	A	A	A	185	B	195\185	A	H	H	A	A	A	H	A	-	A	H	A	A	H	H	A	H	H	A	A	H	H	A	H	A	H	H	A	A	A
Xtxp65	J	130	A	A	A	A	A	A	A	A	132	B	130\132	A	H	H	A	A	A	H	A	-	A	H	A	A	H	H	A	H	H	A	A	-	H	A	H	A	H	H	A	A	A
Xtxp 225	J	MONOMORPHIC											MONOMORPHIC										MONOMORPHIC										MONOMORPHIC										
Xtxp15	J	224	A	-	A	A	A	A	A	A	221	B	224\221	H	H	B	H	-	A	B	H	-	H	H	H	A	A	A	A	H	A	H	H	H	A	H	H	H	H	A	A	H	A

A allele= KR 192(304)

B allele= IS 18551 (267)

H allele = Heterozygous KR192/IS 18551

O= Off type

-' = Not amplified

Selected plants are in bold font

heterozygotes with their targeted QTLs and associated characters are presented (Table 4.11c).

Table 4.11c List of selected BC_1F_1 introgression heterozygotes with their targeted QTLs and associated characters

No. of selected heterozygous plants	Targeted QTL linkage group	Shoot fly resistance trait associations
4	LG A	Deadhearts I and Oviposition I
2	LG G	Trichome density (upper and lower leaf blade surfaces), Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
5	LG A+G	Trichome density (upper and lower leaf blade surfaces), Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
3	LG A+J	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
1	LG A+E+J	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II

4.4.6 Screening of F_1 and BC_1F_1 population based on recurrent parent 20B(186) with SSR marker

4.4.6.1 Testing hybridity with SSR markers

Ten plants putatively produced from cross 20B(186) × RIL 252(318) were genotyped at seedling stage with four SSR marker loci linked with resistance trait QTLs (Table 4.12a). Only two heterozygous (F_1 hybrid) plants were (plate 4.2) identified and crossed as female with pollen from selfed progeny of recurrent parent 20B(179) to generate BC_1F_1 populations.

4.4.6.2 Genotyping BC_1F_1 populations [20B(186) × RIL 252(318)] × 20B(186), recurrent parent and donor parent with SSR markers for foreground selection

Thirty plants were screened at seedling stage with eleven SSR loci from four linkage groups associated with shoot fly resistance traits (Table 4.12b). Twelve heterozygous plants for one more targeted QTL introgressions were selected and used (plate

Table 4.12a: Genotypic data of hybrid (F_1) 20B(186) x RIL252(318) for foreground selection

	20B(186) x RIL 252(318)									
Plant no.	521	522	523	524	**525**	526	527	528	**529**	530
SSR marker locus										
Xtxp 75(170/155)	A	A	A	NG	**H**	A	A	A	**H**	A
Xtxp 37(190/170)	A	A	NA	NG	**H**	A	A	A	**H**	A
Xisp 10258(190/185)	A	A	A	NG	**H**	A	A	A	**H**	A
Xtxp 141(155/160)	A	A	A	NG	**H**	A	NA	NA	**NA**	NA

A allele= 20B(186)

H allele= heterozygote 20B(186)/RIL252(318)

NG= not germinate

NA= not amplified

Table 4.12b: Molecular data of BC1F1 population [20B(186) × RIL 252(318)] × 20B(186), recurrent and donor parent for foreground selection

			Recurrent parent 20B(186)								DPRIL 252(318)												
Plant #	LG	Fragment size	639	640	641	642	643	644	645	646	F size	647	F size	648	649	650	651	652	653	654	655	656	657
Name of SSR Locus																							
Xtxp 75	A	170	A	A	A	A	A	A	A	A	155	B	170\155	-	H	H	H	H	A	A	H	A	H
Xtxp 37	A	190	A	A	A	A	A	A	A	A	170	B	190/170	A	H	H	H	A	A	-	H	A	H
Xtxp 40	E	MONOMORPHIC											135\135										
Xtxp 36	E	MONOMORPHIC											MONOMORPHIC										MONOMORPHIC
Xisp 10362	E	350	A	A	A	A	A	A	A	A	365	B	350\365	H	H	A	A	A	A	A	H	H	-
Xtxp 312	E	170	A	A	A	A	A	A	A	A	195	B	170\195	A	A	A	A	A	A	A	H	H	H
Xtxp 217	G	MONOMORPHIC											MONOMORPHIC										MONOMORPHIC
Xisp 10263	G	320	MONOMORPHIC								320	-	320\320	MONOMORPHIC									
Xgap 01	G	254	MONOMORPHIC																				
Xtxp 141	G	155	A	A	A	A	A	A	A	A	160	B	155\160	A	A	A	A	H	H	H	H	-	H
Xisp 10258	J	190	A	A	A	A	A	-	A	A	185	B	190\185	H	H	H	A	A	H	-	A	A	A
Xtxp 65	J	130	A	A	A	A	A	A	A	A	132	B	130\132	H	H	H	A	A	H	A	A	A	A
Xtxp 225	J	MONOMORPHIC											MONOMORPHIC										MONOMORPHIC
Xtxp 15	J	223	A	A	A	A	A	A	A	A	221	B	223\221	H	A	A	H	H	H	H	A	A	H

	BC1F1 progeny [20B(186) × RIL 252(318)] × 20B(186)																				
Plant #	748	749	750	751	752	753	754	755	756	757	F size	848	849	850	851	852	853	854	855	856	857
Name of SSR Locus																					
Xtxp 75	A	H	A	A	A	H	H	A	H	A	170	H	H	H	A	A	H	A	A	A	A
Xtxp 37	A	A	A	-	A	A	A	H	A	H	195	H	H	H	A	A	H	A	A	A	A
Xtxp 40																					
Xtxp 36											MONOMORPHIC										
Xisp 10362	A	A	A	A	A	A	H	H	A	H	350	H	H	A	-	A	A	A	A	A	A
Xtxp 312	H	H	H	H	H	H	H	A	A	H	170	H	H	A	H	H	A	H	H	A	A
Xtxp 217											MONOMORPHIC										
Xisp 10263											320	DMORPHIC									
Xgap 01																					
Xtxp 141	-	H	A	A	H	A	H	H	A	A	155	H	H	H	A	H	H	A	A	H	A
Xisp 10258	H	A	H	A	H	A	A	A	H	A	190	H	A	A	A	A	H	A	A	H	A
Xtxp 65	H	A	-	A	H	A	A	A	H	A		H	A	A	-	A	H	A	A	H	A
Xtxp 225											MONOMORPHIC										
Xtxp 15	H	A	A	H	H	A	A	A	A	H		H	A	H	A	A	-	-	A	H	-

A allele= 20B(186)

B allele= RIL 252(318)

H allele= Heterozygous 20B(186)/RIL 252(318)

selected plants are in bold font

4.3,4.4,4.5,4.6) in crossing to advance to the BC_2F_1 generation. The details of introgressed QTLs and their associated resistance characters are listed below for these 12 selected BC_1F_1 plants (Table 4.12c).

Table 4.12c List of introgressed plant with the targeted QTL and characters associated

No. of heterozygous plants selected	Targeted QTL linkage group	Shoot fly resistance trait associations
6	LG A	Oviposition I, Deadhearts I
3	LG E	Oviposition I, Deadhearts I
2	LG A+E	Oviposition I, Deadhearts I
1	LG A+J	Glossiness, Seedling vigor II, Deadhearts I and II, Oviposition I and II

4.4.7 Screening of F_1 and BC_1F_1 populations based on recurrent parent 20B(179) with SSR marker

4.4.7.1 Testing of hybridity with SSR markers

Ten plants putatively produced from cross 20B(179) × RIL 153(248) were genotyped at seedling stage with four SSR markers from three linkage groups (Table 4.13a). Eight heterozygous plants were selected and used for crossing (plate 4.2) as females with pollen from selfed progeny of recurrent parent 20B(179) to advance to the BC_1F_1 generation.

4.4.7.2 Genotyping BC_1F_1 populations [20B(179) × RIL 153(248)] × 20B(179), recurrent parent, and donor parent with SSR markers for foreground selection

Thirty plants of this backcross population were screened at seedling stage with eleven SSR marker loci linked with targeted shoot fly resistance QTLs. On the basis of molecular marker data, 19 plants having appropriate allelic constitutions (heterozygous for one or more target QTL intogressions) were identified (Table 4.13b) and crossed as female with pollen from selfed progeny of recurrent parent 20B(179) to advance to the BC_2F_1 generation (plate 4.3,4.4,4.5,4.6). The details of these 19 selected plants heterozygous with various targeted QTLs (and associated characters) are presented below (Table 4.13c).

Table 4.13a: Genotypic data of hybrid (F_1) 20B9179) x RIL 153(248)for fore ground selection

	20B(179) x RIL 153(248)									
Plant no.	531	532	533	534	535	536	537	538	539	540
SSR marker locus										
Xtxp 75(170/155)	H	H	H	H	H	H	NG	H	NG	H
Xtxp 37(190/170)	H	H	H	H	H	H	NG	H	NG	H
Xisp 10258	-	-	-	-	-	-	NG	-	NG	-
Xtxp 141(155/160)	NA	H	NA	NA	H	H	NG	H	NG	H

H allele= heterozygoe 20B(179)/RIL 153(248)
NG = not germinate
NA = not amplified

Table 4.13b: Molecular data of BC1F1 population [20B(179) × RIL 153(248)] × 20B(179), recurrent and donor parent for foreground selection

	Recurrent Parent 20B(179)										DPRIL 153(248)			BC$_1$F$_1$ progeny [20B(179) × RIL 153(248)] × 20B(179)																													
Plant #	LG	Fragment size	658	659	660	661	662	663	664	665	F size	666	F size	667	668	669	670	671	672	673	674	675	676	767	768	769	770	771	772	773	774	775	776	867	868	869	870	871	872	873	874	875	876
Name of SSR locus																																											
Xtxp 76	A	170	.A	A	A	A	A	A	A	A	155	B	170\155	A	A	H	H	A	H	-	H	A	H	H	A	A	A	A	A	H	H	H	A	H	A	A	H	H	A	H	A	A	H
Xtxp 37	A	190	A	A	A	A	A	A	A	A	170	B	190/170	H	A	H	H	A	A	-	-	H	-	H	H	A	A	A	A	H	A	H	A	H	A	A	H	H	A	H	A	A	H
Xtxp 40	E	135	A	A	A	A	A	A	A	A	138	B	135\138	A	H	A	A	A	H	H	H	A	H	H	-	A	A	-	-	-	H	A	-	H	H	A	-	H	-	A	H	A	
Xtxp 36	E	MONOMORPHIC																																									
Xisp 10362	E	350	A	A	A	A	A	A	A	A	360	B	350/360	A	H	A	A	A	H	H	H	A	H	H	B	A	A	A	H	A	H	A	H	H	H	A	A	H	A	A	H	A	A
Xtxp 312	E	170	A	A	A	A	A	A	A	A	180	B	170\180	-	H	A	H	A	A	H	H	A	A	H	H	A	A	A	H	H	A	A	H	H	A	A	H	A	A	A	H	A	H
Xtxp 217	G	MONOMORPHIC																																									
Xisp 10283	G	320	A	A	A	A	A	A	A	A	340	B	320\340	A	H	H	A	H	H	H	A	H	H	H	A	H	A	H	H	H	A	H	H	H	H	A	A	A	A	H	A	H	A
Xgap 01	G	MONOMORPHIC											254\254																														
Xtxp 141	G	155	A	A	A	A	-	A	A	-	160	B	155\160	A	-	H	H	A	H	H	H	A	A	H	A	A	A	H	H	H	A	H	H	H	H	H	H	H	A	H	H	A	A
Xisp 10258	J	190	A	A	A	A	A	A	A	A	185	B	190\185	H	A	H	H	A	A	A	H	A	H	H	A	A	A	H	A	H	A	A	A	H	A	A	H	A	A	A	H	H	H
Xtxp 65	J	130	A	A	A	A	A	A	A	-	132	B	130\132	A	A	H	H	-	A	A	H	H	H	H	A	A	A	H	A	H	A	A	A	H	H	-	H	-	A	H	H	H	H
Xtxp 225	J	MONOMORPHIC																																									
Xtxp 15	J	223	A	A	A	A	A	A	A	A	221	B	223\221	H	H	B	H	A	A	H	H	H	A	A	A	A	A	A	H	H	H	H	H	H	H	A	A	A	A	H	H	H	-

A allele= 20B(179)
B allele= RIL 153(248)

H allele= Heterozygous 20B(179)/ RIL 153(248)

Selected plants are in bold font

Table 4.13c List of selected QTL introgression heterozygote plants with the targeted QTLs and associated characters

No. of hetero-zygous plants selected	Targeted QTL linkage group(s)	Shoot fly resistance trait associations
3	LG A	Deadhearts I and Oviposition I
5	LG G	Trichomes (upper and lower leaf blade surfaces), Seedling vigor II, Glossiness, Oviposition I and II, Deadhearts I and II
1	LG E	Deadhearts I and Oviposition I
3	LG A+G	Trichomes (upper and lower leaf blade surfaces), Seedling vigor II, Glossiness, Oviposition I and II, Deadhearts I and II
2	LG E+J	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
1	LG A+J	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
1	LG E+G	Trichomes (upper and lower leaf blade surfaces), Seedling vigor II, Glossiness, Oviposition I and II, Deadhearts I and II
1	LG A+E+G	Trichomes (upper and lower leaf blade surfaces), Seedling vigor II, Glossiness, Oviposition I and II, Deadhearts I and II
1	LG A+G+J	Glossiness, Trichome density (upper and lower leaf blade surfaces), Seedling vigor II, Oviposition I and II, Deadhearts I and II
1	LG A+E+J	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II

4.4.8 Screening of F_1 and BC_1F_1 populations based on) recurrent parent KR 192(300) with SSR markers

4.4.8.1 Testing hybridity with SSR markers

Nine plants thought to have been produced from cross KR 192(300) × RIL 252(319) were screened at seedling stage with four SSR marker loci associated with targeted shoot fly resistance QTLs (Table 4.14a). Eight heterozygous plants were identified (plate 4.2) and crossed as females with pollen from selfed progeny of recurrent parent KR 192(300) to generate seed of the BC_1F_1 generation.

Table 4.14a: Genotypic data of hybrid (F_1) KR 192(300) x RIL 252(319) for fore ground selection

	KR 192 (300) x RIL 252 (319)								
Plant no.	**561**	562	**563**	**565**	**566**	**567**	**568**	**569**	**570**
SSR marker locus									
Xtxp 75(170/155)	H	A	H	H	H	H	H	H	H
Xtxp 37(190/170)	H	A	H	H	H	H	H	H	H
Xisp 10258 (200/190)	H	A	H	H	H	H	H	H	H
Xtxp 141	-	-	-	-	-	-	-	-	-

A allele= KR192(300)
H allele= heterozygote KR 192(300)/ RIL252(319)
-'= not amplified

Table 4.14b: Molecular data of BC1F1 population [KR192(300) × RIL 152(319)] × KR192(300), recurrent and donor parent for foreground selection

Plant #	LG	Recurrent Parent KR 192(300) Fragment size	677	678	679	680	681	682	683	684	DPRIL 252(319) Fragment size	685	Fragment size
Name of SSR Locus													
***Xtxp* 75**	A	170	A	A	A	A	A	A	A	A	155	B	170\155
***Xtxp* 37**	A	190	-	-	A	A	A	A	A	A	170	B	190/170
***Xtxp* 40**	E	135	MONOMORPHIC								135		135\135
***Xtxp* 38**	E		MONOMORPHIC										
***Xisp* 10362**	E	360	MONOMORPHIC								360		360\360
***Xtxp* 312**	E	230	A	A	A	A	A	A	A	A	200	B	230\200
***Xtxp* 217**	G	MONOMORPHIC											
***Xisp* 10263**	G	340	A	A	A	A	A	A	A	A	320	B	340\320
***Xgap* 1**	G	252	-	A	A	A	-	A	A	A	254	B	252\254
***Xtxp* 141**	G	155	A	A	A	A	A	A	A	A	160	B	155\160
***Xisp* 10258**	J	200	A	A	A	A	A	A	A	A	190	B	200\190
***Xtxp* 66**	J	130	A	A	A	A	A	A	A	A	132	B	130\132
***Xtxp* 225**	J	MONOMORPHIC											
***Xtxp* 15**	J	224	A	A	A	A	A	A	A	A	221	B	224\221

Plant #	BC$_1$F$_1$ progeny [KR192(300) × RIL 152(319)] × KR192(300) 686	687	688	689	690	691	692	693	694	695	786	787	788	789	790	791
Name of SSR Locus																
***Xtxp* 75**	A	H	H	A	A	A	H	A	A	B	H	H	H	B	A	B
***Xtxp* 37**	A	H	H	A	A	A	H	A	A	B	H	A	A	H	A	B
***Xtxp* 40**																
***Xtxp* 38**									MONOMORPHIC							
***Xisp* 10362**									MONOMORPHIC							
***Xtxp* 312**	A	H	H	B	H	A	H	H	B	B	B	A	H	H	A	A
***Xtxp* 217**									MONOMORPHIC							
***Xisp* 10263**	A	B	A	B	A	A	A	A	H	H	H	A	H	H	A	A
***Xgap* 1**	A	B	A	B	A	H	A	A	H	H	H	A	H	H	H	A
***Xtxp* 141**	A	H	A	H	-	H	-	A	H	B	H	H	B	B	H	A
***Xisp* 10258**	H	H	A	H	A	A	A	A	A	H	B	H	H	H	B	H
***Xtxp* 66**	H	-	A	H	A	H	A	A	-	H	B	H	H	H	H	H
***Xtxp* 225**									MONOMORPHIC							
***Xtxp* 15**	A	H	A	H	A	A	A	H	H	H	B	A	H	B	A	A

Plant #	792	793	794	795	886	887	888	889	890	891	892	893	894	896
Name of SSR Locus														
***Xtxp* 75**	H	B	A	H	A	A	A	H	H	A	A	A	H	A
***Xtxp* 37**	H	B	H	H	A	A	A	H	H	A	A	A	H	A
***Xtxp* 40**														
***Xtxp* 38**			MONOMORPHIC											
***Xisp* 10362**			MONOMORPHIC											
***Xtxp* 312**	B	A	A	H	H	A	H	H	A	H	H	H	A	H
***Xtxp* 217**			MONOMORPHIC											
***Xisp* 10263**	H	B	A	A	A	H	H	H	H	H	H	H	A	H
***Xgap* 1**	H	H	A	A	A	H	H	A	H	H	H	H	H	
***Xtxp* 141**	H	B	H	H	A	H	H	A	A	A	H	H	-	H
***Xisp* 10258**	A	H	H	B	A	H	H	H	A	A	H	A	A	A
***Xtxp* 66**	A	H	H	H	A	H	H	H	A	H	H	A	A	H
***Xtxp* 225**			MONOMORPHIC											
***Xtxp* 15**	H	H	H	A	H	A	H	H	A	H	H	A	H	

A allele= KR 192(300)
B allele= RIL 252(319)
H allele= Heterozygote KR 192/RIL 252(319)
selected plants are in bold font

4.4.8.2 Genotyping BC_1F_1 population [KR 192(300) × RIL 252(319)] × KR 192(300), recurrent parent, and donor parent with SSR marker for foreground selection

Screening at seedling stage of thirty BC_1F_1 individual with eleven SSR marker loci linked to targeted shoot fly resistance QTLs was performed (Table 4.14b). The PAGE separated SSR data revealed nine heterozygous plants (plate4.3,4.4,4.5,4.6) appropriate allelic constitutions. These were crossed as female with pollen from selfed progeny of recurrent parent KR 192(300) to generate BC_2F_1 seed. The details of selected QTL introgress heterozygote plants are presented (Table 4.14c).

Table 4.14c List of introgressed plant with the targeted QTL and characters associated

No. of heterozygous plants selected	Targeted QTL linkage groups	Shoot fly resistance trait associations
4	LG A	Oviposition I, Deadhearts I
3	LG G	Trichome density (upper and lower leaf blade surfaces), Seedling vigor II, Oviposition I and II, Deadhearts I and II
1	LG A+J	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
1	LG G+J	Glossiness, Trichome density (upper and lower leaf blade surfaces), Seedling vigor II, Oviposition I and II, Deadhearts I and II

4.4.9 Parental genotyping with SSR marker primer pairs used for background selection

Initial parental screening with 38 SSR marker loci covering the entire genome except the four regions harboring targeted shoot fly resistance QTLs was carried out before actual genotyping of selected foreground BC_1F_1 plants for background screening. The main objective for screening of parental plants with these 38 SSR marker loci was to detect polymorphism among the parents. That could be used background selection to

speed recovery of recurrent parent genotype in genomic regions distant from the four targeted QTLs. The parental genotyping results (Table 3.13) revealed that SSR markers pairs viz. *Xcup*63 (LGB), *Xtxp*283 (LG B) *Xtxp*59 (LG C) and *Xtxp*17 (LGI), failed to detect polymorphism among the pairs of recurrent and donor parents. The remaining 34 SSR marker loci exhibited polymorphism among at least some of the pairs of parents. However twenty-two SSR marker loci exhibited monomorphism between one to four pairs of parents as given in Table 4.15.

Table 4.15 List of SSR marker loci that are monomorphic between pairs of parents

Name of SSR marker locus	LG	Monomorphic parental line pairs
*Xtxp*25	B	KR 192(304), IS 18551(267)
*Xtxp*296	B	KR 192(304), IS 18551(267); 20B(179), RIL 153(248)
*Xcup*11	C	20B(186), RIL 252(318)
*Xcup*61	C	20B(186), RIL 252(318)
*Xtxp*114	C	KR 192(300), RIL 252(319)
*Xgap*236	C	KR 192(304), IS 18551(267); 20B(186), RIL 252(318); 20B(179), RIL 153(248)
*Xcup*14	C	20B(186), RIL 252(318); 20B(179), RIL 153(248)
*Xgap*10	D	28B(288), RIL 189(312); 20B(186), RIL 252(318); 20B(179), RIL 153(248)
*Xcup*28	D	28B(288), RIL 189(312)
*Xgpsb*50	D	28B(288), RIL 189(312); 20B(186), RIL 252(318); 20B(179), RIL 153(248)
*Xtxp*343	D	KR 192(304), IS 18551(267)
*Xtxp*289	F	20B(186), RIL 252(318)
*Xcup*02	F	20B(186), RIL 252(318); 20B(179), RIL 153(248)
*Xtxp*230	F	KR 192(300), RIL 252(319)
*Xtxp*273	H	28B(288), RIL 189(312)
*Xtxp*47	H	20B(186), RIL 252(318); 20B(179), RIL 153(248)
*Xtxp*105	H	28B(288), RIL 189(312); 20B(186), RIL 252(318); 20B(179), RIL 153(248)
*Xtxp*354	H	20B(186), RIL 252(318)
*Xtxp*17	I	28B(288), RIL189(312); 20B(186), RIL 252(318); 20B(179), RIL 153(248); KR 192(300), RIL 252(319)
*Xtxp*57	I	20B(186), RIL 252(318)
*Xtxp*145	I	28B(288), RIL 189(312)
*Xtxp*317	I	28B(288), RIL 189(312); 20B(186), RIL 252(318)

4.4.10 Background genotyping of BC_1F_1 individuals selected on the basis of foreground selection

A total of 61 BC_1F_1 plants selected through foreground screening and forming five back cross populations, were genotyped with a set of polymorphic SSR markers (Table 4.15) covering the entire genome except the genomic regions harboring targeted shoot fly resistance QTLs (i.e. the regions covered in foreground screening). Approximately two SSR marker loci were selected to cover the top, middle, and bottom portion of each of these six non-target linkage groups. The main objective of background selection was to confirm (and hasten) recovery of the recurrent parent genome. Twelve plants were selected from five backcrossing populations. These twelve plants each carry homozygous recurrent parental alleles (A genotype) at most of the SSR loci and have a few heterozygous loci (H genotype) used for background screening (Tables 4.16, 4.17, 4.18, 4.19, 4.20). Those individuals homozygous for any donor parent allele (B genotype) were rejected as they could only have resulted from failure of backcrossing (i.e., selfing) in the previous generation. The 12 selected individuals (plate 4.7,4.8a,b,4.9a,b,4.10a,b,4.11a,b,4.12,4.13) form five populations. Each had been crossed with their respective recurrent parents to advance this marker-assisted QTL introgression programme to BC_2F_1 generation. Detail regarding selected individuals including targeted QTLs and character associations are presented in Table 4.21.

4.4.11 Genotyping of the five BC_2F_1 populations, recurrent parent, and donor parent with SSR markers for foreground selection

Genotyping of 224 BC_2F_1 plants from five backcross populations with 10 SSR marker loci linked to targeted QTLs associated with shoot fly resistance traits in four linkage groups was performed (Tables 4.22, 4.23, 4.24, 4.25, 4.26).One hundred heterozygous BC_2F_1 plants with appropriate allelic constitutions were selected at the seedling stage (plate 4.14,4.15,4.16,4.17) and crossed (as female parent)with their respective recurrent parents to generate BC_3F_1 populations. Details the number of plants genotyped, number of introgressed plant selected with its targeted QTL are presented in table 4.27.

4.4.12 Genotyping selected BC_2F_1 fore ground plants for background selection

Out of 100 BC_2F_1 selected plants in foreground screening only 68 plants from five back cross populations will be genotyped with a set of polymorphic SSR loci (Table 4.28) covering the entire genome except the region harboring targeted QTLs. This

Table 4.16: Genotyping data for background selection of foreground-selected BC $_1$F$_1$ plants

		(28B(288) x RIL 189(312)) x 28B(288)											28B (288)		RIL 189 (312)	
Fore ground					OK A+J			BEST A				Best E+G+J	Size (bp)		Size (bp)	
marker loci	LG	617	710	711	719	811	812	814	815	816	817	818		RP1		DP1
***Xbp* 75**	A	A	H	H	H	A	H	H	H	H	H	A	170	A	155	B
***Xbp* 37**	A	A	H	H	H	A	H	H	H	H	H	A	180	A	172	B
***Xbp* 40**	E	H	H	H	A	-	H	A	-	A	-	-	135	A	138	B
***Xisp* 10362**	E	H	-	H	A	H	H	A	H	A	H	H	370	A	360	B
***Xbp* 312**	E	H	H	H	H	H	H	A	A	A	H	H	165	A	185	B
***Xisp* 10263**	G	-	-	-	-	-	-	-	-	-	-	-		A	-	B
Xgap1	G	H	H	-	A	-	A	A	-	A	-	H	252	A	254	B
***Xbp* 141**	G	H	H	H	A	H	A	A	H	H	A	H	160	A	165	B
***Xisp* 10258**	J	H	H	H	H	A	H	A	H	H	A	H	190	A	185	B
***Xbp* 55**	J	H	H	H	H	A	H	A	-	H	A	H	130	A	132	B
***Xbp* 15**	J	-	H	H	H	H	H	H	-	A	H	H	225	A	223	B
Background marker																
locI																
***Xbp* 26**	B	A	A	-	A	B	B	A	A	A	A	-	130	A	140	B
***Xbp* 298**	B	A	A	A	A	H	A	H	H	H	H	A	210	A	200	B
***Xbp* 296**	B	A	A	H	H	H	H	A	H	H	H	A	150	A	170	B
***Xcup* 11**	C	A	B	-	A	B	B	-	-	B	A	A	163	A	162	B
***Xbp* 228**	C	-	A	A	H	H	-	H	H	H	H	A	240	A	250	B
***Xbp* 31**	C	B	-	A	H	B	H	H	A	B	B	A	240	A	225	B
***Xcup* 61**	C	B	-	-	H	H	A	H	H	-	H	A	199	A	196	B
***Xgap* 236**	C	H	-	A	-	H	A	H	A	-	H	-	180	A	175	B
***Xcup* 14**	C	-	-	-	-	A	H	-	A	-	A	A	211	A	207	B
***Xbp* 343**	D	A	H	H	-	H	A	A	A	-	A	A	145	A	155	B
***Xgap* 206**	F	A	A	A	A	A	A	A	A	A	A	A	110	A	120	B
***Xbp* 289**	F	-	-	H	H	A	A	H	H	A	A	A	275	A	290	B
***Xcup* 02**	F	A	A	H	H	H	H	H	A	B	-	H	210	A	200	B
***Xbp* 258**	F	A	A	H	H	H	H	H	H	H	H	A	220	A	223	B
***Xbp* 230**	F	-	A	H	-	H	A	H	-	-	H	A	170	A	190	B
***Xbp* 321**	H	B	A	A	A	B	A	A	A	-	A	A	200	A	210	B
***Xbp* 47**	H	B	B	B	A	B	A	A	B	B	A	A	290	A	280	B
***Xbp* 57**	I	A	H	H	A	H	H	A	A	H	A	A	245	A	250	B
***Xbp* 265**	I	H	H	H	H	H	A	A	H	B	A	A	200	A	220	B
***Xbp* 274**	I	H	H	H	-	H	A	A	H	A	A	A	325	A	335	B
***Xbp* 6**	I	-	A	B	A	B	B	A	H	B	-	A	115	A	80	B
Total A background		9	10	6	8	3	11	10	9	4	11	18		21		0
Total H background		3	4	9	8	12	6	9	9	5	7	1		0		0
Total B background		4	2	2	0	6	3	0	1	6	1	0		0		21
Total - background		5	5	4	5	0	1	2	2	6	2	2		0		0
Grand total background		21	21	21	21	21	21	21	21	21	21	21		21		21

A allele= 28B(288)
B allele= RIL 189(312)
H allele= Heterozygote
- Not amplified
seletced plants are in bold font

Table 4.17: Genotyping data for background selection of foreground-selected BC $_1$F$_1$ plants

		(KR 192(304) x IS18551(267)) x KR 192(304)														KR 192 (304)		IS 18551 (267)	
Fore ground		Best A+G										Best A+G				Size (bp)		Size (bp)	
Marker loci	LG	629	630	633	729	731	732	736	737	830	832	833	834	835	836		RP2		DP2
Xtxp 75	A	H	H	H	H	H	H	-	H	H	H	H	H	H	H	170	A	155	B
Xtxp 37	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	190	A	170	B
Xtxp 40	E	A	H	A	A	H	-	H	-	A	A	A	H	H	A	135	A	138	B
Xisp 10362	E	MONOMORPHIC			-	-	-	-	-			MONOMORPHIC				360	A	360	B
Xtxp 312	E	A	H	A	A	H	H	A	H	A	A	A	H	A	A	230	A	180	B
Xisp 10263	G	H	H	A	-	H	H	H	A	H	H	H	A	A	H	340	A	320	B
Xgap1	G	H	-	A	A	H	H	H	-	H	A	H	A	H	H	252	A	254	B
Xtxp 141	G	H	H	A	H	H	H	H	A	H	A	A	A	A	H	155	A	160	B
Xisp 10268	J	A	H	A	H	A	H	H	A	H	H	A	H	H	A	195	A	185	B
Xtxp65	J	A	H	A	H	A	H	H	A	H	H	A	H	H	A	130	A	132	B
Xtxp15	J	H	H	-	H	A	A	A	H	A	H	H	H	A	A	224	A	221	B
back ground marker loci																			
Xtxp 256	B	A	A	H	H	H	H	A	A	H	H	H	H	A	A	185	A	188	B
Xcup 11	C	A	A	A	A	A	H	A	A	A	A	H	A	A	A	165	A	170	B
Xtxp 228	C	-	A	A	A	A	H	A	A	A	A	H	A	A	A	245	A	225	B
Xtxp 31	C	H	A	A	-	A	A	A	H	H	A	A	A	H	A	210	A	225	B
Xcup 32	C	-	A	A	A	A	A	A	A	A	A	A	A	-	A	145	A	155	B
Xcup 61	C	A	A	A	A	A	H	A	A	-	A	H	A	A	A	196	A	199	B
Xtxp 114	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	220	A	240	B
Xcup 14	C	-	A	A	-	H	A	H	A	A	H	A	-	A	A	205	A	210	B
Xgap 10	D	A	B	A	B	-	B	B	B	A	A	B	A	A	B	300	A	250	B
Xtxp 21	D	H	H	A	A	A	A	H	-	-	-	A	H	H	A	180	A	170	B
Xcup 28	D	A	A	B	H	H	H	B	B	A	B	B	A	B	H	160	A	164	B
gpsb 050	D	H	H	A	H	H	H	A	H	A	A	A	H	A	H	245	A	210	B
Xgap206	F	A	A	H	H	A	A	H	H	A	A	A	H	A	H	125	A	110	B
Xtxp 289	F	A	H	H	H	A	A	A	A	A	A	A	A	-	A	290	A	275	B
Xcup 02	F	A	A	A	B	B	-	B	A	-	H	H	H	H	A	195	A	205	B
Xtxp 258	F	A	A	H	A	H	H	A	H	A	A	A	A	H	A	225	A	160	B
Xtxp 321	H	A	A	A	B	B	B	-	A	A	-	A	B	-	B	210	A	200	B
Xtxp 47	H	A	H	A	H	A	H	H	A	A	H	A	A	A	A	290	A	260	B
Xtxp 273	H	A	B	B	A	B	A	B	A	A	A	A	A	A	A	205	A	190	B
Xtxp 108	H	-	-	-	H	-	A	A	H	A	A	A	H	H	H	295	A	285	B
Xtxp 210	H	-	-	A	H	A	H	A	A	A	A	H	-	H	H	201	A	205	B
Xtxp 364	H	-	A	B	B	H	B	A	A	A	A	A	A	H	H	165	A	155	B
Xtxp 67	I	H	H	H	H	H	H	H	H	A	H	H	H	A	A	250	A	240	B
Xtxp 145	I	H	A	-	H	-	-	H	H	A	H	H	A	A	A	215	A	240	B
Xtxp 317	I	-	A	H	H	A	H	H	-	A	H	H	H	A	A	165	A	145	B
Xtxp 286	I	H	A	H	H	A	H	H	H	A	A	A	A	A	A	205	A	195	B
Xtxp 274	I	H	A	H	H	A	H	-	H	A	A	A	A	A	A	330	A	320	B
Xtxp 6	I	A	A	A	A	A	H	H	H	A	A	A	-	B	A	110	A	80	B
Total A background		**14**	19	15	9	15	9	13	14	**23**	18	17	16	16	20		28		0
Total H background		**7**	5	8	13	7	14	9	10	**2**	7	9	8	7	6		0		0
Total B background		**0**	2	3	4	3	3	4	2	**0**	1	2	1	2	2		0		28
Total - background		**7**	2	2	2	3	2	2	2	**3**	2	0	3	3	0		0		0
Grand total background		**28**	28	28	28	28	28	28	28	**28**	28	28	28	28	28		28		28

A allele= KR 192(304)
B allele= IS 18551(267)
H allele= heterozygote
- not amplified
selected plants are in bold font

Table 4.18: Genotyping data for background selection of foreground-selected BC $_1$F$_1$ plants

	[20B(186) x RIL 252(318)] x 20B(186)												20B (186)		RIL 252 (318)	
Fore ground			A					Best E					Size (bp)		Size (bp)	
Marker Loci	LG	649	651	655	656	657	754	757	848	849	850	853		RP3		DP3
Xtxp 75	A	H	H	H	A	H	H	A	H	H	H	H	170	A	155	B
Xtxp 37	A	H	H	H	A	H	A	H	H	H	H	H	190	A	170	B
Xtxp 40	E	MONOMORPHIC											135		135	
Xisp 10362	E	H	A	H	H	-	H	H	H	H	A	A	350	A	365	B
Xtxp 312	E	A	A	H	H	H	H	H	H	H	A	A	170	A	195	B
Xisp 10263	G	MONOMORPHIC											320		320	
Xgap1	G	MONOMORPHIC											254		254	
Xtxp 141	G	A	A	H	-	H	H	A	H	H	H	H	155	A	160	B
Xisp 10258	J	H	A	A	A	A	A	A	H	A	A	H	190	A	185	B
*Xtxp*65	J	H	A	A	A	A	A	A	H	A	A	H	130	A	132	B
*Xtxp*15	J	A	H	A	A	H	A	H	H	A	H	-	223	A	221	B
back ground marker loci																
Xtxp 25	B	B	B	B	B	B	B	A	B	A	-	B	175	A	140	B
Xtxp 298	B	H	A	H	H	A	H	H	A	H	A	H	210	A	190	B
Xtxp 296	B	A	A	B	B	A	A	A	A	B	A	A	170	A	166	B
Xtxp 31	C	A	A	A	A	A	A	-	-	-	A	H	222	A	219	B
Xtxp 114	C	-	H	A	H	-	H	A	A	-	A	A	230	A	233	B
Xtxp 21	D	-	A	A	A	H	H	-	B	H	-	H	173	A	169	B
Xtxp 27	D	H	-	A	A	H	H	H	A	A	H	H	310	A	300	B
Xtxp 343	D	H	H	H	A	A	A	H	H	H	H	A	150	A	155	B
I206	F	A	A	H	H	-	A	A	A	A	A	H	110	A	120	B
Xtxp 258	F	A	A	A	H	-	A	H	-	H	H	A	195	A	185	B
Xtxp 230	F	A	A	-	-	H	A	H	A	H	H	-	175	A	195	B
Xtxp 321	H	A	A	-	B	B	B	A	A	B	B	-	210	A	200	B
Xtxp 273	H	A	H	H	H	H	H	-	H	H	A	A	200	A	195	B
Xtxp 210	H	H	A	A	H	A	A	A	A	H	H	A	203	A	205	B
Xtxp 146	I	H	H	A	A	A	A	H	A	H	H	A	210	A	235	B
Xtxp 265	I	H	H	A	A	H	A	H	A	H	A	A	200	A	195	B
Xtxp 274	I	H	H	A	A	H	A	H	A	H	A	A	325	A	320	B
Xtxp 6	I	-	-	-	A	H	H	A	-	H	H	A	95	A	80	B
Total A background		7	9	9	8	6	10	7	11	3	8	10		18		0
Total H background		7	6	4	6	7	6	8	2	11	7	5		0		0
Total B background		1	1	2	3	2	2	0	2	2	1	1		0		18
Total - background		3	2	3	1	3	0	3	3	2	2	2		0		0
Grand total background		18	18	18	18	18	18	18	18	18	18	18		18		18

A allele= 20B(186)
B allele= RIL 252(318)
H allele= heterozygotes
- not amplified
selected plants are in bold font

Table 4.19 : Genotyping data for background selection of foreground-selected BC $_1$F$_1$ plants

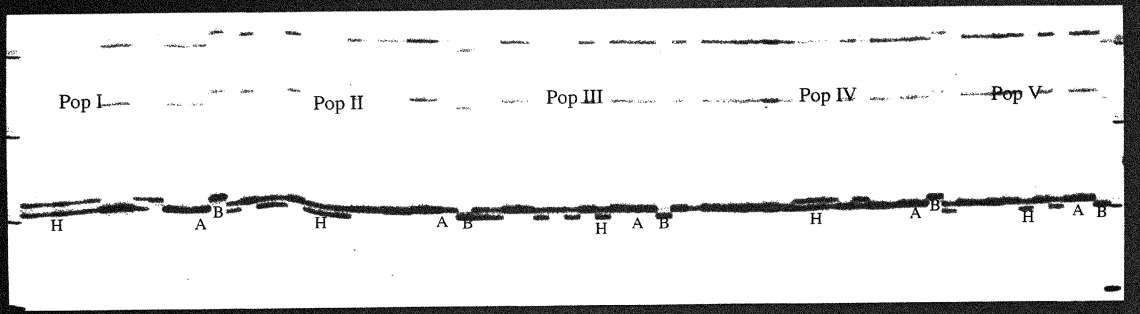
		[20B(179) x RIL 153(248)] x 20B(179)																20B (179)		RIL 153 (248)	
Fore ground		BEST A+G						OK A+G+J						OK A		OK E+J		Size (bp)		Size (bp)	
Marker loci	LG	668	**669**	670	673	674	767	772	**773**	776	867	868	870	**871**	873	**874**	876		**RP4**		**DP4**
Xtxp 75	A	A	**H**	H	-	H	H	A	**H**	A	H	A	H	**H**	H	**A**	H	170	A	155	B
Xtxp 37	A	A	**H**	H	-	-	H	A	**H**	A	H	A	H	**H**	H	**A**	H	190	A	170	B
Xtxp 40	E	H	**A**	A	H	H	H	-	**-**	-	H	H	A	**H**	A	**H**	-	135	A	138	B
Xisp 10362	E	H	**A**	A	H	H	H	H	**A**	H	H	H	A	**H**	A	**H**	A	350	A	360	B
Xtxp 312	E	H	**A**	H	H	H	H	H	**H**	H	H	A	H	**A**	A	**H**	H	170	A	180	B
Xisp 10263	G	H	**H**	A	H	A	H	H	**H**	H	H	H	A	**A**	H	**A**	A	320	A	340	B
Xgap1	G	MONOMORPHIC																			
Xtxp 141	G	-	**H**	H	H	H	H	H	**H**	H	H	H	H	**H**	H	**H**	A	155	A	160	B
Xisp 10258	J	A	**H**	H	A	H	H	A	**H**	A	H	A	H	**A**	A	**H**	H	190	A	185	B
Xtxp65	J	A	**H**	H	A	H	H	A	**H**	A	H	H	H	**-**	H	**H**	H	130	A	132	B
Xtxp15	J	H	**B**	H	H	H	A	H	**H**	H	H	H	A	**A**	H	**H**	-	223	A	221	B
Back ground marker loci																					
Xtxp 25	B	A	**A**	-	A	A	A	A	**-**	A	-	-	-	**A**	-	**A**	A	173	-	177	B
Xtxp 298	B	H	**H**	A	A	H	H	A	**A**	A	A	H	A	**A**	H	**H**	-	210	A	200	B
Xcup 11	C	-	**-**	A	-	-	H	A	**H**	A	A	H	H	**A**	H	**H**	H	175	A	185	B
Xtxp 228	C	H	**A**	A	H	A	H	A	**H**	A	A	H	H	**A**	-	**H**	H	225	A	245	B
Xtxp 31	C	A	**A**	A	A	A	A	A	**A**	A	A	A	A	**A**	A	**-**	A	221	A	219	B
Xcup 32	C	A	**A**	A	H	A	-	A	**A**	B	A	A	A	**A**	A	**A**	A	154	A	152	B
Xcup 61	C	A	**A**	A	H	A	A	A	**H**	A	-	H	H	**A**	H	**H**	H	199	A	196	B
Xtxp 21	D	H	**A**	A	-	A	-	A	**H**	-	H	H	H	**H**	A	**A**	H	175	A	180	B
Xcup 28	D	A	**-**	B	B	B	A	B	**A**	A	A	B	A	**A**	B	**A**	B	165	A	160	B
Xtxp 27	D	A	**A**	A	A	A	A	A	**-**	A	A	A	A	**A**	A	**A**	A	315	A	330	B
Xgap 206	F	A	**A**	A	H	A	H	H	**A**	H	H	A	H	**A**	A	**A**	A	110	A	120	B
Xtxp 289	F	H	**H**	A	H	A	A	A	**-**	A	H	H	A	**H**	A	**H**	A	275	A	290	B
Xtxp 258	F	H	**A**	H	H	H	H	A	**A**	A	A	A	A	**H**	A	**H**	A	190	A	194	B
Xtxp 230	F	A	**A**	H	H	H	-	A	**A**	A	A	A	A	**H**	-	**H**	A	175	A	195	B
Xtxp 321	H	B	**A**	A	A	A	A	B	**-**	-	A	A	B	**A**	B	**A**	A	210	A	200	B
Xtxp 273	H	A	**H**	A	A	A	H	H	**A**	A	A	A	A	**A**	A	**H**	H	216	A	219	B
Xtxp 210	H	H	**A**	A	A	A	A	A	**H**	A	A	A	H	**H**	H	**H**	-	203	A	205	B
Xtxp 67	I	A	**A**	H	A	A	A	A	**A**	H	H	H	H	**H**	A	**A**	A	240	A	250	B
Xtxp 145	I	A	**A**	A	H	A	-	A	**A**	H	H	H	A	**H**	A	**A**	A	215	A	240	B
Xtxp 317	I	A	**A**	-	A	A	A	A	**A**	A	A	A	A	**A**	A	**A**	A	155	A	160	B
Xtxp 265	I	A	**A**	A	A	A	A	A	**A**	H	H	H	A	**H**	A	**A**	A	200	A	210	B
Xtxp 274	I	A	**A**	A	A	A	A	A	**A**	H	H	H	A	**H**	A	**A**	A	325	A	335	B
Xtxp 6	I	A	**A**	A	H	A	H	H	**A**	B	B	H	H	**A**	A	**A**	A	95	A	115	B
Total A background		15	**18**	17	11	18	12	18	**14**	14	13	10	13	**14**	14	**12**	15		22		0
Total H background		6	**3**	3	9	3	7	3	**5**	5	7	11	8	**9**	4	**9**	5		0		0
Total B background		1	**0**	1	1	1	0	2	**0**	2	1	1	1	**0**	2	**0**	1		0		23
Total - background		1	**2**	2	2	1	4	0	**4**	2	2	1	1	**0**	3	**2**	2		1		0
Grand total background		23	**23**	23	23	23	23	23	**23**	23	23	23	23	**23**	23	**23**	23		23		23

A allele= 20B(179)
B allele= RIL 153(248)
H aleele= heterozygote
- not amplified
selectd plants are inbold font

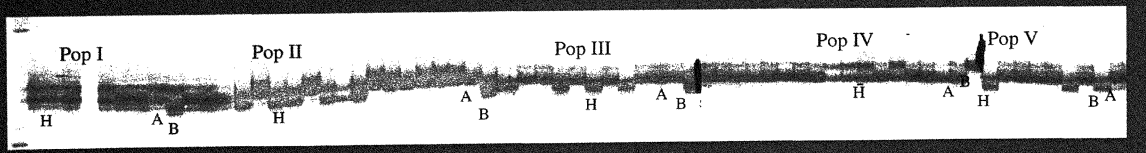
Table 4.20: Genotyping data for background selection of foreground-selected BC $_1$F$_1$ plants

	(KR 192(300) x RIL 252(319) x KR 192(300)										KR 192 (300)		RIL 252 (319)	
Fore ground					Best A+J					BEST G	Size (bp)		Size (bp)	
Marker loci	LG	688	692	887	**889**	890	892	893	894	**895**		RP5		DP5
Xtxp 75	A	H	H	A	H	H	A	A	H	**A**	170	A	155	B
Xtxp 37	A	H	H	A	H	H	A	A	H	**A**	190	A	170	B
Xtxp 40	E	MONOMORPHIC									135		135	
Xisp 10362	E	MONOMORPHIC									360		360	
Xtxp 312	E	H	H	A	H	A	H	H	A	H	230	A	200	B
Xisp 10263	G	A	A	H	**H**	H	H	H	A	H	340	A	320	B
Xgap1	G	A	A	H	**A**	H	H	H	H	H	252	A	254	B
Xtxp 141	G	A	-	H	**A**	A	H	H	-	H	155	A	160	B
Xisp 10258	J	A	A	H	H	A	H	A	A	**A**	200	A	190	B
Xtxp65	J	A	A	H	H	A	H	A	A	H	130	A	132	B
Xtxp15	J	A	A	A	H	A	H	A	H	H	224	A	221	B
Back ground marker loci														
Xtxp 25	B	A	B	A	**A**	B	A	A	A	**A**	150	A	140	B
Xtxp 298	B	A	B	B	**A**	-	A	A	A	**A**	180	A	190	B
Xtxp 296	B	H	B	A	**A**	A	H	H	-	H	170	A	165	B
Xcup 11	C	A	B	H	H	A	A	H	A	H	165	A	175	B
Xtxp 228	C	A	H	H	H	A	A	H	A	H	245	A	225	B
Xtxp 31	C	H	A	H	H	A	H	H	A	H	210	A	220	B
Xcup 32	C	H	-	A	**A**	A	H	H	-	H	145	A	155	B
Xcup 61	C	A	H	H	H	A	-	H	A	H	196	A	199	B
Xgap 236	C	H	-	A	**A**	A	H	H	B	**A**	176	A	172	B
Xcup 14	C	A	A	A	**A**	A	A	A	A	**A**	205	A	210	B
Xgap 10	D	B	B	-	**A**	B	B	B	A	**A**	295	A	250	B
Xtxp 21	D	H	H	A	**A**	H	A	H	A	H	180	A	170	B
Xcup 28	D	H	B	A	**A**	-	H	A	-	**A**	160	A	1164	B
gpsb 060	D	A	H	A	H	H	H	A	A	**A**	245	A	210	B
Xgap 206	F	H	H	A	H	A	H	H	H	**A**	125	A	120	B
Xtxp 289	F	H	B	H	H	H	A	H	H	H	290	A	275	B
Xcup 02	F	A	H	H	H	H	H	H	H	H	195	A	205	B
Xtxp 258	F	A	B	H	H	H	A	H	H	H	225	A	190	B
Xtxp 321	H	B	B	B	**A**	A	A	B	B	**A**	210	A	200	B
Xtxp 47	H	H	H	H	H	H	H	H	H	**A**	280	A	260	B
Xtxp 273	H	A	A	A	**A**	A	A	A	A	**A**	205	A	195	B
Xtxp 105	H	H	-	-	**A**	A	-	H	H	**A**	295	A	285	B
Xtxp 210	H	H	H	A	**A**	A	A	-	H	-	201	-	205	B
Xtxp 354	H	H	H	A	-	A	A	H	H	**A**	170	A	155	B
Xtxp 67	I	-	A	A	**A**	A	H	A	H	**A**	250	A	240	B
Xtxp 146	I	H	A	A	**A**	A	H	A	H	**A**	215	A	235	B
Xtxp 317	I	B	A	A	**A**	A	B	A	-	**A**	157	A	153	B
Xtxp 265	I	H	A	A	**A**	A	H	A	H	**A**	220	A	190	B
Xtxp 274	I	H	A	A	**A**	A	H	A	H	**A**	330	A	320	B
Xtxp 6	I	H	H	A	-	A	H	A	A	**A**	100	A	80	B
Total A background		10	8	18	**18**	20	12	12	12	**19**		29		0
Total H background		16	10	8	**10**	6	14	15	12	**10**		0		0
Total B background		3	9	2	**0**	2	2	2	2	**0**		0		30
Total - background		1	3	2	**2**	2	2	1	4	**1**		1		0
Grand total background		30	30	30	**30**	30	30	30	30	**30**		30		30

A allele= KR 192(300)
B allele= RIL 152(319)
H allele= heterozygote
- not amplified
selected plants are in bold font



***Xtxp* 265**



***Xtxp* 274**

Plate 4.7. Background genotyping of foreground-selected plants from five BC_1F_1 populations (data for Pop I to V as shown in Tables 4.16, 4.17, 4.18, 4.19 and 4.20, respectively) using SSR loci *Xtxp*265 and *Xtxp* 274

*Xtxp*298

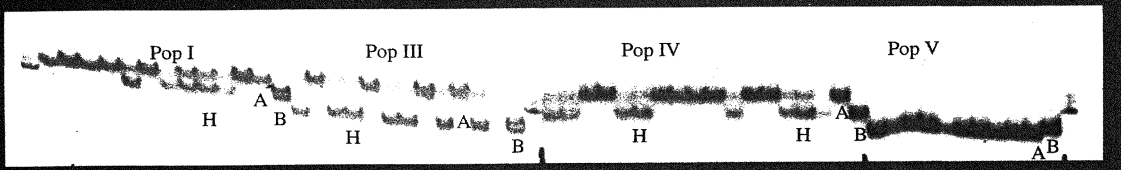


Plate 4.8a. Background genotyping of foreground-selected plants from four BC_1F_1 populations (data for Pop I, III, IV and V as shown in Tables 4.16, 4.18, 4.19 and 4.20, respectively) using SSR locus *Xtxp298*

*Xtxp*57

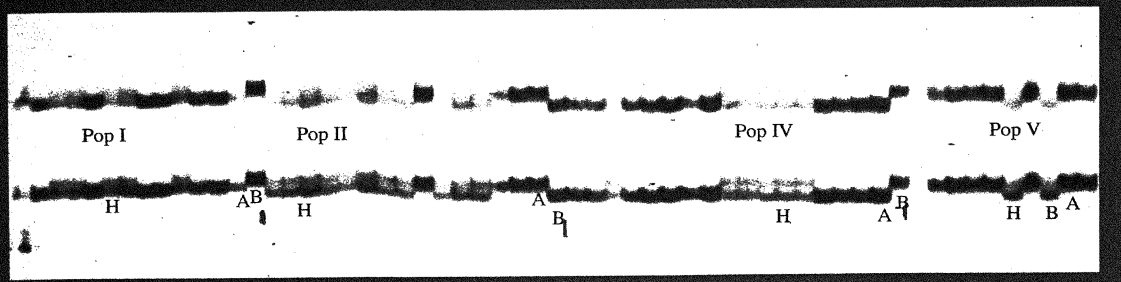


Plate 4.8b. Background genotyping of foreground-selected plants from four BC_1F_1 populations (data for Pop I, II, IV and V as shown in Tables 4.16, 4.17, 4.19 and 4.20, respectively) using SSR locus *Xtxp57*

*Xtxp*289

Plate 4.9a. Background genotyping of foreground-selected plants from three BC_1F_1 populations (data for Pop I, II, and IV as shown in Tables 4.16, 4.17, and 4.19, respectively) using SSR locus *Xtxp*289

*Xtxp*145

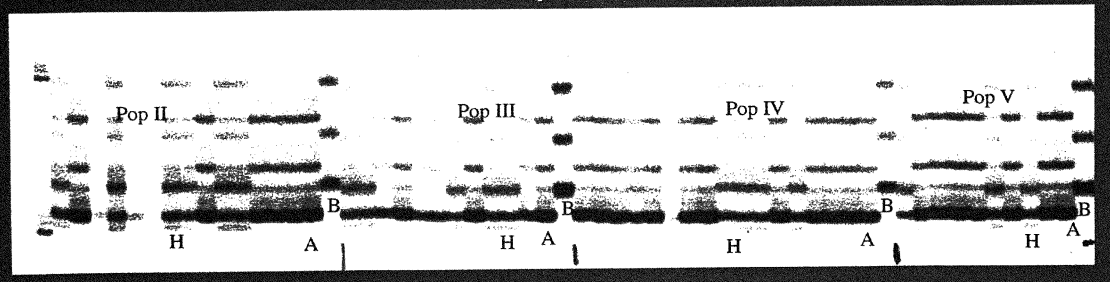


Plate 4.9b. Background genotyping of foreground-selected plants from four BC_1F_1 populations (data for Pop II, III, IV and V as shown in Tables 4.17, 4.18, 4.19, and 4.20, respectively) using SSR locus *Xtxp*145

*Xtxp*31

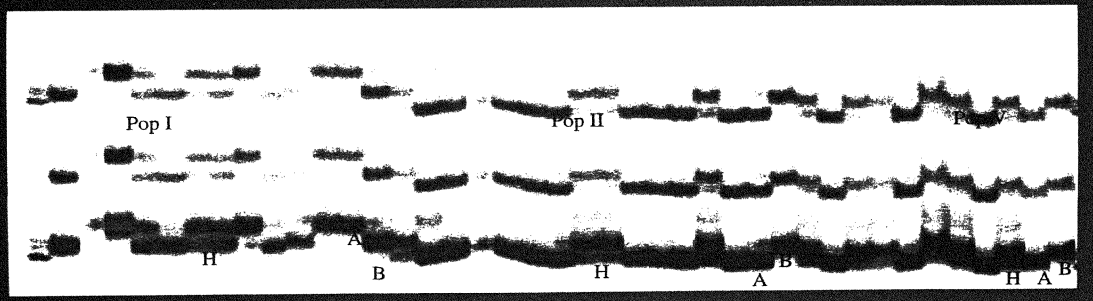


Plate 4.10a. Background genotyping of foreground-selected plants from three BC_1F_1 populations (data for Pop I, II, and V as shown in Tables 4.16, 4.17, and 4.20, respectively) using SSR locus *Xtxp*31

*Xtxp*21

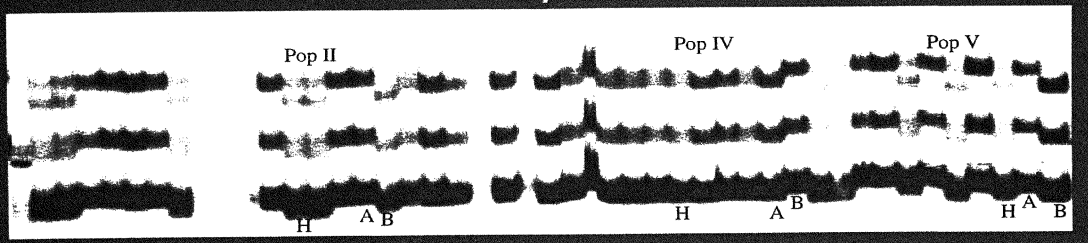


Plate 4.10b. Background genotyping of foreground-selected plants from three BC_1F_1 populations (data for Pop II, IV, and V as shown in Tables 4.17, 4.19, and 4.20, respectively) using SSR locus *Xtxp*21

*Xcup*02

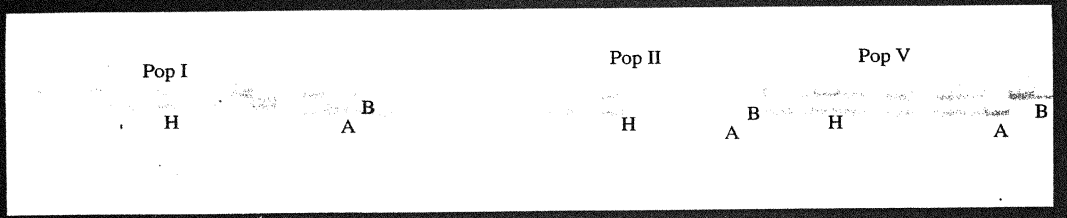


Plate 4.11a. Background genotyping of foreground-selected plants from three BC_1F_1 populations (data for Pop I, II, and V as shown in Tables 4.16, 4.17, and 4.20, respectively) using SSR locus *Xcup02*

*Xtxp*258

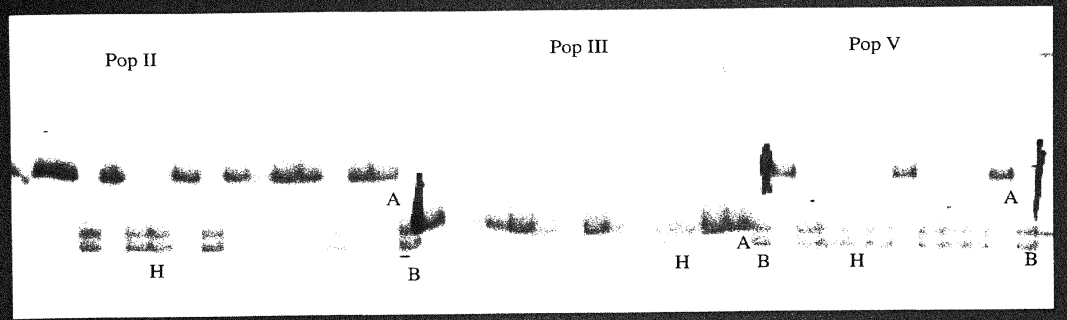


Plate 4.11b. Background genotyping of foreground-selected plants from three BC_1F_1 populations (data for Pop II,III, and V as shown in Tables 4.17, 4.18, and 4.20, respectively) using SSR locus *Xtxp*258

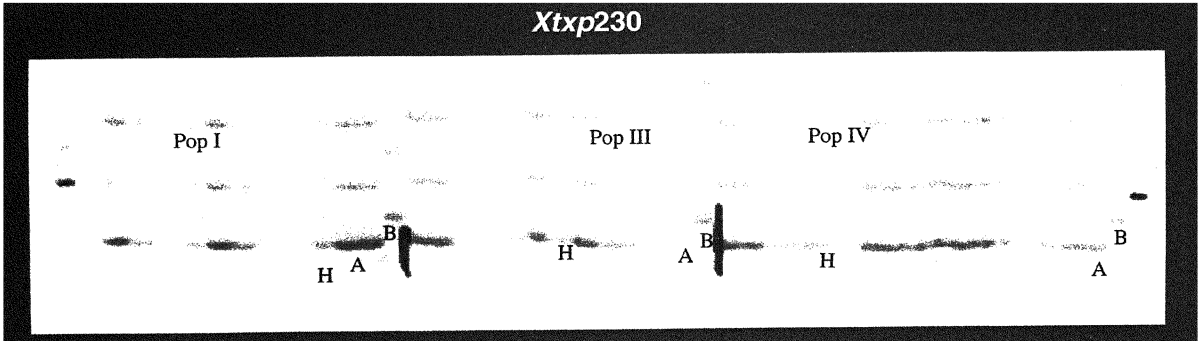


Plate 4.12. Background genotyping of foreground-selected plants from three BC_1F_1 populations (data for Pop I, III, and IV as shown in Tables 4.16, 4.18, and 4.19, respectively) using SSR locus *Xtxp*230

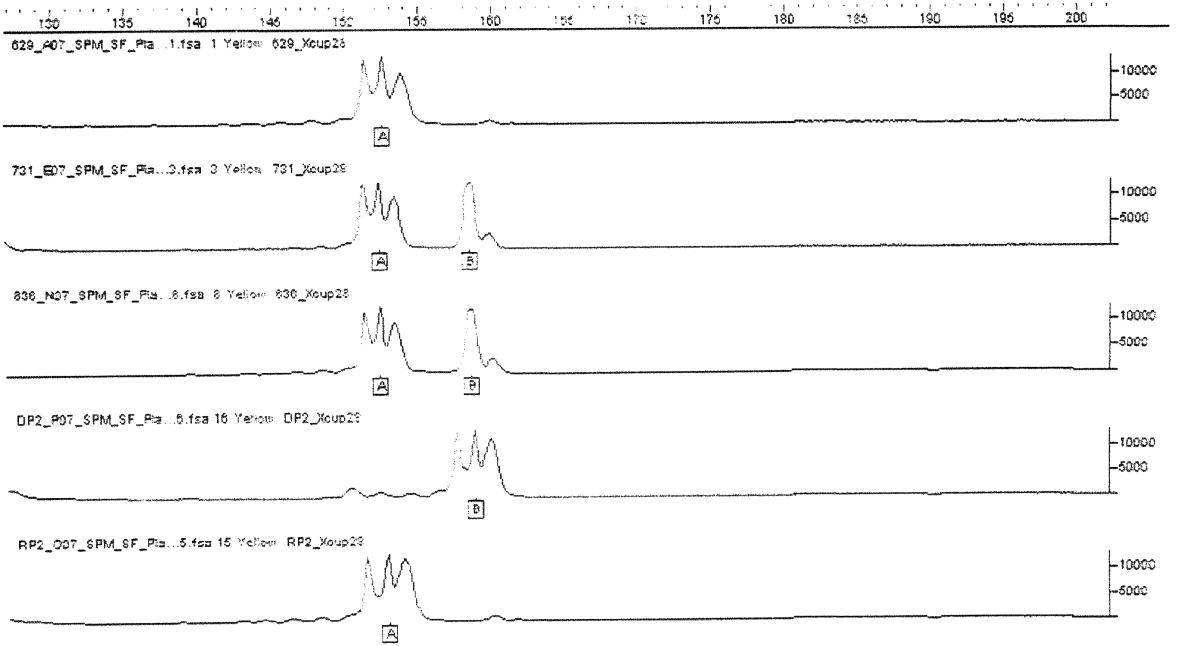


Fig 4.13. Graphical presentation (chromatograph) of selected BC_1F_1 individuals (background screening) with SSR marker *Xcup*28 analyzed using the ABI prism 3700 DNA sequencer.

Table 4.22 Molecular data of BC_2F_1 population [28B(288) × RIL 189(312) × 28B(288)] × 28B(288), recurrent parent and donor parent for foreground selection

	LG	Fragment size	719 A+ J							719 A+ J							814 A							814 A						
Plant no.			1001	1002	1003	1004	1005	1006	1007	1201	1202	1203	1204	1205	1206	1207	1008	1009	1010	1011	1012	1013	1014	1208	1209	1210	1211	1212	1213	1214
SSR locus																														
Xtxp75	A	170/155	H	H	A	H	H	H	H	-	A	H	H	A	A	H	A	H	H	A	H	H	H	A	A	A	A	H	A	A
Xtxp37	A	180/172	H	-	H	H	A	A	H	A	A	B	H	A	A	H	A	H	H	A	H	H	B	A	B	A	A	H	A	A
Xtxp40	E	135/138	-	A	-	A	H	H	H	H	B	H	H	A	A	A	A	A	A	A	A	-	A	A	A	A	A	A	A	A
Xsp10362	E	370/360	-	A	H	A	H	H	B	H	B	H	H	OFF	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Xtxp 312	E	165/185	A	H	H	H	H	H	A	-	H	A	H	OFF	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Xsp10263	G	320	MONOMORPHIC							MONOMORPHIC							MONOMORPHIC							MONOMORPHIC						
Xgap1	G	252/254	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Xtxp141	G	158/165	A	A	A	A	A	A	A	A	A	A	A	OFF	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Xsp258	J	190/185	-	A	H	A	H	A	A	H	A	H	H	A	A	H	A	A	A	A	A	A	-	A	A	A	.	A	A	A
Xtxp65	J																													
Xtxp15	J	225/223	H	A	A	H	A	B	H	A	H	A	B	B	H	H	H	H	A	H	H	H	B	H	A	H	H	H	H	H

A allele= 28B(288)

B allele= RIL 189(312)

H allele= Heterozygote

selected plants are in bold font

Table 4.22 cont.

818 E+G+J														818 E+G+J													28B(288)		RIL189(312)	
1015	1016	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028	1215	1216	1217	1218	1219	1220	1221	1222	1223	1224	1225	1226	1227	1228	F size	RP	F size	OP
A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	170	A	155	B
A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	180	A	172	B
A	H	H	A	H	A	A	A	H	A	A	A	-	A	A	H	A	H	H	-	A	A	A	H	H	H	H	H	135	A	138	B
A	H	H	A	H	A	A	A	H	A	A	A	A	A	A	H	A	H	H	H	A	A	A	A	H	H	H	H	370	A	360	B
A	A	H	A	H	A	A	A	A	A	A	A	A	A	A	H	A	H	H	A	A	A	A	A	A	H	H	H	165	A	185	B
MONOMORPHIC														MONOMORPHIC														320	A	320	B
A	H	A	H	H	-	A	H	H	A	H	A	H	H	H	H	H	H	A	A	H	A	A	H	A	A	H	B	252	A	254	B
A	A	A	A	H	H	A	A	A	A	H	H	H	A	H	A	H	H	A	H	H	H	H	H	A	A	H	B	158	A	165	B
A	A	H	A	A	H	A	A	A	A	H	H	A	A	H	A	H	H	H	A	H	A	H	A	A	A	H	A	190	A	185	B
A	A	A	A	A	A	A	A	-	A	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	225	A	223	B

Table 4.23: Molecular data of BC_1F_{11}(KR 192(304) × IS 18551 (267) × KR 192(304)] × KR 192(304), recurrent and donor parent for foreground selection

	LG	Fragment size	629 A+G							629 A+G							830 A+G												
Plant no.			1029	1030	1031	1032	1033	1034	1035	1229	1230	1231	1232	1233	1234	1235	1036	1037	1038	1039	1040	1041	1042	1043	1044	1045	1046	1047	1048	1049
SSR locus																														
Xbp75	A	170/155	H	B	H	A	H	A	H	H	H	A	H	B	H	H	A	B	H	H	H	B	H	A	A	H	B	B	A	H
Xbp37	A	195/175	H	A	H	H	H	A	A	H	A	A	H	B	A	H	A	H	A	H	H	H	H	H	H	A	H	B	-	A
Xbp40	E	135/138	A	-	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Xsp10362	E	360/360	NONMORPHIC														MONOMORPHIC													
Xbp312	E	230/180	A	A	A	A	-	A	A	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Xsp10263	G	340/320	H	H	H	B	H	H	H	H	H	H	H	H	H	B	A	-	B	A	B	B	H	A	H	B	H	-	H	A
Xgap1	G	252/254	B	H	H	A	H	H	H	H	A	H	H	A	B	B	A	H	B	A	H	A	H	A	H	B	H	H	H	A
Xbp141	G	155/160	H	H	H	H	H	H	H	H	A	H	H	H	B	B	H	H	B	A	H	A	H	A	B	B	H	H	H	A
Xsp10258	J	195/185	-	A	A	A	A	A	A	A	-	A	A	A	A	A	A	H	A	A	A	A	B	A	H	H	B	B	H	H
Xbp15	J	224/221	H	A	H	H	H	H	H	A	H	A	H	B	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A

	LG	Fragment size	830 A+G														KR 192(304)		IS 18551(267)	
Plant no.			1236	1237	1238	1239	1240	1241	1242	1243	1244	1245	1246	1247	1248	1249	F size	RP 2	F size	DP 2
SSR locus																				
Xbp75	A	170/155	H	H	B	H	B	H	H	B	H	H	H	H	A	H	170	A	155	B
Xbp37	A	195/175	A	H	A	H	H	H	B	B	H	H	H	H	H	B	195	A	175	B
Xbp40	E	135/138	-	-	A	-	A	A	A	A	A	A	A	A	A	A	135	A	138	B
Xsp10362	E	360/360															360		360	
Xbp312	E	230/180	A	A	A	A	A	A	A	A	A	A	A	A	A	A	230	A	180	B
Xsp10263	G	340/320	B	B	B	H	H	A	B	B	A	H	H	B	A	B	340	A	320	B
Xgap1	G	252/254	B	B	H	H	H	H	B	H	H	H	H	B	A	A	252	A	254	B
Xbp141	G	155/160	H	B	H	A	B	B	H	H	H	H	H	H	A	H	155	A	160	B
Xsp10258	J	195/185	B	-	B	-	H	H	B	H	H	H	H	H	H	H	195	A	185	B
Xbp15	J	224/221	A	A	A	A	A	A	A	A	A	A	A	A	A	A	224	A	221	B

A allele= KR 192(304)
B allele= IS 18551(267)
H = heterozygote
- not amplified
selected plants are in bold font

Table 4.24: Molecular data of BC_2F_1 [20B(186) × RIL 252(318) × 20B (186)] × 20B(186), recurrent parent and donor parent for foreground selection

	LG	Fragment size	757 E							757 E							20B(186)		RIL 252(318)	
Plant no.			1050	1051	**1052**	**1053**	1054	**1055**	1056	1250	1251	1252	**1253**	1254	1255	1256	F size	RP 3	F size	DP 3
SSR locus																				
Xtxp 75	A	170/155	A	A	**A**	**A**	A	**A**	A	A	A	A	**A**	A	A	A	170	A	155	B
Xtxp 37	A	195/180	H	A	**H**	**H**	A	**A**	H	H	H	A	**A**	A	H	H	195	A	180	B
Xtxp 40	E	135/135	MONOMORPHIC						MONOMORPHIC									A		B
Xisp 10362	E	350\365	A	H	**H**	**H**	A	**H**	A	H	H	A	**H**	H	H	A	350	A	365	B
Xtxp 312	E	170/195	A	A	**H**	**H**	A	**H**	A	A	A	A	**H**	A	A	H	170	A	195	B
Xisp 10263	G	320/320	MONOMORPHIC						MONOMORPHIC								320	A	320	B
Xgap 1	G	254/254	MONOMORPHIC						MONOMORPHIC											
Xtxp 141	G	155/160	A	A	**A**	**A**	A	**A**	A	A	A	A	**A**	A	A	A	155	A	160	B
Xisp 10258	J	190/185	A	A	**A**	**A**	A	**A**	A	A	A	A	**A**	A	A	A	190	A	185	B
Xtxp 15	J	223/221	H	A	**H**	**A**	A	**A**	H	A	H	H	**A**	H	A	A	223	A	221	B

A allele= 20B(186)
B allele= RIL 252(318)
H allele= heterozygote
selected plants are in bold font

Table 4.26: Molecular data of BC_1F_1 [20B(179) × RIL 153(248) × 20B (179)] × 20B(179), recurrent parent and donor parent for foreground selection

	LG	Fragment size	669 A +G							669 A +G							773 A+ G+J											
Plant no.			1057	1058	1059	1060	1061	1062	1063	1257	1258	1259	1260	1261	1262	1263	1064	1065	1066	1067	1068	1069	1070	1071	1072	1073	1074	1075
Name of primer																												
Xbp75	A	170/155	H	A	A	H	A	A	H	A	H	A	H	H	H	H	A	H	H	H	A	H	H	H	H	H	H	A
Xbp37	A	195/180	H	A	A	H	A	A	H	A	H	A	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H	A
Xbp40	E	135/138	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	-	A	A	A	A
Xsp10362	E	370/360	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Xbp312	E	170/180	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	H	H	A	A	H	H	H	H	H	H	A
Xsp10263	G	320/340	H	A	H	H	H	H	H	A	A	A	H	H	A	H	A	H	A	H	H	H	A	A	H	A	A	H
Xgap1	G	254/254	MONOMORPHIC							MONOMORPHIC							MONOMORPHIC											
Xbp 141	G	155/160	H	H	A	A	H	H	H	A	H	H	H	A	A	A	A	H	A	A	A	A	A	A	A	H	A	A
Xsp10258	J	190/185	H	H	H	H	A	A	H	H	H	H	A	H	H	H	H	A	H	H	A	A	H	H	H	A	H	H
Xbp15	J	223/221	H	A	A	A	H	H	A	A	H	A	H	A	A	A	H	A	A	H	H	A	A	H	A	A	A	H

A allele= 20B 179)

B allele= RIL 153(248)

H heterozygous

- not amplified

selected plants are in bold font

Table 4.25 continue...

773 A+ G+J															871 A													871 A			
1076	1077	1264	1265	1266	1267	1268	1269	1270	1271	1272	1273	1274	1275	1276	1277	1078	1079	1080	1081	1082	1083	1084	1278	1279	1280	1281	1282	1283	1284
H	A	A	H	H	A	H	H	A	H	H	H	H	H	H	H	H	H	H	H	A	H	A	H	H	A	H	A	H	H
H	A	A	H	A	A	H	H	H	H	A	A	H	H	H	H	H	H	H	H	A	H	A	H	-	A	A	H	A	H
A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	A	H	A	A	H	H	H	B	H	-	A
A	A	A	A	-	A	-	A	A	-	A	-	A	A	A	A	A	A	-	-	H	A	A	H	H	H	B	H	A	A
H	A	A	H	H	A	A	H	H	A	H	A	A	A	H	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A
H	A	H	H	H	A	A	A	A	A	A	H	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
MONOMORPHIC																MONOMORPHIC							MONOMORPHIC						
H	A	H	H	A	A	A	H	A	H	A	A	A	A	A	A	H	A	A	-	H	A	H	A	H	H	-	H	A	H
H	H	A	H	H	H	A	H	A	H	A	H	A	H	A	H	A	A	A	A	A	A	A	A	A	H	A	A	H	A
H	H	A	H	H	H	H	A	H	H	A	H	H	A	H	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A

Table 4.25 continue...

874 E+J													874 E+J		208(179)		RIL 153(248)	
1085	1086	1087	1088	1089	1090	1091	1285	1286	1287	1288	1289	1290	1291	Fragmer	RP 4	Fragmer	DP 4
A	A	A	A	A	A	A	A	A	A	A	A	A	A	170	A	155	B
A	A	A	A	A	A	A	A	A	A	A	A	A	A	195	A	180	B
A	H	H	A	A	H	H	H	A	A	A	A	A	H	135	A	138	B
A	H	H	H	A	H	H	H	A	-	A	H	-	-	370	A	360	B
A	H	H	H	H	H	H	H	A	A	A	H	A	H	170	A	180	B
A	A	A	A	A	A	A	A	A	A	A	A	A	A	320	A	340	B
MONOMORPHIC							MONOMORPHIC							254		254	
A	A	A	A	A	A	A	A	A	A	H	A	A	H	155	A	160	B
H	H	A	-	H	H	H	A	A	H	A	A	A	H	190	A	185	B
A	A	A	-	A	A	A	A	A	A	A	-	A	A	223	A	221	B

Table 4.26: Molecular data of BC_2F_1 [(KR 192(300) × RIL 252(319) × KR 192(300)] × KR 192(300) , recurrent parent and donor parent for foreground selection

			889 A+J													889 A+J								
Plant no.	LG	Fragment size	**1092**	1093	1094	1095	1096	**1097**	**1098**	1099	1100	**1101**	1102	1103	1104	1105	1292	1293	1294	1295	**1296**	**1297**	**1298**	1299	1300
SSR locus																									
Xtxp75	A	170/155	H	A	A	A	A	H	A	A	A	H	A	A	A	H	A	A	A	A	H	H	H	A	A
Xtxp37	A	195/180	H	A	H	A	H	H	-	H	H	H	A	A	A	H	-	H	A	A	H	H	H	A	A
Xtxp40	E	135/135	MONOMORPHIC														MONOMORPHIC								
Xisp10362	E	360/360	MONOMORPHIC														MONOMORPHIC								
Xtxp312	E	230/200	A	H	A	A	A	A	H	H	H	H	A	A	H	A	H	A	A	H	H	H	A	A	A
Xisp10263	G	340/320	A	A	H	A	A	H	H	A	A	A	H	H	A	H	H	A	H	H	A	H	A	A	A
Xgap1	G	252/254	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Xtxp 141	G	155/160	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Xisp10258	J	200/190	A	H	H	H	A	A	H	A	A	A	A	H	A	A	A	A	A	H	A	H	H	A	A
Xtxp15	J	224/221	H	A	H	A	H	H	H	H	A	A	A	H	H	A	H	H	A	A	A	A	H	A	A

			889 A+J					895 G														KR 912(300)		RIL 252(319)	
Plant no.	LG	Fragment size	**1301**	**1302**	**1303**	1304	1305	**1106**	1107	1108	**1109**	1110	1111	1112	1306	1307	1308	1309	1310	1311	1312	Fragment	RP 5	Fragment	DP 5
SSR locus																									
Xtxp75	A	170/155	A	H	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	170	A	155	B
Xtxp37	A	195/180	A	H	H	B	A	A	H	H	-	A	A	H	A	A	A	A	A	-	H	195	A	180	B
Xtxp40	E	135/135	MONOMORPHIC												MONOMORPHIC							135	A	135	B
Xisp10362	E	360/360	MONOMORPHIC												MONOMORPHIC							360		360	
Xtxp312	E	230/200	H	H	H	A	H	H	A	H	H	A	H	H	A	A	A	B	H	H	A	230	A	200	B
Xisp10263	G	340/320	H	A	-	A	H	H	H	A	H	A	A	A	H	A	H	A	H	H	A	340	A	320	B
Xgap1	G	252/254	A	A	A	A	A	H	-	A	H	A	A	A	-	A	A	A	H	H	H	252	A	254	B
Xtxp 141	G	155/160	A	A	A	A	A	H	A	A	H	A	A	A	A	A scored H	A	A	A	A	A	155	A	160	B
Xisp10258	J	200/190	H	H	H	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	200	A	190	B
Xtxp15	J	224/221	H	A	H	H	A	A	H	A	A	H	H	H	H	A	H	H	H	H	H	224	A	221	B

A allele= KR 192(300)
B allele= RIL 252(319)
H heterozygous
- not amplified
selected plants are in bold font

Table 4.27: Details foreground selection of five BC_2F_1 populations with targeted shoot fly resistance QTL character associations

Name of back cross	No. of plants genotyped	No. of SSR loci used	No. of introgressed plants selected	Targeted QTL linkage group	Shoot fly resistance trait associations
{[28B(288) × RIL 189(312)] × 28B(288)} × 28B(288)	56	10	7	LG A	Ovi I, DH I
			6	LG E	Ovi I, DH I
			4	LG G	Tri (u), Tri (l), SvII, DH I and II
			1	LG J	Glo, Sv I, Ovi I and II, DH I and II
			3	LG E+G	Tri (u), Tri (l), Ovi I and II, Sv II, DH I and II
			1	LG A+J	Glo, Sv II, Ovi I and II, DH I and II
{[KR 192(304) × IS 18551(267)] × KR 192(304)} × KR 192(304)	42	10	3	LG A	DH I and Ovi I
			4	LG G	Tri (u), Tri (l), Glo, Sv II, Ovi I and II, DH I and II
			7	LG A+G	Tri (u), Tri (l), Glo, Sv II, Ovi I and II, DH I and II
{[20B(186) × RIL 252(318)] × 20B(186)} × 20B(186)	14	10	4	LG E	DH I and Ovi I
{[20B(179) × RIL 153(248)]× 20B(179)} × 20B(179)	70	10	20	LG A	Ovi I, DH I
			6	LG E	Ovi I, DH I
			3	LG G	Glo, Tri (u), Tri (l), Sv II, DH I and II, Ovi I and II
			5	LG J	Glo, Sv II, Ovi I and II, DH I and II
			3	LG A+G	Tri (u), Tri (l), Sv II, DH I and II, Ovi I and II, Glo
			5	LG A+J	Glo, Sv II, DH I and II, Ovi I and II
			3	LG A+G+J	Glo, Tri (u), Tri (l), Sv II, DH I and II, Ovi I and II
{[KR 192(300) × RIL 252(319)] × KR 192(300)} × KR 192(300)	42	10	7	LG A	Ovi I and DH I
			2	LG G	Tri (u), Tri (l), Sv II, DH I and II, Ovi I and II, Glo
			5	LG J	Glo, Sv II, Ovi I and II, DH I and II
			1	LG A+J	Glo, Sv II, Ovi I and II, DH I and II

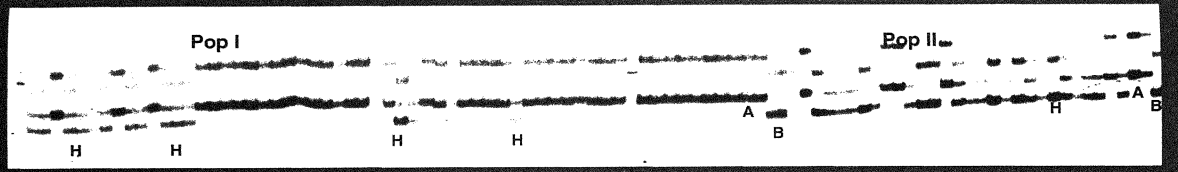


Plate 1

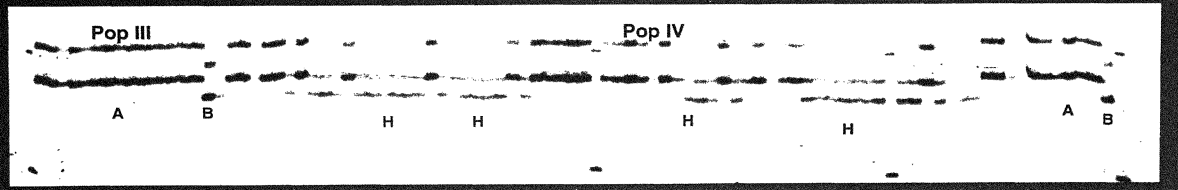


Plate 2

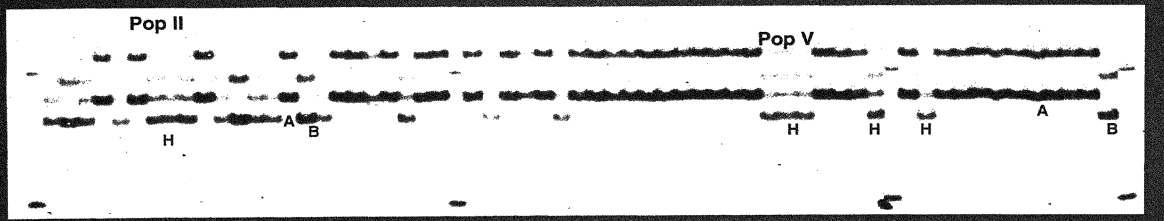


Plate 3

Plate 4.14 Genotyping of five BC_2F_1 populations (data for Pop I to V as shown in Tables 4.22, 4.23, 4.24, 4.25 and 4.26, respectively) for foreground screening using SSR locus *Xtxp75* on three different plates (Plate 1, Plate 2 and Plate 3)

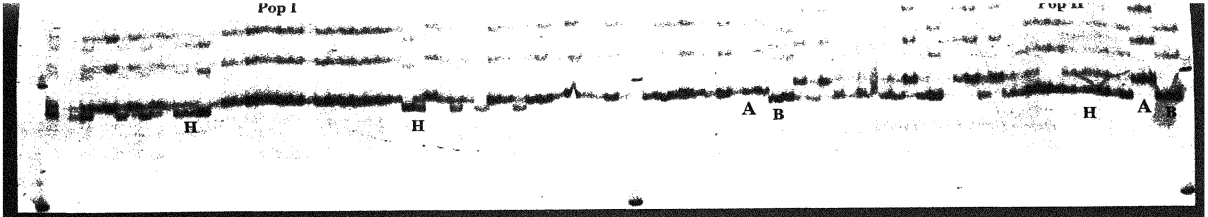


Plate 1

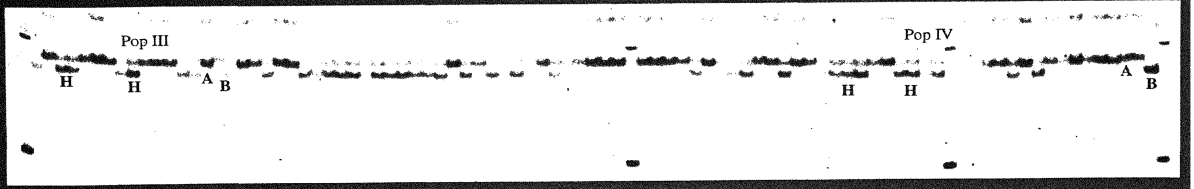


Plate 2

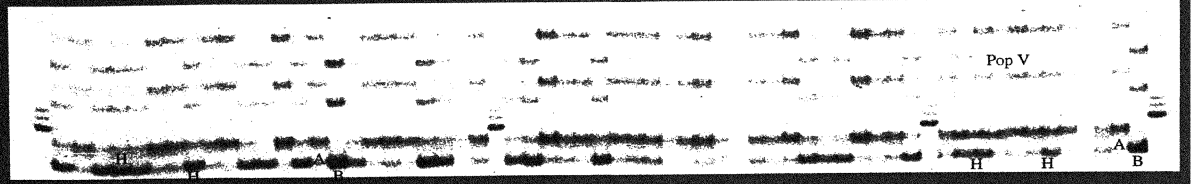


Plate 3

Plate 4.15 Genotyping of five BC_2F_1 populations (data for Pop I to V as shown in Tables 4.22, 4.23, 4.24, 4.25 and 4.26, respectively) for foreground screening using SSR locus *Xtxp37* on three different plates (Plate 1, Plate 2 and Plate 3)

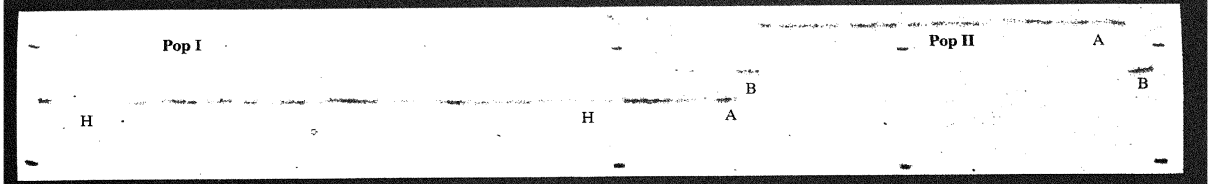


Plate 1

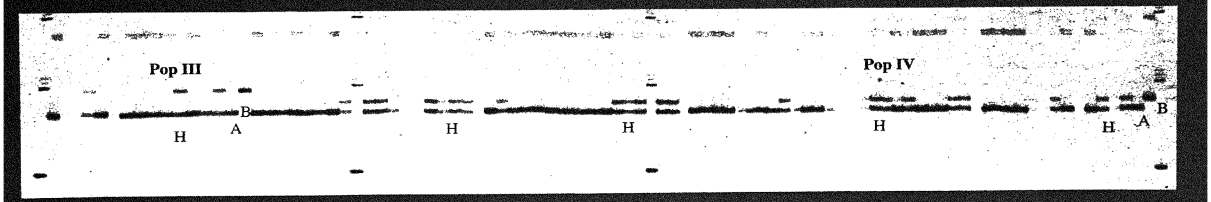


Plate 2

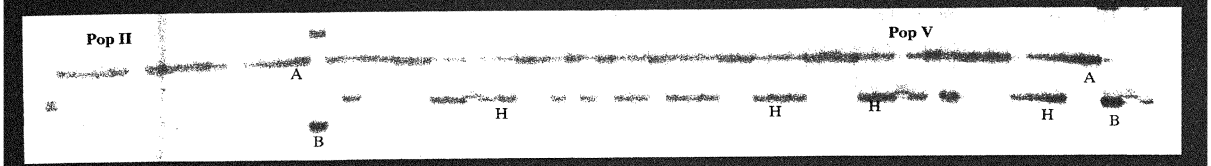


Plate 3

Plate 4.16. Genotyping of five BC_2F_1 populations (data for Pop I to V as shown in Tables 4.22, 4.23, 4.24, 4.25 and 4.26, respectively) for foreground screening using SSR locus *Xtxp312* on three different plates (Plate 1, Plate 2 and Plate 3)

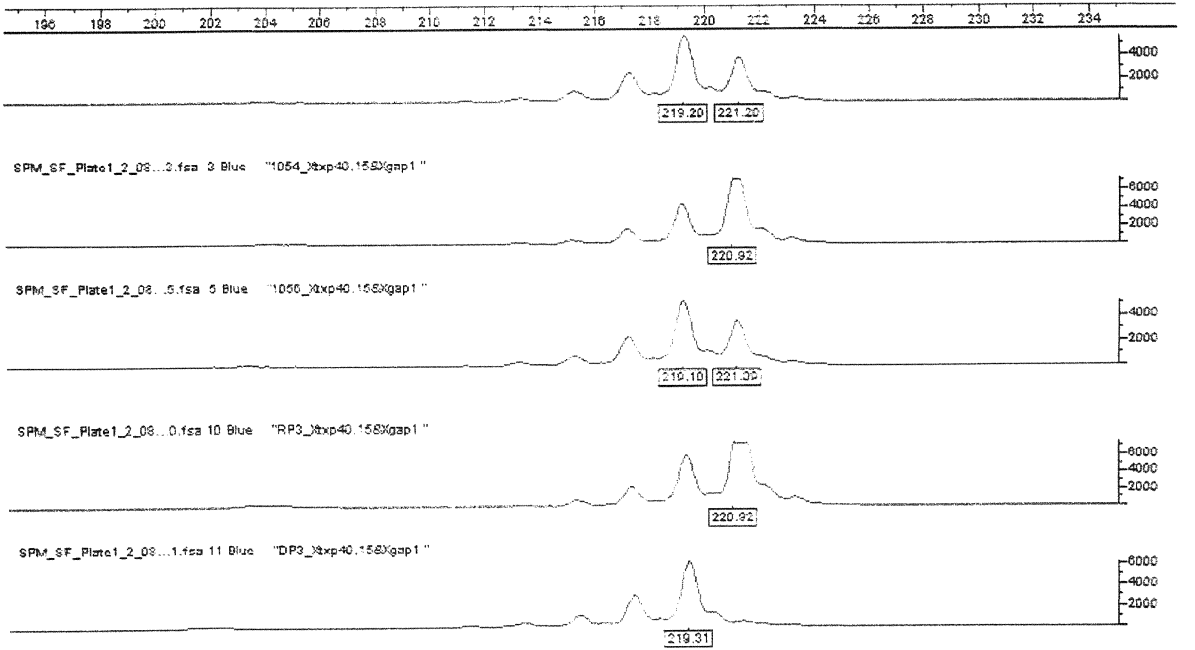


Fig 4.17. Graphical presentation (chromatograph) of BC_2F_1 population screening with SSR marker *Xtxp*15 analyzed using the ABI prism 3700 DNA sequencer.

background screening will be restricted to the loci that were heterozygous and or not amplified in BC_1F_1 generation background screening.

Table 4.28 Details of BC_2F_1 fore ground selected plants chosen for back ground screening

Back cross population	Plants selected	Targeted QTLs
BC_2F_1 {[28B(288) × RIL 189(312) × 28B(288)]} × 28B(288)	18	5 LG A 4 LG E 4 LG G 1 LG J 3 LG E+G 1 LG A+J
BC2F1 [KR 192(304) × IS 18551(267) × KR 192(304)] × KR 192(304)	12	2 LG A 3 LG G 7 LG A+G
BC2F1 [20B (186) × RIL 252(318) × 20B(186)] × 20B(186)	04	4 LG E
BC2F1 [20B (179) × RIL 153(248) × 20B(179)] × 20B(179)	24	3 LG A 4 LG E 3 LG G 3 LG J 3 LG A+G 5 LG A+J 3 LG A+G+J
BC2F1 [KR 192(300) × RIL 252(319) × KR 192(300)] × KR 192(300)	10	4 LG A 2 LG G 3 LG J 1 LG A+J

DISCUSSION

CHAPTER V
DISCUSSION

Shoot fly is a major insect pest of cultivated sorghum. Adoption of chemical control method is not economically feasible for most of the sorghum-growing farmers. Therefore host plant resistance is itself excellent pest controlling method, and when integrated with other methods of insect control offers a sound approach to deal with insect pest. This approach holds great potential for sorghum, which is known to be poor mans crop. Shoot fly resistance involves number of component traits, which are quantitative in nature and influenced by G × E interaction. There fore the phenotypic selection for this trait is difficult. Marker-assisted selection could increase efficiency of breeding of such traits. Efforts are being made in this study to carry out experiments on genetic diversity analysis, QTL mapping and marker-assisted selection for shoot fly resistance in sorghum. The discussions on results obtained are presented below objective wise.

5.1 Application of SSR markers in diversity analysis of sorghum insect resistance germplasm accessions

Genetic resources have evolved as a product of domestication, intensification, diversification and improvement through conscious and unconscious selection by countless generations of farmers. These landraces and improved cultivars provide the basic and strategic raw materials for crop improvement the world over for present and future generations (Mangala Rai, 2002).

Sorghum has an immense range of genetic resources, with much of the genetic variability available in the African regions where domestication first occurred, and in the Asian regions of early introduction. In Africa, the genetic variability occurs as cultivated species, wild crop progenitors and wild species (de Wet and Harlan, 1971; Gebrekidan, 1982). Landraces and wild relatives of cultivated sorghum from the centers of diversity have been rich sources of resistance to new pathogens, insect pests and other stresses such as high temperature and drought, as well as sources of traits to improve food and fodder quality, animal feed and industrial products. However, this natural genetic diversity is under threat through natural selection, the destruction of habitats, by the spread of agricultural practice, and adoption of improved varieties.

To prevent the extinction of landraces and wild relatives of cultivated sorghum the collection and conservation of sorghum germplasm was accelerated about four

decades ago. Since then, germplasm collection and conservation has become an integral component of several crop improvement programs at both national and international levels (Rosenow and Dalhberg, 2000).

Analysis of the extent and distribution of genetic variation in a crop are essential in understanding the evolutionary relationship between accessions and to sample genetic resources in a more systematic fashion for breeding and conservation purposes. Traditionally, taxonomists classified genetic resources in sorghum based mainly on morphological markers (Harlan and de Wet, 1972; Murty *et al.*, 1967;). Harlan and de Wet (1972) used a small number of easily recognizable traits, including grain shape, glumes, and panicle shape, to partition variability in cultivated sorghums into five races and 10 intermediate (hybrid combinations of the major races) forms. The morphological traits used by Harlan and de Wet (1972) are conditioned by a relatively small number of genes (Doggett, 1988). However, several complex quantitative traits, which are related to habitat adoption and particular end use of the crop, exhibit enormous variability among sorghum accessions within each race (de Wet *et al.*, 1976). Thus, classifying germplasm accessions based solely on a few discrete morphological characteristics may not provide an accurate indication of the genetic divergence among the cultivated genotypes of sorghum. Since both natural and human selection have contributed to genetic differentiation in sorghum (Murty *et al.*, 1967), landraces of the same race grown in different habitats may have greater genetic dissimilarity than those of different races from the same habitat.

Biochemical and molecular markers are now widely used as tools to assess the validity of taxonomic classification in cop plants. Allozyme markers have been used extensively to evaluate the extent and pattern of genetic variation in sorghum (Aldrich *et al.*, 1992; Morden *et al.*, 1989, 1990). Allozymes did not clearly separate the various races of cultivate and wild sorghum. Instead, these markers showed some degree of differentiation related to geographic area of origin. Because allozymes only measure variation at a very limited number of sites, these results may not reflect overall patterns of genetic variation throughout the genome (Aldrich *et al.*, 1992).

Restriction fragments length polymorphism (RFLP) and RAPD markers can overcome the limitation of allozymes because they have the potential to identify a large number of polymorphism with good coverage of the entire genome (Melchinger, 1993). These techniques have been used to characterize genetic diversity and phylogenetic relationship in sorghum (Aldrich and Doebley, 1992; Cui *et al.*, 1995;

Deu *et al.*, 1994; Tao *et al.*, 1993). However, these studies provided conflicting results concerning the degree of differentiation among cultivated races of sorghum. These studies also assessed genetic diversity either in a relatively small number of accessions (Aldrich and Doebley, 1992; Cui *et al.*, 1995; Tao *et al.*, 1993) or in non-random accessions selected on the basis of a prior information from other studies (Due *et al.*, 1994). Thus, extensive random sampling from the world collection of sorghum germplasm resources may allow a less biased assessment of the genetic diversity of the crop. Recently microsatellite or SSR (Simple Sequence Repeat) loci, which correspond to tandomly repeated DNA with a very short repreat unit have been introduced as powerful genetic markers in plants (Morgante and Olivieri 1993; Powell *et al.*, 1996a). Comparative studies in crop plants have shown that microsatellite markers are more variable than most other molecular markers (Powell *et al.*, 1996a ; Taramino and Tingey, 1996; Pejic *et al.*, 1998) and provide a powerful methodology for discriminating between genotypes (Yang *et al.*, 1994; Russel *et al.*, 1997; Bredmeijer *et al.*, 1998). SSRs are a highly useful class of such PCR-based genetic markers. Although costly to develop relative to some other classes of genetic marker, once developed their analysis is both easy and inexpensive. They are co-dominant, occur in high frequency, and can display a high level of polymorphism even among closely related accessions. Their high information content and other favorable characteristics made then excellent genetic markers for many types of investigation including marker-assisted selection and finger printng of germplasm collections (Brown *et al.*, 1996).

The analysis of genetic diversity and relatedness between individuals within a species or between different species or populations, is a central task for many disciplines of biological science. Genetic diversity and phylogenetic studies were initially conducted using qualitative and quantitative traits, which are mostly morphological. Using various statistical methods such as analysis of variance (ANOVA), covariance, and diversity measures such as Mahalnobis D^2 statistic, metroglyph analysis and Principal Components Analysis.These analyses are mostly based on quantitative traits that are highly influenced by environmental effects and require tedious statistical procedures. Molecular markers are being widely used in various areas of plant breeding as important tools for evaluating genetic diversity and determining cultivar identity (molecular fingerprinting). Establishment of a molecular marker and phenotypic assessment database of crop germplasm will help breeders to

trace down the origins and degrees of relatedness of many landraces and cultivars. Considering the potential of molecular markers crop breeders can extend their hands to use these to supplement other tools currently exploited in their crop breeding activities.

In this study, we tried to assess the genetic diversity of a set of 91 elite sorghum germplasm accessions using SSR markers. The set includes 12 shoot fly resistant accessions, 15 stem borer resistant accessions, 9 accessions resistant to both shoot fly and stem borer, 17 midge resistant accessions and 38 agronomically elite recurrent parents that were used at ICRISAT to initiate large-scale marker-assisted backcross program for the stay-green component of terminal drought tolerance from donors B35 and E36-1, from which QTLs for this trait have been previously mapped (Subudhi *et al.*, 2000; Haussmann *et al.*, 2002).

Genomic DNA isolation was done by the CTAB method. DNA of the 91 sorghum accessions were then genotyped using 21 SSR primer pairs that detected loci distributed over 9 of the 10 linkage groups in the sorghum nuclear genome. The NTSYS statistical software package was used for cluster analysis. Jaccard's similarity coefficient between each pair of accessions was used to construct a dendrogram using the unweighted paired group method with arithmetic averages (UPGMA).

5.1.1 Poly acrylamide gel electrophoresis (PAGE)

PCR products from 20 SSR primer pairs and template DNA samples from 91 sorghum accessions were separated electrophoretically using six percent denaturing polyacrylamide gels. The allelic composition of each genotype was determined by scoring silver-stained gels of the separated amplified products. Twenty out of 21 SSR primer pairs used provided amplification products, while 11 out of these 20 revealed high levels of polymorphism (> 0.5) among the 91 sorghum accessions. A total of 69 alleles were detected by silver staining. An average 3.45 fragments were amplified per SSR locus among the 91 sorghum accessions studied.

The polymorphic information content (PIC) value range observed for these SSR loci was 0.13 to 0.83. The highest level of polymorphism was found with the primer pair for SSR locus *Xgap*84 (0.83), followed by those for *Xtxp*15 (0.82) and *Xtxp*320 (0.77). The lowest polymorphism was found with the primer pair for SSR locus *Xcup*62 (0.13) (Table 4.1). These results in agreement with Smith *et al.* (2000) and Kamala *et al.* (2005) using the SSR molecular marker system in different sorghum germplasm accessions.

5.1.2 PAGE dendrogram

Jaccard's coefficient of similarity for pairs of the 91 sorghum accessions studied ranged from 0.28 to 1.00. The dendrogram for the genetic similarity between accessions based on PAGE-generated SSR genotypic data showed clustering for geographic origin, sorghum races, raw germplasm versus elite breeding lines and specific traits such as insect resistance. The accessions studied were broadly grouped into clusters representing 4 of the 5 sorghum races (*Durra*, *Caudatum* (including elite material derived from *Zera Zera* landraces), *Bicolor*, and *Guinea*). These results agree with those observed previously by Tao et al. (1993) and Oliveria et al. (1996) using other molecular marker systems. The 91 sorghum accessions studied diverged into 20 clusters at the 50% level of similarity (Fig 4.2). Among these the largest was cluster 4 (18 accessions) was followed by cluster 12 (13 accessions) and cluster 13 (12 accessions). However, some of the clusters (cluster 5, 7, 9, 10, 14 and 20) accommodated only a single accession each and clusters 1, 3, and 6 accommodated only 2 accessions each.

Cluster 4 included 18 genotypes, most of which exhibit resistance to sorghum shoot fly and spotted stem borer. All sorghum genotypes in this cluster originated from the *Durra* race. This group contains genotype IS 18551, which has been used as the resistant parent in development of two ICRISAT shoot fly resistance mapping populations. Maiti et al. (1984) reported most of the shoot fly resistant accessions belong to *Durra* group and some others to taxonomic groups such as *Guinea*, *Caudatum* and *Bicolor*. Premkishore and Jotiwani (1979), Sharma et al. (1983), Prem kishore (1984 reported most stem borer resistance sources belonged to the *Durra* group among sorghums of Indian origin, followed by *Caudatum*, *Conspicuum* (a subgroup within the *Guinea* race), *Caffrorum* (a subgroup within the *Kaffir* race), *Roxburghii* (a subgroup within the *Guinea* race) and *Nervosum* (a subgroup within the *Bicolor* race).

These stem borer resistance sources are tall, late in maturity, susceptible to lodging, photoperiod sensitive, low yielding and possess moderate to high degrees of resistance. IS 21121, IS 2265, IS 2312, IS 2195 and IS 2123 from cluster 4 have been identified as sources of resistance against both sorghum shoot fly and spotted stem borer. These results are in agreement with reports by Jothwani and Devies (1979), and Prem kishore and Jotwani (1982.), Most of the genotype pairs in this cluster have

operational bootstrap values greater than 50%, which provides confidence about their clustering.

Cluster 8 contains 4 genotypes representing an intermediate population developed from *Durra* × *Caudatum* crosses. These are all elite breeding lines and known for their combination shoot fly resistance with better agronomic performance. The genotypes from this group could be used as sources for the development of a new mapping population for grain yield and shoot fly resistance.

Cluster 12 was comprised largely of ICRISAT-bred improved breeding lines having sorghum midge resistance in agronomically superior *Caudatum* background with *Zera Zera* landrace parentage and excellent grain quality. According to Rosseto et al. (1975); Sharma et al. (1992) and (1993a), TAM 2566, AF 28, DJ 6514 are stable sources of resistance to sorghum midge. Jothwani and Davies (1979) and Sharma and Davies(1981) reported that most of identified midge resistance sources belong to the *Caudatum / Negricans-ZeraZera, Caudatum/Kaffir (Hegari) / Durra-Negricans/ Caudatum-Bicolor* groups of sorghum.

Cluster 11 and 13 consisted of largely recurrent parents for the stay-green backcrossing program and hence were comprised largely of improved *Caudatum* race materials including *Zera Zera* landrace derivatives. According to Hash et al. (2003) marker-assisted selection for QTLs controlling the stay-green trait (a component of terminal drought tolerance) in sorghum is in progress at ICRISAT Patancheru. Six QTLs of relatively large effect from donor parent B35, which have independently mapped by two or more groups of earlier workers, are targeted in this program with agronomically elite genetically diverse sorghum varieties R16, ICSV 111, IRAT 204, and ISIAP Dorado as recurrent parents.

Cluster 1 had only two genotypes, 296B and HC 260. Elite hybrid maintainer line 296B is susceptible to insect pests but is a potent combiner for high grain yield. It has been used extensively in the *kharif* season hybrid breeding program in India. This elite line has been used as the susceptible parent in a shoot fly resistance QTL mapping population developed at ICRISAT.

Cluster 6 contains genotypes BTx263 and Supanburi 11. Both genotypes are susceptible to sorghum shoot fly. BTx263 was used as the susceptible parent for the first ICRISAT sorghum RIL population developed for QTL mapping of shoot fly resistance. Cluster 16 had three genotypes: LS 1 and LS 2 originated from the Peoples' Republic of China, and the third genotype, i.e. Malisor 84-7, was developed

from *Guinea* race crosses to *Caudatum* material in ICRISAT's breeding program in Mali. All three are potential recurrent parents for the stay-green marker-assisted backcrossing program. Single genotype clusters 19 and 20 appear to represent the grassy *Bicolor* race of sorghum.

Molecular genetic diversity analysis was carried out on 91 sorghum accessions differing in agronomic eliteness and the level of resistance in several insect pests using the allelic information from 20 SSR loci as revealed by silver-staining of PAGE-separated PCR products. This analysis revealed that the accessions studied are genetically quite diverged, with sorghum accessions showing midge, shoot fly, and stem borer resistance clustering in different groups. In addition, clusters of agronomically superior recurrent parents have been identified that are genetically quite divergent from each of these insect resistant clusters. However, some of the midge, shoot fly and stem borer resistant accessions cluster separately indicating that these accessions might contain new allelic variants that should be exploited in applied breeding programs. This information will be useful to identify parents for use in marker-assisted backcross introgression of insect resistance QTLs from the currently available mapping populations (in some cases from more agronomically elite pedigree-derived insect resistant breeding lines) as well as for identifying additional parental line pairs to use in developing new mapping populations to detect additional insect resistance QTLs.

5.1.3 Capillary electrophoresis (ABI)

The genotypes studied using separation of PCR products on PAGE were also assessed for their polymorphism using automated capillary electrophoresis (ABI 3100/ABI 3700 sequencing machine). A total of 118 alleles generated by 20 SSR primers were detected. On average 5.1 fragments were amplified per SSR locus. Thirteen out of 20 (65 %) of SSR primer pairs detected high levels of polymorphism with PIC values >0.5. The PIC values observed ranged from 0.21 to 0.81. The highest level of polymorphism was found with primer pairs for SSR locus *Xtxp*320 (0.81) and the lowest polymorphism was found with primer pairs for SSR locus *Xcup*60 (0.21) (Table 4.1). Thus, the most of the polymorphic groups of sorghum SSR markers did not show substantial changes across the two PCR product separation and visualization systems.

5.1.4 ABI dendrogram

The Jaccard's coefficient of similarity ranged from 0.21 to 1.00 among pairs of the 91 sorghum accessions studied. Marker alleles detected in the ABI-generated data sheets grouped the 91 sorghum genotypes into 28 clusters at the 50% level of similarity. When compared to the dendrogram from the PAGE-generated data sheet, the number of clusters detected with ABI-generated data sheet was comparatively higher (Fig 4.3). This may be due to the greater sensitivity of the automated sequencer, which allows it to detect SSR alleles differing by smaller numbers of repeat units so that it can effectively indicate higher levels of polymorphism.

The largest cluster was cluster 17, which consisted of 14 genotypes. Many of the genotypes of this cluster show midge fly resistance and are agronomically elite lines selected as a potentially recurrent parents for the ICRISAT marker-assisted breeding program for the stay-green component of terminal drought tolerance. All accessions in this cluster are agronomically elite *Caudatum*-type breeding lines and released varieties.

Twelve and seven genotypes were grouped in 3rd and 4th clusters, respectively. These originated from the *Durra* race and possess moderate to high levels of resistance to sorghum shoot fly and spotted stem borer. Actually the two above-mentioned clusters based on the ABI-generated data sheet (3 and 4) formed a single cluster in PAGE-generated data sheet. The use of a single representative from ABI-generated cluster 3 and another from cluster 4 as resistant parents in two new mapping populations targeting shoot fly and/or spotted stem borer resistance would seem to be a reasonable starting point.

Cluster 14 contains six improved genotypes, most of them with sorghum shoot fly resistance and some of them with sorghum midge resistance. Cluster 15, which could be designated as a cluster of agronomically superior midge resistant breeding lines, included five genotypes. Nearly all genotypes in clusters 14 and 15 were developed at ICRISAT-Patancheru from crosses designed to introgressed insect resistance into elite *Zera Zera* landrace background materials having a superior agronomic characteristics and excellent grain quality. Single selected genotypes from each of these two clusters could be used for the development of two new mapping populations targeting QTLs for grain yield, grain quality and insect resistance.

Compared to the clustering pattern obtained from the PAGE-generated data set many more genotypes formed single-genotype clusters at 50% similarity when the

ABI-generated data set was used. Among these single-genotype clusters, many of them originated from different countries; *i.e.*, Suphanburi11 came from Thailand, Godamhuman originated from Sudan, and IS 18581 and IS 23637 are Nigerian breeding lines. By and large most of the clusters that appeared from the PAGE-generated SSR marker data set were separated further and their positions relative to other clusters changed moderately in the dendrogram based upon the ABI-generated SSR marker data set. This is expected as the ABI should give a more accurate picture than PAGE because of its superior ability to detect the smaller polymorphisms between the genotypes. For example, except for a very large cluster of related breeding products that are insect resistant, all clusters detected based on the PAGE-generated marker data set were separated into distinct sub-groups by the ABI-generated SSR marker data set. If we look at around the 40% level of similarity, both the PAGE- and ABI-generated data sets detect 12 clusters, but the positions of the genotypes within the clusters were slightly modified by the superior sensitivity of PCR product separation on the ABI machines. At the same time if we look the clustering pattern around the 70% level of similarity, both of the systems classified the accessions into a larger number of clusters which indicates that the 91 genotypes studied were well diverged in their genetic makeup.

5.1.5 Implication for sorghum breeding

The information provided by this study about the diversity/similarity of the germplasm from different sources of region should prove extremely useful. The results obtained can find using in heterosis breeding and in selecting parental lines for specific breeding goals related to combining insect resistance with high grain yield and mitigation of drought stress. Dendrograms generated from both the PAGE- and ABI-derived molecular marker allele data sets provide useful information regarding the relatedness of materials of similar and distinct geographical origin, and of genotypes with varying level of agronomic eliteness, particularly with sources of insect resistance that might be exploited in conventional or marker-assisted plant breeding programs.

1. Development of new mapping population(s) for shoot fly resistance by crossing 296B, BTx623 and/or ICSV 88032 as the susceptible female parent and an improved shoot fly resistant male parent (i.e. ICSV 705 and/or ICSV 708.

2. For spotted stem borer, three highly susceptible genotypes are available BTx623, ICSV 745 and ICSV 88032. New mapping population(s) for spotted stem borer

resistance can be developed by crossing one of them as a female parent with genetically distinct stem borer resistant parents such as IS 2367, IS 4756, PB 15881-3 and IS 18577, using one of the latter group as the male parent.

3. For development of a mapping population for both shoot fly and stem borer resistance, three highly susceptible genotypes are available (i.e. BTx623, 296B and ICSV 88032), and any one of them can used as a female parent in a cross to a shoot fly and stem borer resistant parent taken from any of the other clusters (i.e. ICSV 700 and/or ICSV 714) and as male parent.

4. For development of new mapping populations for resistance to sorghum midge fly, susceptible female parent selected from among 296B, BTx623, PB 15881-3 and ICSV 714 can be crossed with a genetically distinct midge resistant male parent available from other clusters such as AF 28, TAM 2566, ICSV 88032 and/or DJ 6514.

5. Almost all genotypes belonging to ABI clusters 17, 18 and 19 are elite recurrent parents in marker-assisted backcrossing programmes initiated at ICRISAT, in which genes for the stay-green component of drought tolerance are being introgressed from trait donors B35 and E 36-1. As genotypes within any one of these three clusters are similar, it should be cost effective to reduce the number of recurrent parents that are actually advanced in each of these clusters.

5.2 Phenotyping of RILs derived from cross 296B × IS 18551 for components of resistance to sorghum shoot fly

Shoot fly is major insect pest of sorghum. Shoot fly resistance is a complex trait, involving a number of component traits, each of which are quantitative in nature and influenced by G × E interaction. Therefore, direct phenotypic selection for this trait will be difficult. Despite efforts made over the last two decades by utilizing the existing cultivated sources of resistance, the level of resistance achieved so far in elite backgrounds is limited. Marker-assisted selection could increase efficiency of breeding for such traits. As an initial step in this program, genomic regions associated with resistance and its components are to be detected.

The present study, involving recombinant inbred lines (RILs) obtained from cross 296B (susceptible) × IS 18551 (resistant), was undertaken to detect and estimate the effects of quantitative trait loci (QTL) for sorghum defense mechanisms for shoot fly oviposition and deadheart incidence; and to identify simple sequence repeat (SSR) markers linked to deadheart incidence QTL(s) for possible introgression

of these QTL(s) from more resistant donor genotypes into susceptible, agronomically elite breeding lines.

5.2.1 Phenotypic and genotypic variation

Characterization of phenotypic and genotypic variation of complex traits like shoot fly resistance is a pre-requisite to application of molecular genetic knowledge to broaden our understanding of their genetic control.. Shoot fly resistance traits are quantitative in nature. Genetic expectations of means and variances were obtained for these resistance traits using 259 RILs evaluated along with their parents. The genetic variability was assessed under two levels of shoot fly infestation in this set of RILs derived from a cross between resistant and susceptible inbred lines. Estimates of genetic variance components thus obtained have been used to compare the heritabilities of different resistance components.

The pooled analysis of variance for different components of shoot fly resistance (Table 4.4) revealed highly significant genotypic (G), environmental (E), and G × E interaction effects. The analysis not only depicts the variability that existed in the two screening environments, but also reflects the presence of genetic variability among the tested genotypes for shoot fly resistance and its component characters. Highly significant differences detected among the RILs and phenotypic differences recorded between the parents for various resistance traits suggested that sufficient variability exists in the experimental material for the purposes of this study.

Based on the varying range of phenotypic values for deadhearts (%) II in susceptible parent 296B in the two screening environments, these environments were categorized as having high shoot fly pressure (late *kharif*) and optimum shoot fly pressure (*rabi*). The phenotypic values for deadhearts (%) II in the susceptible parent were 96% and 77% in the late *kharif* and *rabi* screening environments, respectively. Borikar *et al.* (1982b) observed that selection of shoot fly tolerant genotypes was effective under optimum shoot fly pressure with 67% to 75% seedling mortality on susceptible control entries, while Rana *et al.* (1975) suggested that selection for shoot fly resistance was effective under conditions when mortalities ranged from 6.7% to 67%.

According to Rao *et al.* (1974), the level of seedling mortality in a field crop due to shoot fly deadhearts is a function of the intensity of insect infestation, plant growth rate and inherent genotypic differences. It appeared that the extent of deadhearts observed was primarily related to the level of shoot fly pressure. As the

rate and level of shoot fly population build-up varies with season and location, sorghum genotypes also exhibit variable degrees of shoot fly damage in different environments. Hence the screening of experimental material during the present study was done in two different screening environments. This provides opportunity to study the genetics of adaptation for shoot fly resistance by genotypes. In previous genetic study of shoot fly resistance in three different levels of shoot fly infestation (Borikar and Chopde, 1980), it was indicated that variation within and between genotypic groups became more apparent under high levels of shoot fly infestation. Considering the level of shoot fly damage in terms of deadhearts (%) II, the *rabi* screening environment (E2) then should be considered as more favorable for selection of shoot fly resistant and/or tolerant genotypes.

In general, the two parents (296B and IS 18551) differed significantly for all observed important shoot fly resistance traits (Table 4.2). Lander and Botstein (1989) indicated that the ability to map QTLs underlying a quantitative trait depends on the magnitude of phenotypic difference existing between parents of the mapping population. The differences observed between the parents involved in this study satisfy this requirement.

Parental performance and RILs mean performance for various shoot fly resistance components (Table 4.2 and Table 4.3) are discussed in following paragraphs.

5.2.1.1 Glossiness

Parental performance and RIL mean performance for glossiness was consistent across both screening environments, indicating consistent and reliable evaluation of this trait. Jayanthi *et al.* (1999) also reported that glossiness expression was stable across seasons. The wide range of phenotypic values and high genotypic variance (Table 4.4) for glossiness indicates that selection for this trait will be effective. Expression of differences in glossiness between the parents was greater in *kharif* than in *rabi*. These results corroborate those of Agrawal and Abraham (1985) and Jayanthi *et al.* (1999).

5.2.1.2 Seedling vigor

Significant differences in parental means was observed for this character in the *rabi* screening environment. Wide variations was observed for this character among the RIL means in the same season. The mean RIL values for character indicate that most of RILs had moderate vigour and were less susceptible to shoote fly.

5.2.1.3 Oviposition incidence (%)

The phenotypic values of parents for oviposition (%) varied significantly across the two screening environments indicating environmental influence on shoot fly egg laying. Parental mean values and RIL ranges for oviposition (%) II indicated greater shoot fly pressure in the *kharif* season screening environment than in the *rabi* season screening. Variability observed for oviposition depends on the level of shoot fly pressure prevailing in screening environments and on the breeding material screened. The efficiency of the ovipositional non-preference mechanism of shoot fly resistance is not stable and it is ineffective at high levels of shoot fly pressure (Singh and Jotwani, 1980a; Borikar *et al.*, 1982a; Sharma *et al.*, 1997). The range of the phenotypic values for oviposition (%) in this RIL population clearly indicate that the shoot fly population pressure was optimum in *rabi* screening environment, which was expressed in terms of wide ranges of phenotypic value for this trait. Borikar *et al.* (1982b) reported higher variability when the material was tested under optimum shoot fly population level.

5.2.1.4 Deadhearts incidence (%)

The most direct measure of shoot fly damage is that recorded in terms of deadhearts incidence (%). The phenotypic value of parents for deadhearts incidence (%) varied significantly across two screening environments. Parental mean values and RIL mean ranges for this traits indicated higher shoot fly pressure in the *kharif* season screening environment than in the *rabi* season screen. Variation in phenotypic values of RILs for deadhearts (%) was lower in *kharif* season than in *rabi* season. Therefore, the variability observed for this trait in the rabi season screening is likely to be of greater importance for selecting resistant and/or tolerant genotypes. Borikar et al. (1982b) reported that estimates of variability were higher for seedling mortality when the material was screened under optimum shoot fly population levels. The results obtained for deadhearts (%) in this study confirms that the screening of the RIL population in *rabi* season was done under optimum shoot fly population.

5.2.1.5 Seedling height (cm)

Significant differences in parental mean and RIL ranges for seedling height I observed in the *rabi* screening environments indicated that the behavior of this character is under genetic control. Sharma *et al.* (1977) pointed out the predominance of fixable genes in F2 population for seedling height; RILs are regarded as an immortalized F2 with all the genetic variation fixed (Burr *et al.*, 1988).

5.2.1.6 Trichome density

The parental mean values exhibited a consistent trend for trichome density (both upper and lower surface of leaf blade) across both screening environments. However, RIL population ranges indicated that trichome density was greater in *rabi* season than in *kharif* season. This indicates that the character is under genetic control with some environmental influences in its expression in different seasons. Maiti and Gibson (1983) also indicated that expression of trichomes was comparatively lower in *kharif* than in *rabi* season. In both seasons trichome density on upper leaf blade surface is greater than lower leaf blade surface. Similar observations were recorded by Gibson and Maiti (1983), Borikar and Chundurwar (1989), and Sajjanar (2002).

5.2.1.7 Time to 50% flowering, plant height and grain yield

Parental mean values for these characters revealed that 296B was later in flowering, shorter in height, and lower yielding than IS 18551 in both screening environments. Susceptible parental genotype 296B was heavily infested by shoot fly, producing large numbers of tillers. The time to 50% flowering recorded for 296B takes into account time taken for formation of these tillers also. As the tillers are later in flowering than main culms, flowering 296B was observed to be later than it would have been had the trial been protected from damage by shoot fly.

The range of values for RIL means indicated that selection for flowering could better be done in the *kharif* season due to a wider range of flowering time in this environment; while for plant height wide variation in phenotypic values was observed in both screening environments. Wide variation in phenotypic values for grain yield was also observed in both screening environments.

5.2.1.8 Overall recovery score and aphid damage score

Significant differences in parental means were observed for both characters in the *kharif* screening environment; however, no significant difference in parental means for either character were observed in the *rabi* screening environment. The value of RILs means indicated that larger numbers of RILs having high recovery score and low aphid damage were observed in the *rabi* screening environment than in the *kharif* season screen. Wide variation in phenotypic value for these characters were observed in both screening enviorments.

5.2.1.9 Pigmentation (scale), midge incidence score and agronomic score

Significant differences in parental means were observed for these three characters in the *rabi* screening environment. Wide variations were observed for these characters

among the RIL means in the same season. The mean RIL values for these characters indicate that most of RILs had non-tan foliage pigmentation, were susceptible to midge fly, and had moderate agronomic adaptation.

In general, parental and RIL mean values revealed wide variation in phenotypic values for shoot fly resistance and its component traits in both screening environments. Resistance in terms of oviposition non-preference is due to the component traits that prevent egg laying. This is due to a combination of characters expressing in favorable directions. It was previously reported that leaf color, texture, width (Raina, 1981) and hairiness (Bapat and Mote, 1982b) were important factors in selection of the oviposition substrate by female shoot flies. The wide variation observed in this RIL population for the shoot fly resistance component traits like glossiness; seedling vigor and trichome density indicates that these traits can be used as simple criteria for selection of resistant genotypes.

5.3 Analysis of variance

The analysis of variance for different shoot fly resistance components and related traits observed in this study revealed that genotypic variances were significant for all the observed traits in both of the individual screening environments as well as in the across-season analysis. The genotypic variances for shoot fly resistance component traits like glossiness, oviposition (%) I and II, and deadhearts (%) II was greater than the corresponding variances for G × E interaction. This indicated that these resistance component traits are mainly under genetic control, but that there are significant effects of environment in expression of these traits. Glossiness was mainly under genetic control in agreement with earlier reports (Tarumoto, 1980; Agrawal and House, 1982; Jayanthi *et al.*, 1999); while for trichome densities (both upper and lower leaf surfaces) the genotype variance was greater than G × E variance, indicating these traits are mainly under genetic control but that there are significant effects of environment in their expression. Jayanthi *et al.* (1999) too observed the season effect on expression of trichome density.

5.4 Inheritance of components of shoot fly resistance

The continuous distribution of RILs for various shoot fly resistance component traits observed in this study revealed that most of the traits studied were polygenic. According to Menendez and Hall (1995), the absence of discrete segregating classes for a trait suggests that its inheritance should be determined either by a large number of genes with small effects or a few major genes with substantial environmental

effects. The observations made in the present study are supported by previous workers findings that resistance to *Atherigona soccata* is quantitatively inherited (Agrawal and Abraham, 1985) and polygenically controlled (Goud *et al.*, 1983; Halalli *et al.*, 1983). Sharma *et al.* (1977) and Borikar and Chopde (1980) observed continuous variation in different generations and indicated that shoot fly resistance is due to gradual accumulation of resistance alleles at many genes.

The genetic analysis of components of shoot fly resistance is discussed below.

5.4.1 Broad-sense heritability

Heritability is a useful quantitative statement of the relative importance of heredity and environment in determining the expression of the character (Allard, 1960). The estimates of heritability help the plant breeder in selection of genotypes from diverse genetic populations. Effective selection can be achieved when additive effects are substantial and environmental effects are small, so that heritability estimates are high.

5.4.1.1 Glossiness

Consistently high heritability estimates observed for glossiness in two individual screening environments and moderate estimates across these same two test environments indicate that contributions to phenotypic variance due to environmental factors and G × E interaction are less than genotypic factors as evident from the values of genotypic and G × E variances (Table 4.4). The G × E variance component is, however, significant indicating the complex nature of glossiness. The QTL analyses results revealed a significant epistatic interaction (additive × additive) for this trait with presence of multiple loci controlling the expression of this trait. Glossiness has previously been reported to be controlled by a single recessive gene (Tarumoto, 1980; Agrawal and House, 1982; Jayanthi *et al.*, 1999), while Agrawal and Abraham (1985) indicated that the seedling glossiness intensity is quantitative in nature and controlled by both additive and non-additive genes. The current study also revealed the complex inheritance of seedling glossiness score.

5.4.1.2 Oviposition incidence (%)

Oviposition incidence recorded low to moderate but consistent operational heritability estimates in the two individual screening enviornments and also at different stages of observation .However operational heritability estimates for this trait across seasons for both observation stages were low indicating a prominent role of screening environment and/or genotype × environment interaction in expression of the trait. This observation corroborates those by Halalli *et al.* (1983), Borikar and Chopde

(1982), and Borikar *et al.* (1982b), who indicated that estimates of heritability for oviposition were high when the material was tested under optimum shoot fly population levels. This also confirms the utility of screening test material under optimum insect population levels so that selection for ovipositional non-preference will be effective.

5.4.1.3 Deadhearts incidence (%)

Operational heritability estimates observed for this trait varied from low to moderate and were consistent at both observation stages. However the heritability estimates obtained from the across season analysis were low. These results are in agreement with the results obtained by Halalli *et al.* (1982). According to Blum (1969a), seedling mortality is dependent on the intensity of insect infestation and hence any data on sorghum reaction to shoot fly must be interpreted with reference to the shoot fly population level. Borikar and Chopde (1982) also observed that genetics of deadhearts (%) is most influenced by shoot fly population level. The consistent estimates for heritability observed in individual screening environments in this study, suggests high to optimum insect population levels, which is also revealed by lower heritability values in the across-season analysis. This observation corroborates that by Borikar *et al.* (1982b), who reported that estimates of heritability were moderate to high for seedling mortality when the material was tested under optimum shoot fly population levels

.5.4.1.4 Trichome density

Consistently high heritability estimates ($h^2 > 0.95$) were recorded in both screening environments for trichome density on both upper and lower leaf surfaces, while across-season analyses revealed lower heritability estimates for trichome densities of both leaf surfaces ($h^2 = 0.51$ for upper and $h^2 = 0.49$ for lower leaf surfaces). This indicates the role of environmental factors and G × E factors in expression of this trait. Sharma *et al.* (1977), Gibson and Maiti (1983), and Tarumoto (1980) studied the nature of gene action for non-preference and each found that the presence of trichomes was governed by a single recessive gene; however, the inheritance of trichome density appeared to be more complex in the current study. The results obtained in this study reveals that there is a strong seasonal effect on the expression of trichome density. These results are in agreement with those of Jayanthi *et al.* (1999) and Sajjanar (2002).

5.4.1.5 Time to 50% flowering and plant height

Operational heritability estimates were high and consistent for time to 50% flowering (days) and plant height (cm) in both screening environments. However, in the across-season analyses a moderately lower estimate of heritability was recorded for time to 50% flowering and a high estimate of heritability was recorded for plant height. This indicates these characters are under genetic control and that there is a moderate level of interaction of genotype with the environment in expression of these traits. This observation agreed with the observation of higher genotypic variances than G × E interaction variances for these two agronomic traits.

5.4.1.6 Overall recovery score and aphid incidence score

Moderate operational heritabilities were recorded for these traits in individual screening environments and low to moderate operational heritability estimates were obtained in the across-season analysis for these traits. This suggests that shoot fly population pressure, aphid density and/or other seasonal effects contribute to expression of these traits.

5.4.1.7 Grain yield (g\ plot)

Moderate to low operational heritability was recorded for grain yield under conditions of moderate to severe shoot fly pressure in individual screening environments, and a low operational heritability estimate was obtained in the across-environment analysis. However, the genotypic variances was higher than G × E variances, indicating grain yield is indeed under genetic control although there is substantial interaction with the environment in expression of this trait.

5.5 Transgressive segregation

In the absence of epistasis and in the presence of linkage equilibrium, the mean of RILs will be the midparental value (average of two parents) (Jinks and Pooni, 1981). Epistasis leads to asymmetry in the distribution of derived inbreds relative to the initial inbred parental means. In other words deviation of the mean of the population-derived inbreds from the midparental value (either positive or negative) indicates the presence of epistasis. In the present study, an attempt has been made to elucidate the genetic constitution of parental inbreds 296B and IS 18551, and the nature of gene action involved in controlling shoot fly resistance components in the RILs based on means (of the parents and their derived RILs) and the appearance of transgressive segregants.

In general, for traits with RIL means less than midparental values, the proportion of RILs outside the low scoring parent was greater than that outside the higher scoring parent, and vice versa. The expectation of equal frequencies of inbreds lying outside the parental limits of P1 and P2 was not observed in any case. This shows that for each trait observed there were epistatic interactions influencing trait expression. Further the occurrence of transgressive segregants indicates that the two parental lines of the RIL population both carried desirable and undesirable alleles at various proportions of loci governing the various traits observed.

5.5.1 Glossiness intensity

Continuous distribution, from high intensity of glossiness (*i.e.* score 1) to non-glossiness (*i.e.* score 5), with an apparent valley in the frequency distribution graph between scores 3.5 to 4.0 (Fig 4.4A) indicated the involvement of major genes controlling the glossiness trait. A previous study reported that the glossiness character is controlled by single recessive gene (Tarumoto, 1980). A major gene will have major effects that will be larger than those arising from non-heritable agencies; its effects will be precipitated in phenotypic expression of a trait. The presence of such major genes is also supported by the consistency of high heritability estimates observed for glossiness in the individual screening environments (Table 4.6).

The observation that no transgressive segregant RILs were recorded with phenotypic scores falling outside the high intensity (low score) of glossiness of the resistant parent indicates that the alleles for this trait are predominantly in coupling phase. The positive deviation of the RIL population mean for glossiness from the midparental value indicates the presence of epistasis for this trait. Agrawal and Abraham (1985) also indicated that the seedling glossiness intensity is quantitative in nature, controlled by both additive and non-additive genes. The high mean value of the RILs, approaching that of high scoring parent (296B) indicates that the frequency of RILs with high scores (indicative of low intensity of glossiness) was greater than that of highly glossy (Fig 4.4A) individuals (indicated by low glossy scores). This could be explained by the fact that as the number of epistatically interacting genes controlling the trait increases the probability of obtaining individuals homozygous for favorable alleles for high glossiness intensity (low score) at all the concerned loci will be reduced.

5.5.2 Oviposition incidence (%)

It was observed in the current study that, for oviposition II, the RIL mean did not differ significantly from that of resistant parent IS 18551. The RIL population mean deviated from midparental values towards that of resistant parent for this trait. Favourable transgressive segregants were observed for this trait at both observation stages. However, the proportion of RILs out-side the parental mean of the low scoring parent IS 18551 was comparatively higher for oviposition II than oviposition I. This suggested that the trait is mainly under the control of additive as well as additive × additive genetic effects. Borikar and Chopde (1981b) also indicated the predominance of additive gene action for oviposition incidence.

5.5.3 Deadhearts incidence (%)

The continuous distribution of the RIL population for deadhearts incidence indicated the qualitative nature of this trait. The transgressive segregants were observed for this trait at both observation stages. The proportion of RILs lying outside the low scoring resistant parent IS 18551 was comparatively higher for deadhearts II than for deadhearts I. The RIL population mean for deadhearts incidence were observed near to be close to the midparental value indicating predominance of additive as well as additive × additive gene action for this trait. Borikar and Chopde (1981b) also indicated the predominance of additive gene action for deadhearts incidence.

5.5.4 Trichome density (upper leaf blade surface)

The continuous distribution of the RIL population for trichome density on the upper surface of seedling leaf blades indicated the quantitative nature of this trait. The positive deviation of the RIL population mean from the midparental value indicates the presence of epistasis as Jinks and Pooni (1976) have reported that any deviation of the population mean from the midparental value reveals presence of epistasis. Sharma *et al.* (1977) and Tarumoto (1980) reported that presence of trichomes was recessive in nature, but the inheritance of trichome density was complex. The appearance of transgressive segregation in this RIL population might due to complementation of favorable and unfavorable alleles received from both parents. Due to complementation of positive and negative alleles in the F1 and subsequent recombination events, individual RILs with higher than the parental proportion of favorable alleles have been observed as transgressive segregants having trichome density higher than that of the higher scoring parent. The presence of additive gene action supplemented by additive × additive gene interaction in these RILs might be

main reason for occurrence for these transgressive segregants with trichome density higher than the high scoring parents.

5.5.5. Trichome density (lower leaf blade surface)

It was observed that the mean value of RIL population was on par with the midparental value for trichome density on the lower surface of seedling leaf blades. The distribution was observed to be skewed towards trichomelessness. This deviation of the RIL population mean from the midparental value points out the presence of epistasis for this trait. The occurrence of transgressive segregant RILs with value higher than the higher scoring parent indicates that favorable alleles were contributed by both parents, for the presence of RILs with larger number of favorable alleles than high scoring parent IS 18551. This in turn could be attributed to additive and additive × additive gene action.

5.5.6 Plant height (cm)

The high mean plant height of RILs may be due to transgressive segregants with values lying outside the taller parent IS 18551. The low frequency of such transgressive segregants indicates that the most of the alleles for greater plant height might be associated in coupling phase. However, some favorable parental alleles might be in repulsion phase resulting in limited opportunity for occurrence of transgressive segregants.

5.5.7 Overall recovery score

It was observed that the mean value of the RIL population was at par with the midparental value for this trait. A low proportion of transgressive segregants was observed with a few RILs having values lying outside the high recovery score parent IS 18551. The proportion of transgressive RILs was greater for individuals with values lying outside the low recovery score parent 296B. The appearance of high numbers of transgressive segregant RILs for low overall recovery score might be due to the complementation of more unfavorable alleles received from both parents. It indicates that presence of additive gene action supplemented by additive × additive gene interaction.

5.5.8 Aphid damage score

The positive deviation of the RIL population mean from the midparental value indicates the presence of epistasis. Transgressive segregants were observed outside the limits of both the high and low scoring parents. However the proportion of RILs lying outside the low scoring (more resistant) parent IS 18551was comparatively

higher. The high frequency of such transgressive segregants indicated that a low number of parental alleles might be in coupling phase. This in turn indicated the possible role of additive × additive gene interaction in control of this trait.

5.5.9 Grain yield

The two parents of this RIL population differ significantly for grain yield under conditions of moderate to severe shoot fly pressure. The presence of epistasic gene action for grain yield was revealed by negative deviation of the RIL population from the midparental value. The appearance of transgressive segregants might be due to the accumulation of unfavorable alleles for lower grain yield in the RILs during the process of inbreeding and also some amount of epistasic gene action

5.6 QTL mapping

5.6.1 Glossiness

For glossiness, three QTLs were detected in the *kharif* screening environment (E_1), whereas two QTLs were detected in the *rabi* screening environments (E_2) and two QTLs were detected in the across-environment analysis (Table 4.7 and 4.8). One QTL on LG J explaining 37% of observed phenotypic variance in late *kharif*, 21% in *rabi*, and 34% across these two screening environments, and would be considered a major QTL for this trait. The favorable allele for this QTL originates from the IS 18551 parent. The identification of a QTL explaining a high proportion of the phenotypic variance indicates a strong association between genotype and phenotype. According to Terwilliger (2001), if the test locus genotype–phenotype relationship is strong, the power of QTL identification is solely a function of the strength of linkage relationships.

The identification of a major QTL for glossiness explaining a high proportion of observed phenotypic variance in different screening environments confirms the high heritability (Table 4.6) of this trait and low influence on it by environment and G × E interaction (Table 4.4). This trait has been reported to be controlled by a single recessive gene (Tarumoto, 1980), but the current results indicate its inheritance is more complex in the (296B × IS18551)-derived RIL population. The low frequency of highly glossy RILs does not indicate that the trait is controlled by a single recessive gene (in which case a 1:1 segregation for homozygous glossy and non-glossy RILs would have been expected), but instead that it is controlled by epistatic interactions involving several loci. Agarwal and Abraham (1985) indicated that the intensity of seedling glossiness is quantitative in nature, controlled by both

additive and non-additive genes. This observation can be explained by the presence of multiple QTLs controlling this trait and a significant additive × additive interaction component in their final simultaneous effect. This study indicates that glossiness is controlled a major QTL on LG J, accounting for 34% of the phenotypic variation, and one minor QTL on LG G accounting for 8% of the phenotypic variation in the across-seasons analysis. Over all, the two QTLs mapped in across-season analysis of these two screening environments explained 31% of genetic variation after correcting for non-significant QTL × environment interactions. This suggests the presence of unmapped genetic effects in areas on the genetic map that are presently poorly covered by SSR markers.

Two environment-specific glossiness QTLs were detected in the *kharif* screening environment, and mapped on LG E and LG H, respectively. One minor QTL for glossiness detected on LG G was co-localized with important oviposition, deadhearts, trichome density (upper and lower surfaces of seedling leaf blades) and grain yield QTLs, indicating that glossiness is indeed an important component in the interaction between shoot fly and sorghum and should be targeted for marker-assisted selection for shoot fly resistance. When compared with the previous study to map putative QTLs for shoot fly resistance by Folkertsma *et al.* (2005, unpublished) in a (BTx623 × IS 18551)-derived RIL population, the present study also located the same major QTL originating from IS 18551 for glossiness on LG J. This confirms that this could be a region with a candidate gene for shoot fly resistance. In both these studies, resistant parent IS 18551 contributed the additive genetic effects for increased glossiness. Glossiness has been described as one of the major factors determining sorghum resistance against shoot fly (Omori *et al.*, 1983). The positive correlation between glossiness score and deadhearts incidence; and between glossiness score and oviposition signifies the importance of low glossiness score (*i.e.*, high intensity of glossiness) in imparting resistance of sorghum to shoot fly and should be targeted for marker-assisted selection of shoot fly resistance (Folkertsma *et al.*, 2005; unpublished).

5.6.2 Seedling vigor I and seedling height I

For seedling vigor I and seedling height I, phenotypic observations were not recorded in the late *kharif* screening environment (E_1). Three seedling vigor I and two seedling height I QTLs, respectively, were detected in the *rabi* screening environment. The three seedling vigor I QTLs together explained 24% of the observed phenotypic

variance and the QTL mapped on LG B appears to be the most important QTL as it alone explained 11% of the observed phenotypic variance in seedling vigor I. The two QTLs for seedling height I explained together 14% of observed phenotypic variance. For these two characters one common QTL has been mapped on LG B in marker interval *Xtxp*01-*Xtxp*348. Favorable additive genetic effects at this QTL are contributed by resistant parent IS 18551.The relatively low correlation between vigor and shoot height on one hand, and deadhearts incidence I and II on the other hand, and the absence of the seedling vigor and shoot height QTL co-localizing with any deadhearts QTL indicates that seedling vigor and seedling height (at least as assessed in this study) are of limited relevance to improvement of shoot fly resistance in sorghum, which is in agreement with the findings of Folkerstma *et al.* (2005 unpublished).

5.6.3 Oviposition incidence (%)

Oviposition (%) expressed as the percentage plants of a genotype with shoot fly eggs, is highly correlated with deadhearts, as expected. This indicates that there is a direct relationship between the percentage of the plants with eggs and the percentage of the plants showing deadhearts. Two and four QTLs for oviposition I and II, respectively, were detected across environments, with significant QTL × environment interaction observed for the oviposition I QTL detected on LG F and the oviposition II QTLs detected of LG F and LG G. The two QTLs for oviposition I together explained 6% of genetic variation for this trait, with the two-QTL model having a peak LOD value of 3.9. The four oviposition II QTLs together explained 15% of the genetic variation for this trait, with a major QTL on LG G. In the *rabi* screening environment a common QTL for oviposition I and II was mapped on LG F. The oviposition I QTLs mapped on LG C and LG F are co-localized with oviposition II QTLs in the across-environment analysis. In addition, two more oviposition II QTLs were detected in the across-environment analysis, which mapped on LG G and LG J. The QTL for oviposition detected on LG G in the across-environment analysis co-localized with a deadhearts QTL, a major QTL for trichome density (upper and lower surfaces of seedling leaf blades) and a minor QTL for grain yield under conditions of optimum to high shoot fly pressure. In addition, the QTL for oviposition detected on LG J in the across-environment analysis co-localized with the major QTL for glossiness intensity. The presence of an environment-sensitive QTL for oviposition II was detected on LG E (*kharif* screening environment E1). Variation in phenotypic values for oviposition I

and II across the two screening environments might account for the significant QTL × environment interaction observed for this pair of traits. Both parental lines contributed favorable alleles for oviposition. According to Ramie *et al.* (1998) and Agrama *et al.* (2002,) correlated traits often have QTLs mapping to the same chromosomal locations. Two different types of correlation between the traits have been observed in the current study. Correlation between different evaluation times for a specific trait and correlation between different traits for a specific evaluation timet or across different evaluation times. High correlation between different evaluation times for the same trait indicates that the expression of the trait at different evaluation times is under control of similar genes and therefore QTLs detected for different evaluation times of this should be co-localized.

5.6.4 Deadhearts incidence (%)

No QTL was detected for deadhearts I; however, two QTLs, one each on LG F and LG G, were mapped for deadhearts II across the two screening environments. Significant QTL × environmental interactions were observed for both of the detected QTLs for deadhearts II. The two QTLs together explained 11% of the genetic variation for this trait with a possible major QTL on LG F itself explaining 12% of the observed phenotypic variation. This QTL on LG F was detected in the across-environment analysis as well as in both of the individual screening environments. This QTL on LG F for deadhearts II co-localized with an oviposition QTL and a QTL for trichome density of the lower surface of the leaf blade in the across-environment analyses. Another important QTL for deadhearts II mapped on LG G in the across-environment analysis and in the *rabi* screening environment, and this co-localized with a major QTL for trichome density (upper and lower leaf blade surfaces) and a minor QTL for overall recovery score and grain yield. For oviposition and deadhearts incidence several QTLs were mapped but only a few QTLs were co-localized in the two individual screening environments and few mapped for both traits in the across-season analysis. More over, no QTLs was found to be a major contributor to phenotypic variation for either of these traits. When compared with the previous studies where QTLs for oviposition and deadhearts incidence were co-localized on LG J in the (BTx623 × IS 18551)-derived RIL population (Folkerstma *et al.*, 2005 unpublished), it can be clearly observed that the genomic regions harboring these QTLs were not mapped with SSR markers in the RIL population used in the current

study so that it has not yet been possible to detect these QTLs in both of the IS 18551-derived RIL populations.

5.6.5 Trichome density

One QTL on LG G accounting for 29% (in E1), 15% (in E2) and 30% (across environments) of observed phenotypic variation of trichome density on the upper surface of the leaf blade was detected (Tables 4.7 and 4.8). Moreover, this QTL on LG G for upper leaf trichome density co-localized with a major QTL for trichome density on the lower leaf surface explaining nearly 27% of the phenotypic variation in the latter trait across the two screening environments, pointing to similarities in genetic control of trichomes densities on either side of the sorghum seedling leaf blade. Observations from this study indicated that the QTL on LG G is involved in the control of trichome density on both sides of the seedling leaf blade. The importance of trichomes in connection with shoot fly resistance has been reported by several research groups (Blum, 1968; Maiti and Bidinger, 1979; Maiti *et al.*, 1980; Taneja and Leuschner 1985). Gibson and Maiti (1983) and Maiti and Gibson (1983) indicated that presence of abaxial trichome control by a single recessive gene. However, Halalli *et al.* (1982) reported that trichome density is under the control of both additive and non-additive genetic effects. The QTL mapped on LG G for both upper and lower trichome density in this study corresponds to the QTL mapped in the BTx623 × IS 18551 population (Folkerstama et al., 2005 unpublished). This major QTL mapped on LG G for both upper and lower trichome densities is co-localized with a minor QTL for glossiness, as well as QTLs for oviposition, deadhearts, and grain yield across the two testing environments. The one minor QTL for trichome density detected on LG F co-localized with a minor QTL for deadhearts and oviposition across the two testing environments. A QTL was detected across environments on LG F for trichome density of the lower surface of the seedling leaf blade; however, the same QTL was not detected in either individual screening environment. Similarly, one minor QTL for trichome density of the lower leaf surface was detected on LG C in the *rabi* screening environment, but the same QTL was not detected in the across-environment analysis. The high frequency of transgressive segregant RIL individuals with high trichome densities clearly indicated that this character is not under control of a single recessive gene. Also, the portion of phenotypic variation explained by the putative QTLs detected is low and there is a strong possibility that other QTLs are influencing the trait and can be detected if

genomic region not yet covered by SSR markers can be added to the linkage map for this RIL population. Even though the role of higher trichome density in reducing shoot fly oviposition and deadhearts incidence had been previously documented by several researchers (Blum, 1968; Maiti and Bidinger, 1979; Maiti *et al.* 1980; Taneja and Leuschner, 1985; Karanjkar *et al.*, 1992), the observations of the current study strengthen the case for using this trait as a selection criterion in sorghum shoot fly resistance breeding.

5.6.6 Plant height (cm)

One QTL for plant height was detected in each of the *kharif* and *rabi* screening environments, and in the across-environment analysis. The detected QTL was mapped to a common position on LG I, and accounted for 17.% (in *kharif*),15% (in *rabi*) and 16% (across these two environments) of the observed phenotypic variation for plant height (Tables 4.7 and 4.8). This QTL for plant height that mapped on LG I co-localized with a QTL for midge resistance in the *rabi* screening environment, with the allele for greater height associated with higher midge damage score. The chromosomal region to which this QTL mapped could be near to the *D*w2 dwarfing gene locus. The additive genetic effects for increased plant height were contributed by alleles from taller parent IS 18551 for both screening environments.

5.6.7 Time to 50% flowering

Two QTL each for the *kharif* (E1) screening environment and across-environment analyses, and one QTL in the *rabi* (E2) screening environment were detected for time to 50% flowering. One common QTL mapped on LG A were detected in E1, E2 and the across-environment analyses. In addition, one QTL mapped on LG E was detected in the across-environment analysis, but the same QTL was not detected in either of the individual screening environments. One QTL for this trait mapped on LG I in the *kharif* screening environment, but the same QTL was not detected in the across-environments analysis. The two QTLs detected in the across-environment analysis together explained 16% of the genetic variation. The major QTL for this trait mapped on LG A, which alone explained 18% of the observed phenotypic variation, had a peak LOD value of 9.15. Both QTLs exhibited non-significant QTL × environmental interaction. Favorable additive genetic effects for this trait were contributed by IS 18551 alleles for the QTL on LG A, and by those from 296B for the QTLs on LG E and LG I. The QTL mapped on LG A for time to 50% flowering might be mapped near the *ma*3 locus, which contributes to early flowering. The QTL mapped on LG A

for this trait co-localized with QTLs of seedling vigor I, pigmentation and agronomic score in the cooler *rabi* screening environment and with a QTL for grain yield that was detected in the across- enviroment analysis.

5.6.8 Overall recovery score

Two QTLs were detected for this trait in across environment analysis and four QTLs were detected in the late *kharif* screening environment. No QTL was detected in the *rabi* environment. One common QTL, which mapped on LG E was detected in the *kharif* and across-environment analyses. One additional QTL mapped on LG J was detected only in the across-environment analysis. Three other QTLs were detected in the *kharif* screening environment, one each mapped on LG B, LG C and LG G. These three QTLS were not detected in the across-environment analysis. The two QTLs from the across-environment analysis together explained 9% of genetic variation, with the QTL mapped on LG E explaining 6% of observed phenotypic variation. This latter QTL co-localized with QTLs for glossiness intensity, oviposition II, and grain yield in the *kharif* screening environment, and with a QTL for aphid damage score in across-season analysis. In addition, the QTL mapped on LG J for overall recovery score also co-localized with a QTL for aphid damage score in across-environment analysis.

In both cases the alleles associated with lower overall recovery score were also associated with lower aphid damage score. One QTL mapped on LG G detected for this trait in the *kharif* screening environment co-localized with QTLs for both oviposition II and deadhearts II, and the major QTL of trichome density (upper and lower leaf surface). Favorable additive genetic effects for better overall recovery were contributed by alleles from shoot fly resistant parent IS 18551.

5.6.9 Aphid damage score

Two QTLs were detected for this trait in the across-season analysis. These two QTLs were mapped on LG E and LG J. One common QTL mapped on LG J was detected in both individual screening environments and across these environments. However, the QTL mapped on LG E in across-season analysis, could not be detected in the *rabi* screening environment. The two detected QTLs across seasons together explained 19% of genetic variation for this trait while the major QTL mapped on LG J for explained 20% of observed phenotypic variation and had a LOD peak value of 9.3. The QTL mapped on LG E exhibited significant QTL × environment interaction. The favourable additive genetic effects for both of these QTLs were contributed by IS 18551 alleles. The QTL mapped on LG E for aphid damage score co-localized with

glossiness intensity, oviposition II, overall recovery score and grain yield QTLs in the *kharif* screening environment.

5.6.10 Grain yield

Three QTLS were detected for this trait under conditions of moderate to severe shoot fly pressure in the across-season analysis. These mapped each on LG A, LG G and LG I, and together explained about 12% of genetic variation for this trait in the RIL population. All of these three QTLs exhibited non-significant Q × E interaction. The favorable additive genetic effects were all contributed by alleles from resistant parent IS 18551. The QTLs mapped on LG G and LG I in the across-environment analysis were also detected in the *kharif* screening environment. However, the grain yield QTL mapped on LG A in the across-environment analysis was not detected in either individual screening environment. In addition, a QTL for grain yield mapped on LG E in the *kharif* screening environment was not detected in the *rabi* and across-environment analyses. Similarly, a QTL mapped on LG C in the *rabi* screening environment was not detected in either the *kharif* or across-environment analyses. The QTL mapped on LG A for grain yield in the across-environment co-localized with time to 50% flowering, with the allele for early flowering associated with higher grain yield. The QTL mapped on LG G for grain yield co-localized with QTLs for glossiness intensity, oviposition II, deadhearts II and a major QTL for trichome density on upper and lower surfaces of seedling leaf blades. The QTL mapped on LG I for grain yield co-localized with a seedling height QTL detected in the *rabi* screening environment. The QTL mapped on LG E for grain yield in the late-*kharif* environment co-localized with QTLs for glossiness intensity and oviposition II in the late *kharif* season and QTLs for overall recovery score and aphid damage score in the across-seasons analysis. The QTL mapped on LG C for grain yield in the *rabi* screening environment co-localized with a QTL for midge damage score in that same *rabi* screening environment.

5.6.11 Pigmentation score

Pigmentation score was not recorded in the 2002 late-*kharif* screening environment. Two QTLs were detected for this trait in the *rabi* screening environment, one each mapped on LG A and LG I and explaining 7% and 12% observed phenotypic variance, respectively. The additive genetic effects for darker foliage color were contributed by alleles from IS 18551, as expected given that 296B has lighter tan foliage color. The QTL mapped on LG A for this trait co-localized with QTLs for

seedling vigor I, time to 50% flowering and agronomic score, in the vicinity of the *Y* locus for yellow seed color. The QTL detected on LG I co-localized with a QTL for time to 50% flowering in the longer-daylength *kharif* screening environment, which maps in the vicinity of the *Rs*1 locus governing seedling color.

5.6.12 Midge damage score

Two QTLs were detected in the *rabi* screening environment. One each mapped on LG C and LG I, explaining 6% and 16% of observed phenotypic variance, respectively. The favourable additive genetic effects for low midge score were contributed by 296B. The QTL mapped on LG C for midge damage score co-localized with a grain yield QTL in this environment, while the QTL mapped on LG I for this trait co-localized with a QTL for plant height that was detected in both the *kharif* and *rabi* screening environments as well as in the across-environment analysis.

5.6.13 Agronomic score

One QTL was detected in *rabi* screening environment explaining 6% of the observed phenotypic variance in agronomic score. The additive effects for desirable agronomic score were contributed by the 296B allele. This QTL for agronomic score, which mapped on LG A, co-localized with QTLs for seedling vigor, time to 50% flowering, and pigmentation score in this *rabi* screening environment.

5.7 Correlation between phenotypic traits

Correlated traits often have QTLs mapping to the same chromosomal locations (Veldboom *et al.*, 1994; Xiao *et al.*, 1996). According to Hemamalini *et al.* (2000) co-segregation may be due to tight linkage, pleiotropy or a causal relationship between the traits. Two different types of correlations between traits have been observed in the current study: correlations between different evaluation times for a specific trait and correlations between different traits for a specific evaluation time or across different times. High correlations between different evaluation times for the same trait indicate that expression of the trait at different evaluation times of observation is under control of similar genes and therefore QTLs detected for different evaluation times of the same trait should co-localize (Folkertsma *et al.*, 2005; unpublished). Correlation between upper and lower leaf surface trichome densities was high in the current study. The high correlation (0.82) between these two traits suggests similarity in genes involved in the expression of the density of trichomes on both sides of seedling leaf blades. This suggestion is further supported by a common major QTL on LG G. Co-localization of QTLs of different traits will have implications for marker-assisted

breeding approaches to transfer traits from a donor genotype to a more elite recipient genotypes. Robust phenotyping, genotyping and QTL mapping will help to identify possible unfavorable pleiotropic effects before embarking on a marker-assisted backcross project.

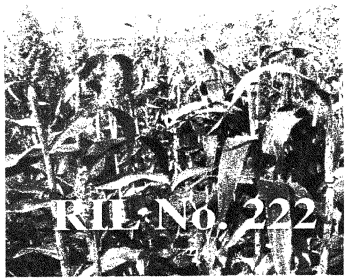
5.8 RILs with potentially useful trait combinations

The across-season means of the RILs obtained from phenotypic screening for shoot fly resistance component traits (Table 5.1) revealed that there exist some RILs that are either statistically superior to or on par with the resistant parent IS 18551 for low deadhearts incidence. These RILs were scored for plant pigmentation and for overall agronomic adaptation. The visual scores for plant pigmentation and overall agronomic adaptation revealed that only a few individual RILs comparable or superior to IS 18551 for deadhearts incidence coupled this with better agronomic adaptation. Among them non-tan RILs 97 and 174 exhibited better adaptation for *rabi* season while for *kharif* adaptation tan RILs 222 and 223 were found better. All RILs with better performance for deadhearts incidence than IS 18551 were scored (based on their marker genotypes) for the presence or the absence of the putative QTLs (Table 5.1) mapped for important shoot fly resistance component traits. These QTLs were said to be present (score 1) when the flanking markers to the targeted QTLs were homozygous for the donor parent allele. When one or more flanking markers were heterozygous for the IS 18551 allele, otherwise the targeted QTL was said to be absent (score 2). Non-amplified and off-type alleles were scored 3. All RILs with better performance for deadheart incidence than the resistant parent were found to harbor one or more important putative QTLs (plate5.1) for shoot fly resistant component traits. RILs 130, , 208 and, 222 each had all of the QTLs mapped for the most direct measure of shoot fly resistance, i.e., the shoot fly deadhearts incidence trait, which was reflected in terms of their exhibition of deadhearts incidence at par to lower than IS 18551 across the two screening environments. RILs 130 and 179 were non-tan, *rabi*–adapted lines intermediate to their parents for plant height. These two RILs can be used for the *rabi* season A/B-line improvement program. Intermediate tan-foliaged RILs 208, 222 and 223 have *kharif* adaptation and harbor most of the putative QTLs imparting resistance to shoot fly. These lines can be used for development of A/B-line pairs for shoot fly resistance and yield and also for varietal development programs targeting the *kharif* season.(plate).

Table 5.1: Recombinant inbred lines (RILs) with shoot fly resistance, measured in terms of deadhearts (%) incidence, comparable to resistant parent IS 18551 and good agronomic adaptation

RIL nos.	Deadhearts (%) II		Pigment-ation score	Phenotypic score		Glossiness		Oviposition (%) II	Trichome density (upper leaf surface) (no./microscopic field)		Trichome density (lower leaf surface) (no./microscopic field)	
	Mean	Mapped QTLs*		Late *Kharif*	*Rabi*	Mean	Mapped major QTL*		Mean	Mapped QTL*	Mean	Mapped QTL*
21	64.2	3	2.3	I (MT)	A (D)	3.4	2	75.5	95	3	50	3
46	56.3	3	3.7	I	A (D)	2.6	1	63.6	111	3	53	3
47	62.3	3	1.3	B	I (MT)	3.0	1	74.0	114	3	49	3
51	62.6	3	1.0	I (MT)	I (D)	2.6	1	66.0	46	3	15	3
82	64.0	3	1.0	I (T)	I (MT)	2.6	1	72.4	99	3	36	3
97	62.1	2	1.0	I (T)	B (MT)	2.5	1	66.0	85	2	40	2
130	62.4	1	1.3	A	I (MT)	2.2	1	67.1	92	1	32	1
174	62.9	2	1.0	I (T)	I (D)	2.6	1	74.0	172	2	72	2
179	59.8	1	1.0	A	I (D)	1.3	2	65.6	122	1	41	1
208	63.6	1	3.3	I (MT)	I (D)	3.3	1	73.3	168	1	82	1
222	64.2	1	3.7	A (D)	I (MT)	2.4	1	71.0	96	1	46	1
223	59.2	1	3.2	I (D)	I (MT)	1.6	2	67.0	67	1	26	1
IS 18551	62.6	1	1.1	B	B	1.0	1	72.6	153	1	74	1
296B	92.6	2	4.0	A	A	5.0	2	93.0	0.0	2	0	2
SE ±	2.83					0.2		3.13	4.22		2.45	

*= The status of the QTL (in case of glossiness major QTL) mapped for the concerned shoot fly resistance trait in the individual RIL, indicated by 1= absent; 2= present; 3= non-availability of marker data. Pigmentation score : 1= non-tan, highly pigmentated, *rabi* type and 5= tan, non-pigmentated, *kharif* type. Phenotypic score: the individual RILs were scored for their phenotypic appearance like A= 296B-like plant type, B= IS 18551-like plant type and I= intermediate plant type. The letters in parentheses indicate the plant height of RILs: D= dwarf, MT= medium tall, T= tall.



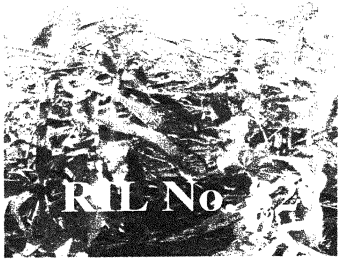
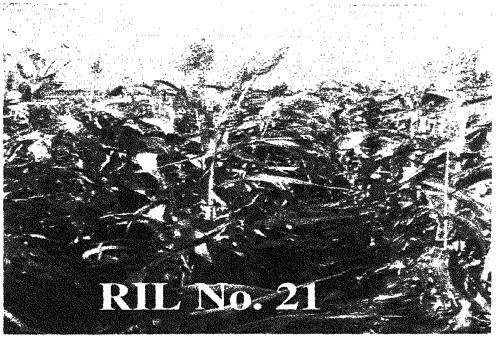


Plate 5.1 Agronomically desirable RILs with shoot fly resistance (measured in terms of deadhearts incidence) comparable to resistant parent IS 18551.


RIL No. 46


RIL No. 223


RIL No. 21


RIL No. 208

The glossy trait, which is characteristic of most of the *kharif* and *rabi* season landrace sorghum grown in India (Blum, 1972; Rao *et al.*, 1978), is reported to be associated with shoot fly resistance (Bapat et al., 1975; Taneja and Leuschner, 1985; Omeri *et al.*, 1983). Interestingly, six RILs (47, 51, 82, 97, 130, 174) combining agronomically desirable plant type with dark pigmented (non-tan) foliage, and intermediate *rabi* adaptation were found to harboring (plate5.1) the major QTL for high glossiness intensity. These RILs may be used as donors for transferring the glossiness trait to *rabi*-adapted breeding lines. Similarly, intermediate tan foliaged, agronomically desirable and *kharif*-adaptated RILs 46, 208 and 222 can be used as donors for transfer the glossiness trait to *kharif* breeding lines. As glossiness intensity was observed to have a high broad-sense heritability, it can easily be manipulated through conventional breeding practices and fixed in breeding lines.

5.9 Implication for breeding approaches

Following implications for breeding approaches can be drawn from the present study.

1. Shoot fly resistant component traits like glossiness, seedling vigor, trichome density, oviposition % and deadhearts % were observed to have moderate to high heritability. These traits can be used as reliable parameters for large-scale screening of germplasm and breeding material aimed at improving shoot fly resistance.
2. Expression of glossiness is little affected by season and it is observed to be a reliable component parameter of shoot fly resistance. Glossiness can be used as a selection criterion for shoot fly resistance screening and fixed in breeding material following conventional breeding procedures. RILs 47, 51, 82, 97, 130, and 174 with intermediate height and pigmented foliage, and RILs 46, 208, and 222 with non-pigmented (tan) foliage, intermediate height, all harbor the major glossiness QTL and can be used as donors for transferring high glossiness intensity in to more agronomically elite tan and non-tan plant genotypes.
3. Transfer of shoot fly resistance from donor IS 18551 probably requires more cycles of selections. In the present study shoot fly resistant RILs that are agronomically more desirable than original donor parent IS 18551 were identified with the help of molecular analysis. It is suggested to now utilize these RILs as donor parents in crosses with the most agronomically elite

breeding lines so as to efficiently combine elite agronomic features with shoot fly resistance.

4. RILs 222 and 208 with 296B-like plant type, each harboring 4 QTLs contributing to shoot fly resistance, and having shoot fly resistance comparable to donor parent IS 18551, can be used for sorghum improvement aimed at combining shoot fly resistance with dwarf plant stature, which would be appropriate for seed parents (A/B-pairs) of hybrid cultivars.
5. Non-tan RILs 130 and 179, which each have four putative QTLs for shoot fly resistance and its component traits while having comparable shoot fly resistance with IS 18551, can be used as donors in a marker-assisted breeding program to improve shoot fly resistance in agronomically elite non-tan breeding lines.
6. In the present study the putative QTLs for glossiness (on LG J), trichome density (on LG G), and deadhearts incidence (LG G) mapped to the same locations that had previously been mapped using the RIL population derived from cross BTx623 × IS 18551 (Sajjanar, 2002; Folkertsma *et al.*, 2005 unpublished). This confirms that these genomic regions contain genes contributing to shoot fly resistance.
7. It is suggested that in immediate future additional markers should be added to the linkage map of the RIL population derived from 296B × IS 18551 in the regions with larger inter-marker distance and in the bottom sections of LG F and LG J, which are not well covered in the present study. In addition, more phenotypic screening of this RIL population across seasons and locations should be conducted to measure and quantify more precisely the effect of environment on expression of shoot fly resistance and its component traits.

5.10 Marker-assisted selection for shoot fly resistance in sorghum

In breeding for disease and pest resistance, at present the segregating populations derived from crosses between the resistance sources and otherwise desirable and productive genotypes are selected either at natural disease or pest 'hot-spots' or under artificially created disease and pest nurseries or by infecting individual plants under controlled environment conditions. Although these procedures have given excellent results, they are time consuming and expensive. Besides, there are nearly always susceptible plants that escape attack. Furthermore, the pathogens or the pests have to be maintained either on the host or alternate hosts if they are obligate parasites.

Screening of plants with several different pathogens and their pathotypes, or pests and their biotypes, simultaneously or even sequentially is difficult if not impossible or impractical. Availability of tightly linked genetic markers for resistance genes will help in identifying plants carrying these genes simultaneously without subjecting them to the pathogen or insect attack in early generations. The breeder would require only a small amount of DNA from each of the individual plants to be tested, and this can be obtained without destroying the plant. Using the known set of DNA primer pairs for PCR, the products of the reaction would have to be run out on PAGE gels and the genotypes of the individual plants assessed to predict resistance or susceptibility by the presence or absence of the resistant parent's marker band on the gel. Only the materials in the advanced generations would be required to be tested in disease and insect nurseries. Thus, with MAS, it is now possible for the breeder to conduct many rounds of selection in a year without depending on the natural occurrence of the pest or pathogen, and theoretically without the pest or pathogen as well. However, the presence of different races or biotypes complicates the development and application of molecular marker-assisted selection. Markers developed for one pathotypes or biotype may not have application in other locations in which different pathotypes or biotypes occur unless resistance to these is controlled by the same gene.

The selection of sorghum genotypes for resistance to shoot fly by utilizing one or a few resistance parameters is inefficient because several components are involved in resistance and one or more genes govern each of these resistance components. Further expression of many of these components is influenced by environmental variation, hence shoot fly resistance is a quantitative trait and shows a large amount of genotype × environmental interaction.

Marker-assisted selection has a considerable potential to improve the efficiency of the selection for quantitative traits (Hash and Bramel-Cox, 2000) such as shoot fly resistance, for which expression is sensitive to the testing environment. As the components to resistance to shoot fly are mostly quantitative in nature it is potentially important to identify quantitative trait loci (QTLs) for these from the viewpoint of applied genetics and plant breeding. The ultimate goal of such QTL analysis is to develop tools that are useful for marker-assisted selection. In a practical breeding program the aim is to increase the level of resistance in agronomically elite

backgrounds. If this is successful, these marker-assisted breeding tools can also be helpful in pyramiding genes for hoot fly resistance.

Conventional quantitative genetic studies on shoot fly resistance with different sorghum genetic materials have been reported by many workers. Recently QTL analysis for shoot fly resistance component traits has been done using a set of sorghum recombinant inbred lines (a RIL population) derived from cross BTx623 × IS 18551. Sajjanar (2002) and Folkertsma *et al.* (2005 unpublished) have reported on 252 RILs of a (BTx623 × IS 18551)-derived mapping population that were screened for shoot fly resistance component traits in three environments. The same set of RILs was genotyped using 109 SSR marker loci and QTL analysis performed with the aim of identifying the genomic region associated with shoot fly resistance. Composite interval mapping QTL analysis using PLAB QTL version 1.1 revealed the presence of 28 QTLs detected at least two of the three screening environments. Closely linked markers were identified for four QTLs for deadhearts incidence. In the present study efforts are being made to transfer these four deadhearts incidence QTLs by marker-aided selection from one or more donors to three elite recurrent parent lines (28B, 20B and KR 192) developed at the Sorghum Research Station Parbhani.

Using marker-assisted selection, we able to introgress genomic regions for shoot fly resistance from donor parents (*i.e.*, IS 18551 and RILs 189, 153 and 252 derived from BTx623 × IS 18551) to recurrent parents (28B, 20B and KR 192) over two generations. Markers linked to shoot fly resistant QTL regions to be transferred from donor to recurrent parents were used for foreground selection, where as polymorphic markers evenly distributed over genomic regions to be retained from the recurrent parents were used for background selection. Based on the genotype data, individuals heterozygous (BC_1F_1 and BC_2F_1 generations) for markers spanning shoot fly resistance QTLs were selected during the first step of selection (foreground selection). Among these initially selected individuals, those with background genotypes having minimal presence of donor alleles unlinked to shoot fly resistance QTLs were selected during the second step of selection (background selection).

5.10.1 Criteria for selecting the individuals

Markers, especially foreground markers, were taken into consideration for selection of the individual segregants to be advanced. The individuals scored as 'A', 'H' or 'B' for markers used in foreground selection, and 'A' for most background markers, were selected for generation advance. Individuals scored 'H' at a particular marker locus

are expected to produce progeny segregating 1:2:1 for homozygosity for the recurrent parent allele (scored 'A'), heterozygosity (scored 'H') and homozygosity for the donor parent allele (scored 'B') if they are advanced by selfing, or segregating 1:1 for homozygosity for the recurrent parent allele (scored 'A') and heterozygosity (scored 'H') if they are advanced by backcrossing to the recurrent parent. Presence of 'A' genotypes for background markers and 'H' genotypes for foreground markers flanking a particular shoot fly resistant QTL ensures the recovery of the recurrent parent genome (28B, 20B or KR 192), while advancing introgression of the genomic region contributing to shoot fly resistance traits. Individuals meeting these criteria were selected and advanced to the next generation by selfing and backcrossing. Individuals scored 'A' (for all foreground and background) markers were found to be very similar to their recurrent parents (in fact they should be identical to the recurrent parent except for small introgressions) and could be selected as control entries for use in field trials to assess the efficiency of MAS for the shoot fly resistance component traits.

For selected individuals, the markers scored 'H' and those that did not amplify during the BC_1F_1 background screening were screened again during the next generation. The markers scored 'A' (homozygous for the allele of the recurrent parent) were not tested further in advanced generations because recovery of the recurrent parent genotype at these loci has been completed and their genetic constitution is not expected to change further assuming a negligible rate of mutation and no outcrossing to non-recurrent parent genotypes. Once the recurrent parent genome has been recovered for all the background markers, a generation of selfing and selection for homozygous donor parent marker alleles at loci flanking specific target shoot fly resistance QTLs will be conducted and the selected genotypes then multiplied by selfing prior to being tested multilocationally to evaluate them phenotypically for shoot fly resistance component traits and other agronomic traits. After testing, if the progeny with the introgressed shoot fly resistance QTLs are found to be significantly superior compared with the recurrent parent, they can be released as improved versions of the recurrent parental lines for use in breeding agronomically elite hybrids with improved shoot fly resistance. The improved potential for shoot fly resistance of these new elite parental lines will be due to introgression of shoot fly resistance QTLs by marker-assisted backcrossing.

Twelve SSR marker loci linked to the four targeted shoot fly resistance QTLs were used for genotyping recurrent and donor parents. The details of the parental polymorphism detected at these loci were presented in the Results chapter of this thesis (Table 4.9). After detecting the polymorphism between the recurrent and donor parents, homozygous parental-type plants were selected at seedling stage for subsequent plant-to-plant crosses, which were effected by manual emasculation followed by controlled pollination. Finally, we succeeded to effect five F_1 hybrids involving true-to-type parental plants. Out of these five hybrids, two were based on 20B, two were based on KR 192, and one was based on 28B.

5.10.2 F_1 and BC_1F_1 generations for recurrent parent 28B

- Five plants putatively produced from cross 28B (288) × RIL 189 (312) were genotyped at seedling stage using (Table 4.10a) four SSR loci linked with targeted shoot fly resistance QTLs. Two heterozygous F_1 hybrid plants were selected as a females and crossed with pollen from selfed progeny of the original recurrent parent 28B (288) to produce BC_1F_1 seed.
- Thirty BC_1F_1 plants of the [28B (288) × RIL 189 (312)] × 28B (288) backcross population were screened at seedling stage at loci detected by 11 SSR primer pairs that targeted four shoot fly QTLs (Table 4.10b). Fourteen heterozygous plants (SP nos. 612, 613, 617, 619, 710, 711, 719, 811, 812, 814, 815, 816, 817 and 818) were selected at seedling stage and crossed with the pollen from the selfed progeny of recurrent parent 28B (288) to generate BC_2F_1 populations. According to Tanksely *et al.* (1989), computer stimulation using the tomato as a model showed that by selecting the best plant out of a total of 30 per generation, the whole recurrent genome could be covered in two generations.

5.10.3 F_1 and BC_1F_1 generations for recurrent parent 20B

- Ten plants putatively produced from the plant-by-plant cross 20B (186) × RIL 252 (318) were genotyped at seedling stage with four SSR loci linked with targeted shoot fly resistance QTLs. Two heterozygous F_1 hybrid plants were identified and backcrossed with pollen from the selfed progeny of the original recurrent parent 20B (186) to generate BC_1F_1 populations (Table 4.12a).
- Thirty BC_1F_1 plants of the [20B (186) × RIL 252 (318)] × 20B (186) backcross populations were genotyped at seedling stage with 11 SSR loci

associated with shoot fly resistance traits (Table 4.12b). Twelve plants (SP nos. 649, 650, 651, 655, 656, 657, 754, 757, 848, 849, 850 and 853) heterozygous for one or more targeted QTL introgressions were selected and used as females in backcrosses with selfed progeny of the original recurrent parent 20B (186) to advance to the BC_2F_1 generation.

- Ten plants putatively produced from the plant-by-plant cross 20B (179) × RIL 153 (248) were genotyped at seedling stage with four SSR loci linked with targeted shoot fly resistance QTLs. Eight heterozygous F_1 hybrid plants were selected (Table 4.13a) and used for backcrossing as females with pollen from selfed progeny of the original recurrent parent 20B (179) to advance to the BC_1F_1 generation.
- Thirty plants of these backcross populations [20B (179) × RIL 153 (248)] × 20B (179) were screened at seedling stage with 11 SSR marker loci associated with shoot fly resistance traits (Table 4.13b). Nineteen BC_1F_1 plants heterozygous for one or more targeted QTL(s) introgressions were identified (SP nos. 668, 669, 670, 672, 673, 674, 767, 771, 772, 773, 775, 776, 867, 868, 870, 871, 873, 874 and 876) and backcrossed selfed progeny of the original recurrent parent 20B (179) to advance to the BC_2F_1 generation.

5.10.4 F_1 and BC_1F_1 generations for recurrent parent KR 192

- Ten plants putatively produced from cross KR 192 (304) × IS 18551 (267) were genotyped using four SSR loci linked to targeted shoot fly resistance QTLs (Table 4.11a). All ten plants were identified as heterozygous and backcrossed with pollen from selfed progeny of the original recurrent parent KR 192 (304) to generate BC_1F_1 seed.
- Thirty BC_1F_1 plants of the [KR 192 (304) × IS 18551 (267)] × KR 192 (304) backcross populations were genotyped at seedling stage with 11 SSR loci linked to the four targeted shoot fly resistance QTLs (Table 4.11b). Fifteen heterozygous plants having one or more targeted QTL introgressions were selected (SP nos. 629, 630, 633, 636, 729, 731, 732, 736, 737, 830, 832, 833, 834, 835 and 836) and used as females for backcrossing with pollen from selfed progeny of the original recurrent parent KR 192 (304) to generate BC_2F_1 seed.
- Nine plants putatively produced from cross KR 192 (300) × RIL 252 (319) were screened with four SSR loci at the seedling stage (Table 4.14a). Eight

heterozygous plants were identified and crossed as females with pollen from selfed progeny of the original recurrent parent KR 192 (300) to generate BC_1F_1 seed.

- Genotyping at seedling stage of 30 BC_1F_1 [KR 192 (300) × RIL 252 (319)] × KR 192 (300) individuals with 11 SSR loci linked to targeted shoot fly resistance QTLs (Table4 .14b) identified 9 heterozygous plants (SP nos. 688, 692, 887, 889, 890, 892, 893, 894 and 895), which were selected and crossed with pollen from selfed progeny of the original recurrent parent KR 192 (300) to generate BC_2F_1 seed.

One hundred and fifty plants from five BC_1F_1 populations (described in the above paragraphs) were screened at seedling stage with 11 SSR loci linked with the four targeted shoot fly resistance QTLs. On the basis of molecular data, 69 plants having appropriate allelic constitution (heterozygous for one, two or more targeted QTL introgressions) were identified (Tables 4.10b-4.14b) and crossed as females with pollen from selfed progeny of their respective original recurrent parents to advance to the BC_2F_1 generation. The details of these 69 selected plants heterozygous for various targeted QTLs and the associated characters for the targeted genomic regions are presented in Table 5.2.

Table 5.2 List of selected QTL-introgressed heterozygote plants and associated characters of five backcross (BC_1F_1) populations produced in this study

No. of heterozygote plants selected	Targeted QTL linkage groups	Shoot fly resistance traits associated
20	LG A	Oviposition I, Deadhearts I
5	LG E	Oviposition I, Deadhearts I
10	LG G	Trichome density (upper and lower leaf blade surfaces), Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
4	LG A+E	Oviposition I, Deadhearts I
7	LG A+J	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
8	LG A+G	Trichome density (upper and lower leaf blade surfaces), Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
2	LG E+J	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
1	LG E+G	Trichome density (upper and lower leaf blade surfaces), Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
1	LG G+J	Trichome density (upper and lower leaf blade surfaces), Glossiness, Seedling vigor II, Oviposition II, Deadhearts II
3	LG E+G+J	Trichome density (upper and lower leaf blade surfaces), Glossiness, Seedling vigor II, Oviposition II, Deadhearts II
5	LG A+E+J	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
2	LG A+G+J	Trichome density (upper and lower leaf blade surfaces), Glossiness, Seedling vigor II, Oviposition II, Deadhearts II
1	LG A+E+G	Trichome density (upper and lower leaf blade surfaces), Glossiness, Seedling vigor II Oviposition I and II, Deadhearts I and II

5.10.5 Efficiency of marker-assisted selection

5.10.5.1 Background genotyping of BC_1F_1 individuals selected on the basis of foreground selection

A total of 61 BC_1F_1 plants selected through foreground screening from five backcross populations were genotyped with a set of polymorphic SSR markers (Table 4.21) covering the entire genome except the genomic regions harboring the four targeted shoot fly resistance QTLs.

The main objective of background selection was to confirm and hasten recovery of the recurrent parent genome in these genomic regions distant from the four targeted introgressions. Twelve plants were selected from these five backcross populations, each of which carry homozygous recurrent parent alleles ('A' genotypes) at most of the background SSR loci and have a few heterozygous ('H' genotypes) background SSR loci. Those individuals homozygous for any donor parent allele ('B' genotype) were rejected as they could only have resulted from failure of backcrossing (*i.e.*, selfing) in the previous generation. The 12 selected individuals from five BC_1F_1 populations each had been crossed with their respective recurrent parents to advance this marker-assisted QTL introgression program to the BC_2F_1 generation. Details regarding selected individuals including targeted QTLs, number of SSR loci genotyped as 'A' allele, 'H' allele, and/or 'not amplified SSR loci' in the BC_1F_1 are presented in Table 5.3.

The result of the BC_1F_1 generation background screening (Table 5.3) revealed that in the BC_1F_1 generation for recurrent parent 28B on average 21 SSR marker loci were tested and in the BC_2F_1 of recurrent parent 28B the number of these background markers requiring genotyping reduced to 3-13 per population. Further, in the BC_1F_1 generation for recurrent parent KR 192 (304) on average 28 SSR marker loci /population were tested for background screening, and in the BC_2F_1 generation for this recurrent parent the number of these background markers requiring genotyping reduced to 5-14 per population. Moreover, in the BC_1F_1 generation for recurrent parent KR 192 (300), an average 30 of marker loci/population were tested for background screening and in the BC_2F_1 generation for KR 192 (300) the number of these background markers requiring genotyping reduced to 12 per population. Finally, in the BC_1F_1 generation for recurrent parents 20B (186) and 20B (179), an average 18 and 23 SSR marker pairs/ population, respectively, were tested during background genotyping, and in the BC_2F_1 generation for 20B (186) and 20B (179) the number of

Table 5.3: Details of background screening of BC_1F_1 selected individuals and of BC_2F_1 individuals to be genotyped, with targeted QTLs

Names of the backcross populations	Plant selected (BC_1F_1)	Targeted QTL linkage group	Plants genotyped (BC_1F_1)	No. of polymorphic SSR loci used in background screening for (BC_1F_1)	No of SSR loci genotyped 'A' allele in BC_1F_1	No of SSR loci genotyped 'H' allele in BC_1F_1	No of SSR loci not ampli-fied in BC_1F_1	No of SSR loci to be used in back-ground screening for BC_2F_1	No of genotypes to be back-ground screened in BC_2F_1
28B (288) × RIL 189 (312) × 28B (288)	SP 719	A+J	11	21	8	8	5	13	1
	SP 814	A			10	9	2	11	5
	SP 818	E+G+J			18	1	2	3	12
KR 192 (304) × IS 18551 (267) × KR 192 (304)	SP 629	A+G	14	28	14	7	7	14	6
	SP 830	A+G			23	2	3	5	6
20B (186) × RIL 252 (318) × 20B (186)	SP 757	E	11	18	7	8	3	11	4
20B (179) × RIL 152 (248) × 20B (179)	SP 669	A+G	16	23	18	3	2	5	6
	SP 773	A+G+J			14	5	4	9	11
	SP 871	A		23	14	9	-	9	3
	SP 874	E+J			12	9	2	11	4
KR 192 (300) × RIL 252 (319) × KR 192 (300)	SP 889	A+J	9	30	18	10	2	12	8
	SP 895	G			19	10	1	11	2

these background markers still requiring genotyping reduced to 11 and 5, respectively. The decreasing numbers of background markers still requiring genotyping in the BC_2F_1 generation populations reflects the increasing percentage of recurrent parent alleles fixed in each advancing backcross generation. Hospital *et al.* (1997), based on the stimulation studies, recommended an optimal distance between two adjacent markers of about 5-10 cM. We used much larger intervals between both foreground marker pairs and between adjacent background marker loci. However, we still observed that the frequency of recurrent genotypes among the selected progeny increased as the selection intensity for recurrent genotype increased, as reported by Knapp (1998). Practically speaking, the number of markers that must be used decreases in each successive backcross generation (Table 5.3) because once the recurrent parent allele has been fixed at any given non-targeted locus, it is not necessary to continue screening at that locus in subsequent generations as the locus will remain homozygous for the remainder of the backcross and selfing generations (Moris *et al.*, 2003). Marker-assisted selection has the potential to greatly reduce the cost and time for selecting desirable genotypes with traits of interest (Moris *et al.*, 2003). Marker-assisted selection is more efficient and cost effective than conventional selection for traits with a low heritability and high phenotypic trait effect (Hospital *et al.*, 1997). Using MAS the current study was able to advance through four generations within two years. When conventional breeding strategies are applied, the advancement of the four backcrossing generations with phenotypic selection for shoot fly resistance traits will take at least four years. Conventional breeding schemes feature low cost per unit time during the research stage but require a longer time to complete, where as marker-assisted breeding features high cost during the research stage, but takes less time to complete. Release stage and adaptation stages of conventional and marker-assisted breeding are assumed to be identical in terms of cost as well as duration. From an economic point of view the advantages of MAS thus derives from the fact that the release and adaptation stages move forward in time, so that the benefits from crop varietal improvement reach farmers and consumers earlier (increasing the rate of return on the research investment). This suggests that while MAS needs more initial investment, it is worthwhile in at least certain cases because via the accelerating rate of varietal release, MAS generates large additional economic benefits (Moris *et al.*, 2003).

The fact that MAS technology is so challenging should not be a reason for discouragement, but instead, a wake-up call for more ingenuity, better planning and execution of marker-assisted breeding programs. MAS for quantitative traits is in an important transition phase, and the field is on the verge of producing convincing results. Technology development, including automation, allele-specific diagnostics and DNA chips, will soon make marker-assisted selection approaches based on large-scale screening much more powerful and effective (Young, 1999).

5.10.6 Genotyping of the five BC_2F_1 populations with SSR markers for foreground selection

Screening 224 BC_2F_1 plants from five populations was completed at the seedling stage with 10 SSR marker loci linked to targeted QTLs associated with shoot fly resistance traits in four linkage groups (Table 4.22-4.26). Around 100 heterozygous plants have one or more targeted QTL introgressions were selected and crossed as a female parents with the selfed progeny of their respective recurrent parents to generate BC_3F_1 populations. Details of the numbers of plants introgressed with each combination of targeted QTLs and associated shoot fly resistance traits across these five populations are presented in Table 5.4.

Table 5.4 Details of foreground selection of five BC_2F_1 populations with targeted shoot fly resistance QTL and character association

No. of introgressed plants selected	Targeted QTL linkage group	Shoot fly resistance trait associations
37	LG A	Oviposition I and Deadhearts I
16	LG E	Oviposition I and Deadhearts I
13	LG G	Trichome density (upper and lower leaf blade surfaces), Seedling vigor II, Deadhearts I and II, Oviposition I and II, and Glossiness
11	LG J	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
03	LG E+G	Trichome density (upper and lower leaf blade surfaces), Seedling vigor II, Deadhearts I and II, Oviposition I and II, and Glossiness
07	LG A+J	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
10	LG A+G	Trichome density (upper and lower leaf blade surfaces), Seedling vigor II, Deadhearts I and II, Oviposition I and II, and Glossiness
03	LG A+G+J	Glossiness, Trichome density (upper and lower leaf blade surfaces), Oviposition I and II, Deadhearts I and II

5.10.7 Genotyping of foreground-selected BC_2F_1 plants for background selection

Out of 100 BC_2F_1 plants selected on the basis of foreground screening only 68 plants from five backcross population will be genotyped with a set of polymorphic loci covering the entire genome except regions associated with targeted shoot fly resistance QTLs. This background screening will be restricted to the SSR loci that were heterozygous or not amplified in the BC_1F_1 generation background screening (Table 4.28). Details of the 68 foreground-selected BC_2F_1 plants from five backcross populations chosen for background screening are presented in Table 5.5.

Table 5.5 Details of foreground-selected BC_2F_1 plants chosen for background genotyping from five BC_2F_1 populations with targeted QTL and character associations

No. of plants chosen for background screening	Targeted QTL linkage groups	Shoot fly resistance trait associations
14	LG A	Oviposition I and Deadhearts I
12	LG E	Oviposition I and Deadhearts I
12	LG G	Trichome density (upper and lower), Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
07	LG J	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
03	LG E+G	Trichome density (upper and lower), Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
07	LG A+J	Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
10	LG A+G	Trichome density (upper and lower), Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II
03	LG A+G+J	Trichome density (upper and lower), Glossiness, Seedling vigor II, Oviposition I and II, Deadhearts I and II

5.10.8 Recommendations for future studies

Based on the present study, the following two routes for future pyramiding of shoot fly resistance QTLs in elite agronomic backgrounds are suggested.

1 The first avenue consists of identifying the best BC_3F_1 plants, *i.e.* those showing the highest amount of favorable QTL introgression for shoot fly resistance traits and fixing the favorable recurrent parent alleles in BC_3F_1 generation background screening. Such selected BC_3F_1 plants can then be crossed with other BC_3F_1 plants of different descent but having a common recurrent parent in order to pyramid as many as shoot fly resistance QTLs as possible (each contributing to different traits) within the same genome (selective pyramiding).

2 The second avenue consists of seedling stage genotyping of the BC_3F_1 population for each recurrent parent with linked SSR marker pairs targeting each of the four shoot fly resistance QTLs for foreground selection. Foreground--selected individuals will be background genotyped using a set of polymorphic SSR markers unlinked to targeted QTL regions. Those individuals appearing to fully recover of recurrent parent marker genotype across these background marker loci and phenotypically most nearly identical to their recurrent parent will selfed to fix the introgressed QTL alleles. The resulting segregating progenies will be used for development of near-isogenic lines (NILs). Such plant material should prove useful to study the effects of any given single QTL on the phenotypic value of a plant harboring it. In case the introgressed QTL proves to contribute significantly to the improvement of any given trait the line can then be used directly in hybrid breeding. Further, such QTL-NILs could also be used as donor or recurrent parents in a short series of crosses for QTL pyramiding

3 Phenotyping of the selected individuals for the shoot fly resistance components traits is needed. Introgression of any trait is confirmed phenotypically after several generation of genotyping. In this context, the selected BC_3F_2 genotypes (in 28B, KR 192 and 20B recurrent parent backgrounds) will be evaluated for shoot fly resistance component traits during late *kharif* 2006 and *rabi* 2006/07 seasons. Fine mapping for these shoot fly QTLs will be a practical possibility only once the presence of the different shoot fly resistance QTLs is phenotypically confirmed. Such confirmed shoot fly resistance QTL introgression lines can then be used to to generate ESTs for better understanding of this complex trait.

SUMMARY AND CONCLUSIONS

CHAPTER VI

SUMMARY AND CONCLUSIONS

The present investigation entitled, "Genetic diversity analysis, QTL mapping and marker-assisted selection for shoot fly resistance in sorghum [*Sorghum bicolor* (L.) Moench]" was aimed, 1) to assess genetic diversity by SSR markers in a set of insect resistant lines, 2) to understand the genetics of the shoot fly resistance and locate the chromosomal regions harboring the quantitative trait loci (QTLs) for shoot fly resistance and related component traits, and 3) introgression of shoot fly resistance components traits in agronomically superior genotypes using molecular marker-assisted selection. For all the studies involving the use of SSR markers, DNA was extracted with a modified CTAB method. In the first objective, genetic diversity analysis by SSR markers in a set of sorghum lines, 91 sorghum accessions were genotyped using 20 SSR primer pairs that detected loci distributed over 9 of the 10 linkage groups in the sorghum nuclear genome. PCR amplification of a targeted sequence was conducted in an Applied Biosystem GeneAMP PCR system 9700 thermocycler using a touch down PCR protocol. Amplified products were separated by electrophoresis on 6% non-denaturing polyacrylamide gels, and by capillary electrophoresis using automated DNA sequencers. PCR products separated by PAGE were visualized by silver staining. Intensely amplified specific bands representing the corresponding allelic products of the SSR markers in each accession were scored to produce the PAGE data set. The NTSYS statistical software package was used for cluster analysis of the SSR marker allele data sets from the PAGE and capillary electrophoresis PCR product separation methods. Jaccard's similarity coefficient between each pair of accessions was used to construct dendrograms for each of these two data sets using the un-weighted paired group method with arithmetic averages (UPGMA).

The second objective included phenotyping and mapping of QTL(s) for shoot fly resistance and its component traits in a (296B × IS 18551)-derived RIL mapping population. The 259 RILs along with their two parental lines were evaluated in late *kharif* 2002 and early *rabi* 2004-2005 screening environments with standard shoot fly resistance screening procedure at ICRISAT, Patancheru. Observations for shoot fly resistance and component traits were recorded during phenotypic screening in each environment. The analysis of variance for phenotypic data sets was performed using

the residual maximum likelihood algorithum (ReML), which provides the best linear unbiased predictors (BLUPs) of performance of the genotypes. The BLUPs of 213 uniform RILs, along with their genotypic data from 111 marker loci, were used for QTL analysis. QTL analysis was performed using the composite interval mapping (CIM) method. The required computations were performed using PLABQTL version 1.1, which uses a regression approach.

The third objective included introgression of shoot fly resistance components traits into agronomically superior genetic backgrounds using marker-assisted selection, in which two potential maintainer lines (20B and 28B) and one potential restorer line (KR 192) used as a recurrent parents, and RILs 153, 189 and 252 derived from cross (BTx623 × IS 18551), and IS 18551 (resistant parent) were used as donor parents. Three shoot fly resistance QTLs had been previously mapped in each of these selected RILs, and the resistant parent carries all the four QTLs imparting shoot fly resistance. Eleven primer pairs for SSR loci flanking to the four target shoot fly resistance QTLs from 4 linkage group were used for genotyping the parental population at seedling stage, polymorphic SSR loci were identified, true-to-type parental plants were selected and crosses (plant-to-plant) between recurrent and donor parents were effected. Five hybrids were developed and these five hybrids were advanced for backcrossing. Now these five populations are in BC_2F_1 stage. The genotyping procedure described for the first objective above was also followed in this objective.

The research results and conclusions for each of these objectives are briefly summarized below.

I. Application of SSR markers in diversity analysis of sorghum insect resistant germplasm accessions

1. In this study we tried to assess the genetic diversity of a set of 91 elite sorghum germplasm accessions using SSR markers. The set include 12 shoot fly and 15 stem borer resistant accessions, 9 accessions resistant to both shoot fly and stem borer, 17 midge resistant accessions, and 38 agronomically elite recurrent parents that were in use at ICRISAT to initiate a large-scale marker-assisted backcross program for the stay-green components of terminal drought tolerance from donor parents B35 and E 36-1.
2. In case of PAGE electrophoresis separation of the PCR products for diversity analysis, 20 out of 21 primer pairs used provided amplification products, while

11 out of these 20 revealed high level of polymorphism. A total 69 alleles were detected by silver staining, for an average of 3.5 fragments amplified per SSR locus across the 91 sorghum accessions studied. In case of capillary electrophoresis (ABI) separation of these PCR products, a total of 118 alleles were detected, for an average of 5.1 fragments amplified per SSR locus. Based on capillary electrophoretic separation of their PCR products, 13 out of 20 (65%) SSR primer pairs were able to detect a high level of polymorphism.

3. Jaccard's coefficient of similarity for pairs of the 91 sorghum accessions studied ranged form 0.28-1.00. The dendrograms for the similarity between accessions based on PAGE- and ABI-generated SSR genotype data showed clustering for geographical origins, races and specific traits such as insect resistance.
4. In case of the PAGE dendrogram, the 91 sorghum accessions diverged into 20 clusters at the 50% level of similarity. Among these, the largest cluster was cluster 4 (18 accessions), which was followed by cluster 12 (13 accessions) and cluster 13 (12 accessions). However, some of the clusters (*e.g.* clusters 5, 7, 9, 10, 14, 19 and 20) accommodated only a single accession each, and clusters 1, 3 and 6 accommodated only 2 accessions each. In case of the ABI dendrogram, the 91 sorghum genotypes grouped into 28 clusters at the 50% level of similarity. When compared to the dendrogram from the PAGE-generated data sets, the number of clusters detected with ABI-generated data was moderately higher. This is likely due to the greater sensitivity of the automated sequencer, which allow it to detect SSR alleles differing by smaller numbers of repeated units than was possible with the silver-stained PAGE gels, so that it coulde effectively detect a higher level of polymorphism.
5. The information provided by this study about the diversity/similarity of the germplasm from different sources of origin or region should prove extremely useful for heterosis breeding and in selecting parental lines for specific breeding goals related to combining insect resistance with high grain yield and mitigation of drought stress.
6. This diversity analysis for 91 sorghum accessions revealed that the genotypes studied are genetically quite diverged with sorghum lines showing midge, shoot fly and stem borer resistance clustering in different groups. In addition, a cluster of agronomically superior recurrent parents was identified that is

genetically quite divergent from each of these insect resistance clusters. However, some of the accessions with resistance to midge, shoot fly and stem borer clustered separately, indicating that these lines might contain new allelic variants that can be exploited in breeding program. This information will be useful for identifying parents for marker-assisted backcrossing programmes to introgress insect resistance QTLs from the currently available mapping populations. Further, newly identified agronomically elite and genetically diverse insect resistant breeding lines can be used for developing new mapping populations to detect additional insect resistance QTLs.

II. Phenotyping a set of RILs and identification of QTLs for shoot fly resistance components of the RIL population derived from cross 296B × IS 18551.

1. In general, the mapping population parents (296B and IS 18551) differed phenotypically for all observed parameters of shoot fly resistance.
2. Parental and RILs mean values revealed wide variation in phenotypic values for shoot fly resistance and its component traits in both of the screening environments. Wide variation was observed in the RIL population for shoot fly resistance component traits like glossiness intensity, trichome density (upper and lower surfaces of seedling leaf blades), seedling vigor, oviposition incidence (%), and deadhearts incidence (%). These traits can be used as simple criteria for selection of resistant genotypes.
3. The genotypic variances for shoot fly resistance traits were significant in both of the individual screening environments as well as in the across-season analysis.
4. Glossiness intensity, trichome density (both upper and lower surfaces of seedling leaf blades), oviposition incidence (%), deadhearts incidence (%), and seedling vigor recorded consistent heritability (broad sense) estimates in individual screening environments, but low to moderate heritability estimates in the across-season analyses indicating that these traits are under genetic control but that there is a substantial role of genotype (G) × environment (E) interaction in expression of these traits.
5. RIL population means differed significantly from those of both the parents for important shoot fly resistance component traits like glossiness, oviposition incidence (%), deadhearts incidence (%) and trichome density (upper and

lower surfaces of seedling leaf blades). Transgressive segregants with phenotypic values out side the parental limits were observed for glossiness, oviposition incidence, deadhearts incidence and trichome density.

6. QTL analysis revealed presence of putative QTLs for all important shoot fly resistance and resistance component traits like glossiness, oviposition incidence (%), deadhearts incidence (%), and trichome density. The portion of observed phenotypic variance explained by different putative QTLs varied from 6 to 34%. Glossiness intensity was largely controlled by a major QTL on LG J, accounting 34% of observed phenotypic variation, and one minor QTL on LG G, accounting for 8% of observed phenotypic variation in the across-season analysis. After adjusting for QTL × environmental interaction, these two QTLs explained 31% of genetic variation in glossiness intensity in this RIL population. Resistant parent IS 18551 contributed the additive genetic effects for increased glossiness at both of these QTLs.
7. For oviposition II and deadhearts II incidence (%), two common QTLs (one on LG F and one on LG G) were mapped in the across-season analysis. Both QTLs together explained 17% and 19% phenotypic variation in oviposition II and deadhearts II, respectively, in the across-season analysis. Significant QTL × environmental interactions were observed for both of these QTLs for oviposition II and deadhearts II resistance traits. The QTL mapped on LG G for deadhearts and oviposition co-localized with a major QTL for trichome density (upper and lower surfaces of seedling leaf blades) and a minor QTL of glossiness intensity. The QTL mapped on LG F for deadhearts and oviposition co-localized with a minor QTL for trichome density of the lower leaf blade surface.
8. For trichome density, one QTL was detected on LG G accounting for 30% of observed phenotypic variance in the across-season analysis for trichome density on the upper leaf blade surface. This QTL for trichome density on the upper leaf surface co-localized with a QTL for trichome density on the lower leaf surface that explains nearly 27% of observed phenotypic variance across the two screening environments. This indicates similarity in genetic control of trichome density on either side of sorghum seedling leaf blades.
9. The major QTL for glossiness intensity and minor QTL for oviposition (LG J) and major QTL for trichome density and minor QTLs for glossiness,

deadhearts and oviposition (LG G) detected in this study have previously been mapped at the same location in another sorghum RIL population derived from cross BTx623 × IS 18551. This confirms that these chromosomal regions might be harboring candidate genes contributing to shoot fly resistance of IS 18551.

10. Two aphid resistance QTLs were detected in the across-season analysis. These mapped on LG E and LG J and together explain 26% of observed phenotypic variation in aphid score. The QTL mapped on LG J was a major one, accounting for 20% of observed phenotypic variance and having non-significant Q × E interaction. The favorable additive genetic effects for both aphid resistance QTLs were contributed by IS 18551 alleles.
11. Utilization of RILs no. 47, 51, 82, 97, 130 and 174 (*rabi* season adaptation) and RILs no. 46, 208, 222, and 223 (*kharif* season adaptation) in sorghum improvement programs aimed at improving shoot fly resistance of elite cultivars and hybrid parental lines is likely to be more fruitful than direct use of an agronomically poor source like IS 18551.

III. Introgression of shoot fly resistance component traits in agronomically superior genotypes using molecular marker-assisted selections

1. Flanking SSR marker loci closely linked to four QTLs for shoot fly resistance components in the (BTx623 × IS 18551)-based RIL population and resistant parent IS 18551 were identified in an earlier studies. In the present study efforts are being made to transfer these four QTLs by marker-assisted selection from donor parents (IS 18551, and RIL nos. 189, 153, and 252 derived from BTx623 × IS 18551) to three elite recurrent parental lines (28B, 20B and KR 192) developed at SRS, MAU, Parbhani, Maharashtra, India.
2. Twelve SSR marker loci linked to the targeted shoot fly resistance QTLs from four linkage groups (LG A, LG E, LG G and LG J) were used for genotyping the three recurrent and four donor parents. After detecting SSR marker polymorphism between the recurrent and donor parents at seedling stage, homozygous parental-type plants were selected and subsequent plant-to-plant crosses were effected. Finally we succeeded in developing five F_1 hybrids: *i.e.* 1. 28B (228) × RIL 189 (312), 2. KR 192 (304) × IS 18551 (267), 3. 20B (186) × RIL 252 (318), 4. 20B (179) × RIL 153 (248), 5. KR 192 (300) × RIL 252 (319).

3. Five to ten putative F_1 plants produced from each of hybrid combinations were genotyped at the seedling stage using four SSR loci linked with targeted QTLs. Heterozygous F_1 hybrid plants in each of these combinations were selected and used as a females in crosses with pollen from selfed progeny of their respective recurrent parent to produce BC_1F_1 generation seeds.
4. Around 150 plants from five BC_1F_1 populations were screened at the seedling stage with 11 SSR loci linked with the four targeted shoot fly resistance QTLs (foreground selection). On the basis of this genotypic data, 69 heterozygous plants having one, two or more targeted QTL introgression(s) were identified and crossed as females with pollen from selfed progeny of their respective recurrent parent to produce BC_2F_1 seed.
5. In case of background genotyping of these BC_1F_1 individuals a total of 61 BC_1F_1 plants selected through foreground screening from five backcross populations were genotyped with a set of polymorphic SSR markers covering the entire genome except the genomic regions harboring the four targeted shoot fly resistance QTLs.

   Twelve plants were selected from across these five backcrossing populations, whichcarry homozygous recurrent parent alleles at most of the background SSR marker loci and have only a few heterozygous loci to be used for future background screening. The backcrossed seed of the 12 plants were advanced to raise the BC_2F_1 generation.
6. Around 224 BC_2F_1 plants from five populations were genotyped at the seedling stage with 10 SSR marker loci linked to four targeted QTLs associated with shoot fly resistance traits. Around 100 heterozygous plants have one, two or more QTL introgression(s) were selected and crossed as a female parents with the selfed progeny of theie respective recurrent parents to generate BC_3F_1 seed.
7. Out of 100 BC_2F_1 plants selected in foreground screening only 68 plants from five backcross populations will be genotyped (background selection) with a set of polymorphic SSR loci covering the entire genome except the region of targeted QTLs. This background screening will be restricted to the loci that were either heterozygous or not amplified in the BC_1F_1 generation background screening. On the basis of background and foreground screening data, individuals having essentially full genome of recurrent parent recovered along

with one or more of the four targeted QTLs will be selected. Cross seed of such individuals will be advanced to raise the BC_3F_1 generation for advancement to the BC_3F_2/BC_4F_1 generations.

8. In the BC_3F_1 generation sizable numbers of plants in each backcross population will be genotyped at the seedling stage with 10 SSR markers linked with the four targeted QTLs (foreground selection). On the basis of foreground molecular data heterozygous individuals will be identified that carry one or more of the targeted QTLs. Such individuals will be selfed to fix the favorable allele(s), and segregating progeny will be used for development of near-isogenic lines (NILs) and pyramiding of particular resistance QTL combinations in the genetic backgrounds of each of the three recurrent parents.
9. Phenotyping of shoot fly resistance component traits for the resulting selected homozygous individuals for each near-isogenic line pair is suggested. In this context the selected genotypes in five BC_3F_2 populations will be evaluated for shoot fly resistance component traits in a shoot fly screening nursery in late *kharif* 2006, and *rabi* 2006-2007. Fine mapping for these shoot fly QTLs is possible once the presence of different shoot fly resistance QTLs is phenotypically confirmed. ESTs can be generated subsequently from such confirmed QTL introgression lines in order to improve our understanding of this complex and challenging trait. for the economic benefit of poverty-striken poor sorghum growing farmers in the Semi-Arid Tropics (SAT).

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* Originals not seen

APPENDICES

APPENDIX I: Particular characteristics of sorghum accessions used in this sorghum diversity study

Sl. No	Genotype name	Origin	Shoot fly	Stem borer	Midge	Stay-green	Mapping population parent	Recurrent Parent for Shoot fly resistance	Donor parent
1	BTx623	TAMU, USA	Susceptible	Susceptible	Susceptible	Senescent	Shoot fly		
2	IS 2122			Resistant					
3	IS 2123		Resistant	Resistant					
4	IS 2195	India	Resistant	Resistant					
5	IS 2205	India	Resistant						
6	IS 2265	Sudan	Resistant	Resistant					
7	IS 2312	Sudan	Resistant	Resistant					
8	IS 2367	Nigeria		Resistant					
9	IS 3962	India		Resistant					
10	IS 4756	India		Resistant					
11	IS 5469	India		Resistant					
12	IS 5470	India	Resistant						
13	IS 5480	India	Resistant						
14	IS 5490	India		Resistant					
15	IS 5571	India		Resistant					
16	IS 5658	India		Resistant					
17	IS 17948	India		Resistant					
18	IS 18573	Nigeria (breeding line)		Resistant					
19	IS 18577	Nigeria (breeding line)		Resistant					
20	IS 18579	Nigeria (breeding line)		Resistant					
21	IS 18581	Nigeria (breeding line)		Resistant					
22	IS 22148	India	Resistant						
23	ICSV 700	ICRISAT	Resistant	Resistant	Susceptible		Stem borer and midge fly		Stem borer resistance
24	PB 12779-2	ICRISAT		Resistant					
25	PB 15881-3	ICRISAT		Resistant	Susceptible		Stem borer and midge fly		Stem borer resistance
26	IS 1034		Resistant						
27	IS 2269		Resistant						
28	IS 2291		Resistant	Resistant					
29	IS 18366		Resistant						

Sl. No	Genotype name	Origin	Shoot fly	Stem borer	Midge	Stay-green	Mapping population parent	Recurrent Parent for Shoot fly resistance	Donor parent
30	IS 18551		Resistant				Shoot fly		Shoot fly Resistance
31	IS 22121		Resistant	Resistant					
32	ICSB 457	ICRISAT	Resistant				Shoot fly		Shoot fly resistance
33	ICSV 705	ICRISAT	Resistant						
34	ICSV 707	ICRISAT	Resistant						
35	ICSV 708	ICRISAT	Resistant						
36	ICSV 713	ICRISAT	Resistant						
37	ICSV 714	ICRISAT	Resistant	Resistant	Susceptible		Stem borer and midge		Stem borer resistance
38	PB 15520	ICRISAT	Resistant	Resistant			Stem borer and midge		Stem borer resistance
39	IS 18695				Resistant				
40	IS 19476				Resistant				
41	IS 22806				Resistant				
42	ICSV 197	ICRISAT			Resistant				
43	ICSV 388	ICRISAT			Resistant				
44	ICSV 391	ICRISAT			Resistant				
45	ICSV 730	ICRISAT			Resistant				
46	ICSV 736	ICRISAT			Resistant				
47	ICSV 745	ICRISAT		Susceptible	Resistant		Stem borer and midge		Midge resistance
48	ICSV 746	ICRISAT			Resistant				
49	ICSV 748	ICRISAT			Resistant				
50	ICSV 757	ICRISAT			Resistant				
51	ICSV 88014	ICRISAT			Resistant				
52	ICSV 88032	ICRISAT	Susceptible	Susceptible	Resistant		Stem borer and midge		Midge resistance
53	ICSV 88041	ICRISAT			Resistant				
54	ICSV 89051	ICRISAT			Resistant				
55	ICSV 89054	ICRISAT			Resistant				
56	ICSV 90004	ICRISAT			Resistant				

Sl. No	Genotype name	Origin	Shoot fly	Stem borer	Midge	Stay-green	Mapping population parent	Recurrent Parent Shoot fly resistance	Donor forparent
57	DJ 6514				Resistant				
58	TAM 2566	TAMU			Resistant				
59	AF 28				Resistant				
60	296B	India (hybrid seed parent maintainer)	Susceptible	Susceptible	Susceptible		Shoot fly and grain mold	Stay-green	
61	ICSV 111	ICRISAT						Stay-green	
62	ICSV-LM 90501	ICRISAT LASIP						Stay-green	
63	Macia	ICRISAT						Stay-green	
64	CSV 4 (CS 3541)	India (converted zera-zera landrace)						Stay-green	
65	CSV 14R	India						Stay-green	
66	CSV 8R	India						Stay-green	
67	Godamhuman	Sudan				Senescent		Stay-green	
68	HC 260							Stay-green	
69	ICSB 101	ICRISAT						Stay-green	
70	ICSB 11	ICRISAT						Stay-green	
71	ICSV 401	ICRISAT						Stay-green	
72	ICSV-LM 86513	ICRISAT LASIP						Stay-green	
73	ICSV-LM 89522	ICRISAT LASIP						Stay-green	
74	ICSV-LM 90541	ICRISAT LASIP						Stay-green	
75	LARSVYT	ICRISAT LASIP via SADC						Stay-green	
76	M 148							Stay-green	
77	Malisor 84-7	ICRISAT Mali						Stay-green	
78	Nagawhite							Stay-green	
79	Patancheru local	India						Stay-green	
80	RS 29	India					Grain mold	Stay-green	
81	SDSL 87046	ICRISAT SADC						Stay-green	
82	SDSL 87574	ICRISAT SADC						Stay-green	
83	SDSL 88928	ICRISAT SADC						Stay-green	
84	SPUCV 422							Stay-green	
85	SPV 467	India						Stay-green	
86	SSG 59-2							Stay-green	

Sl. No	Genotype name	Origin	Shoot fly	Stem borer	Midge	Stay-green	Mapping population parent	Recurrent Parent for Shoot fly resistance	Donor parent
87	Swati	India						Stay-green	
88	Madau 131	Indonesia						Stay-green	
89	UPCAS 1-132	Indonesia						Stay-green	
90	Keris	Thailand						Stay-green	
91	Uthong 1	Thailand						Stay-green	
92	Suphanbur 11	Thailand						Stay-green	
93	Suphanbur 160	Thailand						Stay-green	
94	LS 1	PR China						Stay-green	
95	LS 2	PR China						Stay-green	
96	CSV 15	India						Stay-green	

APPENDIX II
Preparation of Stock Solutions

CTAB (Cetyl Trimethyl Ammonium Bromide) (2 %) buffer

CTAB	20 g
1 M Tris	200 ml
5 M NaCl	280 ml
0.5 M EDTA	40 ml
Na2SO3	2.5 g
Distilled water	460 ml

Add mercaptoethanol (0.1 %) fresh while using CTAB (2 %) solution.

Rnase (10 mg/ ml)

Dissolve Rnase in water, place in a tube in a boiling water bath for 10 minutes. Allow this to cool on a bench and store at -20^0c.

Chloroform: isoamyl alcohol (24:1)

Chloroform	240 ml
Isoamyl alcohol	10 ml

Store in dark at room temperature. Make up and dispenses the solution in a fumed cupboard.

Ethanol (70 %)

Absolute alcohol	70 ml
Distilled water	30 ml

NaCl (5 M)

Dissolve 292.2 g NaCl in 750 ml water. Make up to 1 liter with water, filter and autoclave.

Phenol/ Chloroform

Mix equal volume of the buffered phenol and chloroform: isoamyl alcohol (24:1). Store at 4 ^{0}c.

Sodium acetate (2.5 M, pH 5.2)

Dissolve 340.2 g sodium acetate in 500 ml water. Adjust pH to 5.2 with glacial acetic acid and make volume up to 1 liter and autoclave.

Tris HCl (1M, pH 8.0)

Dissolve 121.1 g Tris in 800 ml of water. Adjust pH to 8.0 with conc. HCl make volume up to 1 liter and autoclave.

EDTA (0.5 m, Ph 8.0)

Dissolve 186.1 g Na_2 EDTA.$2H_2O$ in 800 ml water. Adjust pH to 8.0 with Sodium hydroxide pellets. Make up volume to 1 liter and autoclave.

$T_{10}E_1$ buffer

1M Tris HCl pH 8.0	10 ml
1M EDTA pH 8.0	1 ml

And make up to 1 liter with sterile distilled water.

$T_{50}E_{10}$ buffer

1M Tris HCl pH 8.0	50 ml
0.5 M EDTA pH 8.0	20 ml

Make volume up to 1 liter with sterile distilled water.

10X Tris-Borate Buffer (TBE) (per liter)

Tris buffer

Boric Acid

EDTA

108 g Tris base, 55 g Boric acid and 9.3 g EDTA. Add deionised H_2O to 1 liter. The pH is 8.3 and requires no adjustment.

6X Gel loading buffer (0.25 % Bromophenol blue, 40 % sucrose)(10 ml)

Sucrose	4 g
Bromophenol Blue	2.5 ml
dH_2O	up to 10 ml

Store at 4°c.

Ethidium bromide (10 mg/ml)

Dissolve 100 mg ethidium bromide in 10 ml of distilled water; wrap tube in aluminium foil and store at 4°c.

Caution: Ethidium bromide is extremely mutagenic.

Acrylamide / biacrylamide 29:1 (w/w)

Acrylamide	29 g
Biacrylamide	1 g

Water (deionised distilled) up to 100 ml

Store at 4°c for <= 1 month.

Acrylamide/bisacrylamide 29:1 (v/v)

87 ml Acrylamide

3 ml Bisacrylamide

Add deionised distilled water to 300 ml. Solution can be stored up to 1 month at 4°C.

10 %(W/V) Ammonium Per Sulphate

Ammonium per Sulphate 1 g

Water (deionised distilled) 10 ml

Make fresh stock every week and store at 4°C.

TEMED (N, N, N', N'-tetramethylethylenediamine)

Ready made, store between 10 and 30°C (check label flask).

Loading buffer for non-denaturing PAGE (5X)

50 mM EDTA (1 ml of 0.5 M EDTA, pH 8.0)

50 mM NaCl (100 µl of 5 M NaCl)

50% (v/v) glycerol (5 ml)

Make up to 9 ml with sterilized deionised water. Add 10 mg fast orange G dye and adjust the volume to 10 ml. If you are using bromophenol blue and cyanol then less is required.

Binding silane

0.15 ml Bind silane

0.5 ml Acetic Acid

99.35 ml Ethanol

Mix the ingredients and store at 4°C.

100 base pairs ladder (50ng/ml)

100 bp ladder (stock conc.1 µg/µl)	50 µl
Blue (6X dye)	165 µl
T10E1 buffer	785 µl

Repel silane

Ready made, store at 4°C.

Reagents used for the Silver staining for PAGE

0.1 % (w/v) CTAB

2 gram CTAB in 2 liters of distilled deionised water

1 M NaOH

freshly prepared

0.3 % liquid ammonia

wear face mask when handling ammonia, should preferably be done in fume cupboard

Silver nitrate solution (freshly prepared)

2 gram silver nitrate

8 ml 1M NaOH

6-8 ml 25% ammonia.

Dissolve the silver nitrate and NaOH into 2 liters of distilled deionised water. Titrate with ammonia (on a shaker) until the solution becomes clear; add a further 1 ml of ammonia solution.

Sodium Carbonate solution

(freshly prepared, mind that the Sodium Carbonate should not be older than 12 months)

30 g Sodium Carbonate

0.4 ml Formaldehyde

Dissolve the sodium carbonate in 2 liters of distilled deionised water. Add 0.4 ml formaldehyde.

Glycerol solution

30 ml Glycerol into 2 liters distilled deionised water.

Concentrated NaOH solution

40 gram into 1 liter of water.

APPENDIX III

Details on pedigree of 259 $F_{7:8}$ RILs derived from cross 296B × IS18551

(cont...)

Sr.No.	Plot No	Pedigree
1	43101	(296B x IS 18551)-1-1-1
2	43102	(296B x IS 18551)-2-1-1
3	43103	(296B x IS 18551)-3-1-1
4	43104	(296B x IS 18551)-4-1-1
5	43105	(296B x IS 18551)-5-1-1
6	43106	(296B x IS 18551)-6-1-1
7	43107	(296B x IS 18551)-7-1-1
8	43108	(296B x IS 18551)-8-1-1
9	43109	(296B x IS 18551)-9-1-1
10	43110	(296B x IS 18551)-10-1-1
11	43111	(296B x IS 18551)-11-1-1
12	43112	(296B x IS 18551)-12-1-1
13	43113	(296B x IS 18551)-13-1-1
14	43114	(296B x IS 18551)-14-1-1
15	43115	(296B x IS 18551)-15-1-1
16	43116	(296B x IS 18551)-16-1-1
17	43117	(296B x IS 18551)-17-1-1
18	43118	(296B x IS 18551)-18-1-1
19	43119	(296B x IS 18551)-19-1-1
20	43120	(296B x IS 18551)-20-1-1
21	43121	(296B x IS 18551)-21-1-1
22	43122	(296B x IS 18551)-22-1-1
23	43123	(296B x IS 18551)-23-1-1
24	43124	(296B x IS 18551)-24-1-1
25	43125	(296B x IS 18551)-25-1-1
26	43126	(296B x IS 18551)-26-1-1
27	43127	(296B x IS 18551)-27-1-1
28	43128	(296B x IS 18551)-28-1-1
29	43129	(296B x IS 18551)-29-1-1
30	43130	(296B x IS 18551)-30-1-1
31	43131	(296B x IS 18551)-31-1-1
32	43132	(296B x IS 18551)-32-1-1
33	43133	(296B x IS 18551)-33-1-1
34	43134	(296B x IS 18551)-34-1-1
35	43135	(296B x IS 18551)-35-1-1
36	43136	(296B x IS 18551)-36-1-1
37	43137	(296B x IS 18551)-37-1-1
38	43138	(296B x IS 18551)-38-1-1
39	43139	(296B x IS 18551)-39-1-1
40	43140	(296B x IS 18551)-40-1-1
41	43141	(296B x IS 18551)-41-1-1
42	43142	(296B x IS 18551)-42-1-1
48	43148	(296B x IS 18551)-48-1-1
49	43149	(296B x IS 18551)-49-1-1
50	43150	(296B x IS 18551)-50-1-1
51	43151	(296B x IS 18551)-51-1-1
52	43152	(296B x IS 18551)-52-1-1
53	43153	(296B x IS 18551)-53-1-1
54	43154	(296B x IS 18551)-54-1-1
55	43155	(296B x IS 18551)-55-1-1
56	43156	(296B x IS 18551)-56-1-1
57	43157	(296B x IS 18551)-57-1-1
58	43158	(296B x IS 18551)-58-1-1
59	43159	(296B x IS 18551)-59-1-1
60	43160	(296B x IS 18551)-60-1-1
61	43161	(296B x IS 18551)-61-1-1
62	43162	(296B x IS 18551)-62-1-1
63	43163	(296B x IS 18551)-63-1-1
64	43164	(296B x IS 18551)-64-1-1
65	43165	(296B x IS 18551)-65-1-1
66	43166	(296B x IS 18551)-66-1-1
67	43167	(296B x IS 18551)-67-1-1
68	43168	(296B x IS 18551)-68-1-1
69	43169	(296B x IS 18551)-69-1-1
70	43170	(296B x IS 18551)-70-1-1
71	43171	(296B x IS 18551)-71-1-1
72	43172	(296B x IS 18551)-72-1-1
73	43173	(296B x IS 18551)-73-1-1
74	43174	(296B x IS 18551)-74-1-1
75	43175	(296B x IS 18551)-75-1-1
76	43176	(296B x IS 18551)-76-1-1
77	43177	(296B x IS 18551)-77-1-1
78	43178	(296B x IS 18551)-78-1-1
79	43179	(296B x IS 18551)-79-1-1
80	43180	(296B x IS 18551)-80-1-1
81	43181	(296B x IS 18551)-81-1-1
82	43182	(296B x IS 18551)-82-1-1
83	43183	(296B x IS 18551)-83-1-1
84	43184	(296B x IS 18551)-84-1-1
85	43185	(296B x IS 18551)-85-1-1
86	43186	(296B x IS 18551)-86-1-1
87	43187	(296B x IS 18551)-87-1-1
88	43188	(296B x IS 18551)-89-1-1
89	43189	(296B x IS 18551)-90-1-1

(cont...)

Sr. No.	Plot No	Pedigree
95	43195	(296B x IS 18551)-96-1-1
96	43196	(296B x IS 18551)-97-1-1
97	43197	(296B x IS 18551)-98-1-1
98	43198	(296B x IS 18551)-99-1-1
99	43199	(296B x IS 18551)-100-1-1
100	43200	(296B x IS 18551)-101-1-1
101	43201	(296B x IS 18551)-102-1-1
102	43202	(296B x IS 18551)-103-1-1
103	43203	(296B x IS 18551)-104-1-1
104	43204	(296B x IS 18551)-105-1-1
105	43205	(296B x IS 18551)-106-1-1
106	43206	(296B x IS 18551)-107-1-1
107	43207	(296B x IS 18551)-108-1-1
108	43208	(296B x IS 18551)-109-1-1
109	43209	(296B x IS 18551)-109-2-1
110	43210	(296B x IS 18551)-110-1-1
111	43211	(296B x IS 18551)-110-2-1
112	43212	(296B x IS 18551)-111-1-1
113	43213	(296B x IS 18551)-111-2-1
114	43214	(296B x IS 18551)-112-1-1
115	43215	(296B x IS 18551)-112-2-1
116	43216	(296B x IS 18551)-113-1-1
117	43217	(296B x IS 18551)-113-2-1
118	43218	(296B x IS 18551)-114-1-1
119	43219	(296B x IS 18551)-114-2-1
120	43220	(296B x IS 18551)-115-1-1
121	43221	(296B x IS 18551)-115-2-1
122	43222	(296B x IS 18551)-116-1-1
123	43223	(296B x IS 18551)-116-2-1
124	43224	(296B x IS 18551)-117-1-1
125	43225	(296B x IS 18551)-117-2-1
126	43226	(296B x IS 18551)-118-1-1
127	43227	(296B x IS 18551)-118-2-1
128	43228	(296B x IS 18551)-119-1-1
129	43229	(296B x IS 18551)-119-2-1
130	43230	(296B x IS 18551)-120-2-1
131	43231	(296B x IS 18551)-121-1-1
132	43232	(296B x IS 18551)-121-2-1
133	43233	(296B x IS 18551)-122-1-1
134	43234	(296B x IS 18551)-122-2-1
135	43235	(296B x IS 18551)-123-1-1
136	43236	(296B x IS 18551)-123-2-1
137	43237	(296B x IS 18551)-124-1-1
138	43238	(296B x IS 18551)-124-2-1
139	43239	(296B x IS 18551)-125-1-1
140	43240	(296B x IS 18551)-125-2-1
141	43241	(296B x IS 18551)-126-1-1
142	43242	(296B x IS 18551)-126-2-1
143	43243	(296B x IS 18551)-127-1-1

(cont...)

Sr. No.	Plot No	Pedigree
144	43244	(296B x IS 18551)-127-2-1
145	43245	(296B x IS 18551)-128-1-1
146	43246	(296B x IS 18551)-128-2-1
147	43247	(296B x IS 18551)-129-1-1
148	43248	(296B x IS 18551)-129-2-1
149	43249	(296B x IS 18551)-130-1-1
150	43250	(296B x IS 18551)-130-2-1
151	43251	(296B x IS 18551)-131-1-1
152	43252	(296B x IS 18551)-131-2-1
153	43253	(296B x IS 18551)-132-1-1
154	43254	(296B x IS 18551)-132-2-1
155	43255	(296B x IS 18551)-133-1-1
156	43256	(296B x IS 18551)-133-2-1
157	43257	(296B x IS 18551)-134-1-1
158	43258	(296B x IS 18551)-134-2-1
159	43259	(296B x IS 18551)-135-1-1
160	43260	(296B x IS 18551)-135-2-1
161	43261	(296B x IS 18551)-136-1-1
162	43262	(296B x IS 18551)-136-2-1
163	43263	(296B x IS 18551)-137-1-1
164	43264	(296B x IS 18551)-137-2-1
165	43265	(296B x IS 18551)-138-1-1
166	43266	(296B x IS 18551)-138-2-1
167	43267	(296B x IS 18551)-139-1-1
168	43268	(296B x IS 18551)-139-2-1
169	43269	(296B x IS 18551)-140-1-1
170	43270	(296B x IS 18551)-140-2-1
171	43271	(296B x IS 18551)-141-1-1
172	43272	(296B x IS 18551)-141-2-1
173	43273	(296B x IS 18551)-142-1-1
174	43274	(296B x IS 18551)-142-2-1
175	43275	(296B x IS 18551)-143-1-1
176	43276	(296B x IS 18551)-143-2-1
177	43277	(296B x IS 18551)-144-1-1
178	43278	(296B x IS 18551)-144-2-1
179	43279	(296B x IS 18551)-145-1-1
180	43280	(296B x IS 18551)-145-2-1
181	43281	(296B x IS 18551)-146-1-1
182	43282	(296B x IS 18551)-146-2-1
183	43283	(296B x IS 18551)-147-1-1
184	43284	(296B x IS 18551)-147-2-1
185	43285	(296B x IS 18551)-148-1-1
186	43286	(296B x IS 18551)-148-2-1
187	43287	(296B x IS 18551)-149-1-1
188	43288	(296B x IS 18551)-149-2-1
189	43289	(296B x IS 18551)-150-1-1
190	43290	(296B x IS 18551)-150-2-1
191	43291	(296B x IS 18551)-151-1-1
192	43292	(296B x IS 18551)-151-2-1

(cont...)

Sr. No.	Plot No	Peigree
193	43293	(296B x IS 18551)-152-1-1
194	43294	(296B x IS 18551)-152-2-1
195	43295	(296B x IS 18551)-153-1-1
196	43296	(296B x IS 18551)-153-2-1
197	43297	(296B x IS 18551)-154-1-1
198	43298	(296B x IS 18551)-154-2-1
199	43299	(296B x IS 18551)-155-1-1
200	43300	(296B x IS 18551)-155-2-1
201	43301	(296B x IS 18551)-156-1-1
202	43302	(296B x IS 18551)-156-2-1
203	43303	(296B x IS 18551)-157-1-1
204	43304	(296B x IS 18551)-157-2-1
205	43305	(296B x IS 18551)-158-1-1
206	43306	(296B x IS 18551)-158-2-1
207	43307	(296B x IS 18551)-159-1-1
208	43308	(296B x IS 18551)-159-2-1
209	43309	(296B x IS 18551)-160-1-1
210	43310	(296B x IS 18551)-160-2-1
211	43311	(296B x IS 18551)-161-1-1
212	43312	(296B x IS 18551)-161-2-1
213	43313	(296B x IS 18551)-162-1-1
214	43314	(296B x IS 18551)-162-2-1
215	43315	(296B x IS 18551)-163-1-1
216	43316	(296B x IS 18551)-163-2-1
217	43317	(296B x IS 18551)-164-1-1
218	43318	(296B x IS 18551)-164-2-1
219	43319	(296B x IS 18551)-165-1-1
220	43320	(296B x IS 18551)-165-2-1
221	43321	(296B x IS 18551)-166-1-1
222	43322	(296B x IS 18551)-166-2-1
223	43323	(296B x IS 18551)-167-1-1
224	43324	(296B x IS 18551)-167-2-1
225	43325	(296B x IS 18551)-168-1-1
226	43326	(296B x IS 18551)-168-2-1
227	43327	(296B x IS 18551)-169-1-1
228	43328	(296B x IS 18551)-169-2-1
229	43329	(296B x IS 18551)-170-1-1
230	43330	(296B x IS 18551)-170-2-1
231	43331	(296B x IS 18551)-171-1-1
232	43332	(296B x IS 18551)-171-2-1
233	43333	(296B x IS 18551)-172-1-1
234	43334	(296B x IS 18551)-172-2-1
235	43335	(296B x IS 18551)-173-1-1
236	43336	(296B x IS 18551)-173-2-1
237	43337	(296B x IS 18551)-174-1-1
238	43338	(296B x IS 18551)-174-2-1
239	43339	(296B x IS 18551)-175-1-1
240	43340	(296B x IS 18551)-175-2-1
241	43341	(296B x IS 18551)-176-1-1

(cont...)

(cont...)

Sr. No.	Plot No	Peigree
242	43342	(296B x IS 18551)-176-2-1
243	43343	(296B x IS 18551)-177-1-1
244	43344	(296B x IS 18551)-177-2-1
245	43345	(296B x IS 18551)-178-1-1
246	43346	(296B x IS 18551)-178-2-1
248	43348	(296B x IS 18551)-179-2-1
249	43349	(296B x IS 18551)-180-1-1
250	43350	(296B x IS 18551)-180-2-1
251	43351	(296B x IS 18551)-181-1-1
252	43352	(296B x IS 18551)-181-2-1
253	43353	(296B x IS 18551)-182-1-1
254	43354	(296B x IS 18551)-182-2-1
255	43356	(296B x IS 18551)-184-2-1
256	43357	(296B x IS 18551)-185-1-1
257	43358	(296B x IS 18551)-185-2-1
258	43359	(296B x IS 18551)-186-1-1
259	43360	(296B x IS 18551)-186-2-1
260	43361	296 B
261		IS 18551 (Entomology source)

APPENDIX IV. Mean performance of RILs (1-259) and parents (296B, and IS 18551) evaluated under the late kharif screening environments 2002

RIL No.	Glossiness Score	Egg (%) I	Egg (%) II	DH (%) I	DH (%) II	Tri_u	Tri_l	Flower	plht	ORS	AS	GR-Yi
1	4	59.1	88.4	80	89	73.7	29.7	78	207.5	6	6	667
2	3	60.4	94.9	74	93	134.0	54.3	83	180.0	8	6	159
3	3	49.3	91.3	72	88	83.0	28.7	83	165.4	5	7	468
4	4	50.4	89.8	67	88	121.0	31.3	93	266.3	3	6	668
5	3	59	89.7	73	91	109.3	51.0	80	184.2	6	8	550
6	3	47.9	85.6	77	88	113.3	53.0	90	201.7	8	7	316
7	3	62.1	96.9	75	94	2.0	0.3	81	204.2	7	7	178
8	5	58.9	93.2	77	92	105.7	53.0	95	219.6	5	6	434
9	4	48.7	89.3	72	86	18.7	1.0	77	159.6	6	6	808
10	3	69.3	94.8	83	95	77.7	42.3	82	230.8	5	7	420
11	2	50.4	85.4	74	84	163.0	74.3	91	197.5	5	5	669
12	2	52.2	92.3	74	91	105.7	34.7	74	132.5	7	8	479
13	3	55	85	68	89	136.3	41.7	90	151.3	5	7	753
14	3	81	94.5	81	92	82.3	29.0	85	189.6	6	7	580
15	3	62.3	88.4	83	89	26.3	1.7	86	259.2	4	5	672
16	3	61.7	86.3	69	87	161.0	73.3	81	137.9	6	8	585
17	4	57.2	87.1	77	86	100.7	66.0	67	161.7	7	8	224
18	5	60.3	95.7	84	96	187.0	39.0	84	148.3	8	8	87
19	4	70.4	94.4	80	90	20.7	6.3	74	152.5	7	7	396
20	2	58.4	85.9	72	88	4.0	0.0	73	200.0	6	7	523
21	3	57.9	84.1	61	77	83.7	56.7	87	192.5	5	7	430
22	5	74.2	95.7	80	94	0.0	0.0	79	226.7	6	7	448
23	4	67.6	92.6	75	89	18.3	1.7	81	204.2	7	6	478
24	4	61.4	86.6	64	85	160.0	62.3	82	250.0	7	6	452
25	3	53	89.2	74	89	0.0	0.0	83	165.8	6	7	447
26	4	57.7	91.3	73	88	89.7	38.7	86	227.5	5	6	563
27	3	53.9	85.1	68	84	109.0	54.0	86	209.2	4	5	942
28	3	67.5	89.8	76	91	150.0	61.0	76	174.2	4	7	988
29	3	56.6	89.3	75	87	85.3	34.0	89	268.3	4	5	659
30	5	65.9	88.1	75	88	74.0	24.7	87	212.5	6	5	625
31	4	67.5	93.5	79	92	29.3	1.7	85	148.8	7	8	351

RIL No.	Glossiness Score	Egg (%) I	Egg (%) II	DH (%) I	DH (%) II	Tri_u	Tri_l	Flower	plht	ORS	AS	GR-Yi
32	5	70.4	94.6	78	92	0.0	0.0	82	210.4	6	7	520
33	3	64.1	83.3	59	75	156.7	84.0	85	202.9	4	7	686
34	5	64.9	97.1	73	88	81.7	44.0	86	195.0	6	5	598
35	3	65.4	88.7	76	88	70.0	34.7	75	174.2	5	7	384
36	3	58.5	97.9	73	92	73.3	33.3	90	152.5	5	8	575
37	4	56.8	93.6	71	88	25.7	2.7	91	137.5	7	6	620
38	4	67.5	92.6	73	89	18.7	4.3	79	174.2	8	6	590
39	3	64.9	84.4	72	86	113.7	50.3	79	135.4	6	8	554
40	4	56.9	90.8	73	86	45.0	3.7	90	169.6	6	5	282
41	4	67.1	97.5	76	90	44.3	13.7	84	240.0	5	6	488
42	4	58.3	85.2	76	86	177.7	55.0	92	204.2	6	7	306
43	3	64.1	86.5	72	83	26.3	3.7	92	223.3	6	5	419
44	3	59.5	89.5	73	89	111.0	47.0	85	189.6	6	7	332
45	4	70.7	96.4	76	89	74.7	20.7	86	118.3	8	8	298
46	2	53.8	76.5	60	75	89.3	28.3	83	190.4	6	5	508
47	3	66	85.1	62	76	143.7	55.0	81	227.5	5	6	486
48	4	66.3	95.1	69	89	85.3	41.3	80	192.5	6	7	675
49	4	64.3	93.8	84	95	66.7	29.0	78	200.8	6	7	586
50	3	62.1	90.6	77	91	192.3	92.7	85	169.2	6	7	711
51	3	54	75.1	53	73	0.0	0.0	90	165.4	7	7	192
52	3	59.4	85.5	74	86	64.7	25.0	75	204.2	6	6	453
53	3	59.3	89	75	88	66.7	34.3	78	218.8	6	6	533
54	4	60.7	98.7	68	89	138.7	46.7	80	175.8	5	6	772
55	4	63.1	92.4	79	89	88.7	52.0	82	217.5	4	7	815
56	3	66.4	91.3	82	91	77.7	33.3	78	209.2	5	6	700
57	3	61.3	90.3	75	84	90.3	45.3	80	164.6	7	7	644
58	2	62.4	94.2	76	91	35.3	14.7	93	217.5	6	4	420
59	3	62.2	98.9	76	94	104.3	61.3	81	195.8	6	5	434
60	5	55.5	86.5	71	88	66.3	29.0	70	194.2	7	8	165
61	2	50	76.3	61	73	11.7	4.0	85	221.3	5	6	547
62	4	64.2	87.2	76	89	61.7	23.3	76	141.7	7	7	605
63	4	61.1	92.7	72	89	59.3	30.3	78	180.0	6	8	344

RIL No.	Glossiness Score	Egg (%) I	Egg (%) II	DH (%) I	DH (%) II	Tri_u	Tri_l	Flower	plht	ORS	AS	GR-Yi
64	4	67.5	92.3	76	91	44.3	24.7	82	220.0	6	6	451
65	4	63.9	94.2	74	88	99.3	47.7	87	136.7	7	8	298
66	5	70.1	92.1	78	91	118.0	42.3	87	183.8	7	8	251
67	2	60.9	87.8	68	87	100.7	40.7	81	198.3	6	7	445
68	5	69	91.3	83	91	118.3	32.7	86	221.3	6	6	535
69	4	59	91.2	80	90	152.3	50.3	85	240.0	5	6	496
70	4	64.6	91.8	71	88	138.3	53.7	89	250.0	4	5	587
71	4	41.9	90.6	68	90	29.3	7.3	87	175.0	7	8	372
72	4	57.7	89.8	75	88	126.0	54.0	81	194.2	6	8	613
73	5	63	92.3	76	95	34.0	13.0	84	156.3	6	8	531
74	5	61.5	99.2	83	96	95.7	38.3	84	223.3	6	6	309
75	4	63.8	95.3	80	97	25.7	11.0	80	200.0	6	6	743
76	4	56	85.4	67	86	43.7	17.0	80	137.5	6	7	643
77	4	69	88.3	74	85	69.3	32.3	81	234.2	6	6	746
78	5	67.7	94.3	83	94	47.3	24.0	71	155.0	8	8	235
79	4	62.1	91.2	75	92	48.0	21.0	83	132.5	8	8	256
80	3	57.4	93	71	87	181.3	81.7	84	173.3	6	8	725
81	4	59.9	91.8	75	86	89.7	41.3	79	183.3	7	7	424
82	2	64.1	86.2	57	73	111.0	32.3	81	200.8	4	6	796
83	4	64.2	89.8	73	86	135.0	52.0	76	228.3	6	7	395
84	3	66.6	89.7	76	90	79.3	34.0	76	184.6	6	6	525
85	3	66.6	89.4	83	92	0.0	0.0	80	215.0	5	7	579
86	4	78.9	89.6	76	87	73.0	34.0	82	230.8	5	5	840
87	4	56.2	88.3	73	84	58.3	22.7	79	236.7	5	7	572
88	3	56	80.9	63	76	100.7	39.0	79	247.5	4	5	496
89	4	56.2	85.5	71	88	14.7	2.7	82	211.3	6	6	355
90	4	59.8	78.3	69	83	9.7	3.3	79	173.8	7	7	280
91	3	56.4	89.9	66	87	0.0	0.0	83	200.8	7	7	303
92	2	48.9	81	68	81	121.3	55.3	83	227.1	3	5	835
93	3	68.6	94.9	72	91	135.7	38.3	86	224.2	5	7	440
94	3	54.6	85.1	71	84	107.7	70.7	90	217.1	6	7	484
95	3	52.3	93.2	77	90	103.0	50.3	88	221.3	6	6	630

Cont...

RIL No.	Glossiness Score	Egg (%) I	Egg (%) II	DH (%) I	DH (%) II	Tri_u	Tri_l	Flower	plht	ORS	AS	GR-Yi
96	1	60.3	82.8	56	75	111.7	46.0	85	167.5	7	6	326
97	2	67.3	80.9	58	73	43.7	22.7	79	228.3	4	5	955
98	3	64.3	88.2	85	92	122.0	62.3	75	167.9	7	6	519
99	4	53.7	90.1	72	86	2.3	2.7	86	221.3	6	6	249
100	5	65.2	91.4	76	91	43.0	13.3	84	235.0	4	7	550
101	4	49.2	87	65	83	15.7	4.0	79	197.9	6	5	295
102	3	67	88.4	67	85	111.3	22.3	79	210.0	5	6	822
103	4	66.1	90.2	73	87	82.0	48.0	81	141.7	5	8	447
104	3	41.1	84.8	70	88	87.0	33.7	72	168.8	7	8	264
105	5	62.6	92.1	72	87	92.3	30.0	90	205.8	6	7	485
106	5	54.7	90.8	67	90	130.7	37.3	78	180.8	7	8	510
107	3	69.5	90	78	90	37.3	12.0	89	242.9	4	5	675
108	4	63.2	95.3	79	91	155.7	59.7	86	221.7	6	7	282
109	5	66.3	90.5	76	87	135.3	54.3	85	197.9	6	7	309
110	2	44.6	81.3	74	85	151.0	71.3	88	197.1	5	7	719
111	4	73.7	91.3	84	91	9.0	3.7	79	237.5	5	5	356
112	4	65.3	90.2	73	87	0.0	0.0	82	208.3	7	7	386
113	2	56.2	82.1	65	84	17.0	6.7	81	186.7	5	7	647
114	4	59.5	93.1	71	88	6.3	2.0	87	194.2	6	8	693
115	4	58.3	92.8	71	93	213.0	57.3	95	161.3	4	6	355
116	4	66.6	89.8	75	89	56.0	19.0	82	219.2	6	6	817
117	3	67.8	88.4	77	88	188.3	88.7	76	201.7	5	6	550
118	4	49.5	91	79	93	54.7	31.7	89	211.3	5	7	539
119	4	57.4	91	68	88	6.0	2.3	80	213.3	6	7	560
120	3	59	89.2	71	86	135.0	57.3	77	133.3	6	7	909
121	4	70.4	87.6	78	87	57.0	16.7	78	202.5	6	5	571
122	2	61.4	89.8	73	86	133.0	55.7	80	130.0	5	7	792
123	3	65.5	89.7	76	88	0.0	0.0	84	180.8	6	8	683
124	3	66.9	93.8	82	96	84.3	32.0	86	185.0	5	5	904
125	5	57.6	94.2	74	90	116.3	51.3	73	139.6	6	7	419
126	2	54.3	88.6	68	87	4.0	0.0	78	191.7	6	7	384
127	4	65.1	96.8	84	92	0.0	0.0	86	213.3	6	7	264

RIL No.	Glossiness Score	Egg (%) I	Egg (%) II	DH (%) I	DH (%) II	Tri_u	Tri_l	Flower	plht	ORS	AS	GR-Yi
128	3	63.8	89.4	77	90	72.0	22.0	89	240.8	4	7	912
129	5	68.8	93.2	74	89	198.7	92.0	78	168.8	6	7	817
130	1	48	78	56	74	120.7	36.7	85	135.0	6	7	474
131	3	69	91.4	78	93	65.0	18.7	95	236.7	5	6	545
132	4	67.6	91.5	73	89	3.3	0.0	81	202.9	7	7	402
133	3	57.7	83.6	77	89	35.7	8.7	91	283.8	5	4	295
134	3	65.8	91.5	82	89	114.7	31.3	85	206.7	5	5	590
135	4	54	91.5	74	88	41.0	10.0	78	170.8	7	8	192
136	4	56.4	88.8	70	87	31.7	8.0	83	212.5	4	6	512
137	4	64.9	93.3	70	85	23.7	8.0	81	202.5	7	6	322
138	3	63.8	89.9	72	87	109.7	31.0	77	157.1	7	7	757
139	2	48.4	85.4	65	84	116.7	42.3	93	217.9	5	7	364
140	3	55.3	89.8	69	88	186.3	79.0	82	170.4	8	8	263
141	4	65.9	95.9	79	93	45.3	14.7	83	134.6	9	8	91
142	2	75.9	89.9	76	89	53.3	18.3	77	208.3	6	7	290
143	5	65.8	96.9	72	88	23.0	7.0	92	184.2	8	7	107
144	4	69.5	85.4	73	84	16.0	6.0	84	226.7	4	4	614
145	3	61.2	88.5	77	87	110.0	55.3	82	246.3	5	5	563
146	4	64.4	89.6	74	94	153.3	62.3	86	249.4	6	6	382
147	4	54	91.1	71	89	33.7	13.0	81	218.3	5	6	424
148	3	62	81.1	71	82	112.0	41.3	84	192.1	6	7	510
149	4	60.8	92	78	85	119.7	36.7	81	207.5	5	7	681
150	3	58.6	87.8	71	85	148.3	52.0	85	234.2	4	4	189
151	4	54.1	94.3	73	89	65.7	23.0	92	187.5	5	6	800
152	2	64.5	89.5	74	86	150.3	56.0	76	196.7	5	6	774
153	3	55.2	90.2	76	90	194.7	82.7	75	120.4	6	7	802
154	4	63.4	87	78	87	155.0	53.0	92	133.8	7	8	337
155	4	66.6	92.6	79	88	154.0	42.3	86	216.7	6	7	331
156	4	63.3	91.5	79	93	15.3	2.3	69	167.1	6	9	270
157	4	60.2	94.2	79	90	14.7	3.3	78	251.7	5	6	511
158	4	73	89.3	79	91	74.0	32.0	79	162.5	6	7	566
159	3	44.7	90	70	89	0.0	0.0	85	148.8	8	8	314

RIL No.	Glossiness Score	Egg (%) I	Egg (%) II	DH (%) I	DH (%) II	Tri_u	Tri_l	Flower	plht	ORS	AS	GR-Yi
160	3	56.1	94	74	95	33.7	13.3	97	214.2	6	6	136
161	4	62.7	94.9	78	92	18.0	4.7	89	215.0	6	8	317
162	4	72.1	93.2	78	87	109.7	51.0	79	225.8	6	7	640
163	4	61	92.1	67	85	74.3	40.3	77	235.8	5	7	499
164	4	72.1	93.9	77	91	153.3	50.7	79	208.3	7	7	367
165	3	61.7	87	72	88	63.3	25.3	79	189.2	6	7	575
166	3	56.7	90.6	70	87	75.3	29.7	94	148.3	6	7	436
167	4	75.1	80.1	74	88	18.7	5.7	89	159.2	8	6	322
168	2	47.5	83.8	56	69	45.7	16.0	73	155.4	6	7	497
169	3	66.2	86.4	68	88	14.0	5.0	89	253.8	5	6	552
170	4	67.6	94.5	77	88	92.3	47.3	84	232.9	4	7	783
171	4	53.6	90.9	71	89	57.3	19.7	80	222.9	6	7	664
172	4	61.1	87	80	91	143.3	50.7	77	238.8	4	5	462
173	4	62.5	88.2	77	89	61.3	26.0	84	172.1	6	6	331
174	2	55.9	80.3	58	74	166.7	61.7	92	202.5	5	7	383
175	2	54	85.9	78	89	57.3	21.0	90	194.6	6	6	559
176	1	56.7	82.6	57	72	30.3	8.3	86	207.5	4	6	632
177	3	68.8	86	74	82	143.7	56.3	94	210.0	5	7	242
178	4	63.9	93.3	77	86	79.0	23.0	93	204.2	5	7	284
179	1	51.8	78.9	57	70	103.0	31.3	83	136.3	6	6	709
180	3	58.4	92.2	82	93	144.7	55.3	89	203.3	6	7	474
181	3	58.4	87.3	77	92	160.3	67.7	78	199.2	4	7	918
182	2	53.7	87.5	73	85	95.0	33.7	83	210.0	4	6	782
183	4	65.4	93.6	70	89	72.3	17.7	83	186.7	6	6	327
184	4	56.5	91.5	73	88	135.0	35.7	77	199.6	8	9	171
185	2	57.6	82.2	61	74	6.3	2.0	91	237.1	4	6	673
186	4	60.8	88	79	88	5.0	0.0	92	249.6	4	5	675
187	2	60.6	93	78	90	130.0	45.0	77	194.6	3	6	790
188	3	60.9	90.9	73	88	88.7	26.3	88	219.2	6	6	477
189	4	50.6	88.5	70	85	45.3	9.0	83	219.2	6	5	374
190	4	64.2	92.9	82	95	79.0	13.7	83	223.3	6	6	377
191	5	60.2	91.6	73	91	17.7	3.0	86	200.0	7	7	390

RIL No.	Glossiness Score	Egg (%) I	Egg (%) II	DH (%) I	DH (%) II	Tri_u	Tri_l	Flower	plht	ORS	AS	GR-Yi
192	5	60.1	93.9	75	91	16.0	3.0	82	200.8	5	7	628
193	4	61.8	90.2	77	92	78.7	43.0	83	227.5	5	6	663
194	5	64	89.2	71	92	116.7	59.7	89	195.8	6	6	461
195	3	63	93.9	75	92	53.7	22.0	92	141.7	7	7	322
196	4	59.1	93.2	77	90	21.3	8.3	87	214.6	5	6	428
197	3	68.9	94.3	82	95	35.7	18.7	87	197.5	7	7	481
198	4	67.4	93.6	75	90	63.3	26.0	80	162.1	6	6	563
199	3	64.8	95.9	84	88	127.3	28.7	76	210.4	5	6	479
200	4	64.6	86.1	73	85	72.7	37.0	83	213.8	5	6	494
201	4	59.6	89.3	72	90	0.0	0.0	76	165.4	7	8	349
202	4	60.8	93.5	73	88	25.3	11.3	78	155.4	7	8	394
203	4	66	81.8	75	83	66.3	34.0	74	142.5	7	6	585
204	3	63.7	92	79	90	61.0	33.0	69	150.8	7	7	512
205	5	64.5	86.9	77	92	53.7	17.3	90	196.7	6	5	206
206	5	64.4	82.7	66	81	111.7	47.0	86	209.6	5	6	214
207	3	66.5	89.7	73	86	92.3	30.7	86	166.3	6	8	466
208	3	51.7	81.9	54	73	159.7	79.7	84	193.3	5	7	530
209	3	56.6	90	75	91	38.3	21.3	90	242.5	4	5	648
210	2	53.3	84.8	70	88	103.7	47.0	92	233.8	4	4	487
211	4	58.9	92.4	74	88	98.7	43.7	76	188.3	6	7	755
212	4	64.4	92.8	77	88	76.7	39.3	81	196.7	6	7	942
213	5	64.4	92	76	91	54.0	24.0	76	177.5	8	8	356
214	5	66.5	97.5	76	90	11.0	5.0	82	158.8	8	7	409
215	4	63.7	86.7	75	88	23.7	10.7	85	205.4	6	6	466
216	4	54.9	85.4	73	85	76.3	28.0	87	202.1	5	5	506
217	4	60.4	88	75	89	176.3	56.3	88	219.2	6	6	625
218	5	60.6	92.6	78	93	53.3	24.7	85	192.1	6	7	764
219	5	62.6	92.1	69	87	22.7	10.0	90	217.5	6	7	326
220	4	61.6	92.3	75	92	22.7	8.3	88	219.2	6	8	473
221	4	67.1	92.5	74	93	147.3	49.0	93	172.5	4	7	547
222	2	59.7	82.1	58	74	119.0	54.3	82	160.0	4	8	701
223	1	51.9	81.7	57	73	62.0	21.0	90	199.2	5	7	587

RIL No.	Glossiness Score	Egg (%) I	Egg (%) II	DH (%) I	DH (%) II	Tri_u	Tri_l	Flower	plht	ORS	AS	GR-Yi
224	3	55.2	90.4	75	93	92.0	44.0	85	217.5	6	7	427
225	3	53.3	86.7	74	86	3.3	0.7	91	159.6	6	7	757
226	4	62.7	86.6	74	89	133.3	59.7	89	173.3	6	6	701
227	4	47.4	91.7	71	90	98.0	51.3	88	248.3	2	6	803
228	4	62.7	92.3	72	84	71.7	30.0	82	214.2	4	7	586
229	3	65.8	93.2	75	90	78.0	32.3	78	220.4	6	5	440
230	4	55.6	94	78	91	51.0	19.7	87	221.3	7	5	298
231	2	55.8	89.1	73	87	155.0	65.3	86	162.9	6	6	373
232	2	66.3	92.4	74	86	199.0	54.0	89	159.6	7	7	470
233	3	70.3	84.4	75	85	57.7	19.7	71	125.4	6	8	413
234	4	69	91	77	91	17.7	6.0	77	210.0	6	7	489
235	4	67.5	88.4	76	91	41.3	13.7	92	119.2	5	7	636
236	3	61.8	84.4	69	80	149.3	57.7	78	136.7	4	6	930
237	5	67.5	90.8	70	88	90.3	44.7	83	172.9	8	8	268
238	4	57	85.6	70	83	0.0	0.0	85	196.3	7	7	365
239	4	53	91.1	75	88	80.3	40.0	79	151.7	5	7	449
240	4	55.7	91.6	69	90	176.7	72.7	81	141.3	6	8	455
241	4	61.7	88.2	76	93	210.3	60.0	83	210.8	5	7	570
242	4	58.1	93.9	68	86	182.0	88.0	87	225.0	5	7	261
243	4	63.9	87.6	71	84	72.7	33.0	83	223.3	4	5	885
244	5	65.1	97.1	68	85	147.7	62.7	93	220.0	7	7	255
245	5	65.3	89.2	75	91	16.0	5.3	76	216.7	5	7	397
246	3	58.3	94.8	80	90	0.0	0.0	80	221.7	6	7	656
247	2	56.8	83.3	69	85	35.3	13.3	80	246.7	5	6	1120
248	2	55	88.6	77	91	45.7	18.3	78	230.8	4	4	856
249	4	60.1	91.9	69	85	44.7	15.0	88	170.4	6	5	413
250	4	65.9	90.4	73	91	27.3	9.3	71	141.7	6	5	595
251	4	66	91.5	72	89	89.0	43.3	78	143.8	6	7	649
252	3	69.8	97.1	73	92	134.7	64.3	83	194.2	4	7	920
253	3	64.9	90.7	80	90	139.3	73.3	79	200.8	5	6	998
254	3	63.3	87.5	74	88	78.3	24.0	94	235.4	3	5	937
255	4	56.4	88.7	81	89	70.7	37.3	84	205.0	5	7	704

RIL No.	Glossiness Score	Egg (%) I	Egg (%) II	DH (%) I	DH (%) II	Tri_u	Tri_l	Flower	plht	ORS	AS	GR-Yi
256	4	60.7	89.2	76	87	80.0	38.0	88	205.0	5	7	459
257	3	56	90.1	68	90	138.0	57.3	86	188.8	5	7	779
258	3	54.1	91.7	77	92	141.3	63.7	93	165.0	7	8	220
259	3	68	86.4	74	86	121.7	62.3	92	145.0	4	7	976
296B	5	64.5	96	73.3	95	0	0	86	120	7	8	235
IS 18551	1	37.8	77.6	52.5	70	155.67	77.67	88	254.58	3	5	1109
296B	5.0	75.0	98.9	78.9	96.0	0.0	0.0	88.0	109.2	8	8	158
296B	5.0	64.1	95.7	73.0	93.0	0.0	0.0	90.0	105.0	8	8	202
296B	5.0	63.8	90.4	81.7	95.0	0.0	0.0	90.0	109.2	9	8	201
296B	5.0	63.0	95.1	74.7	98.0	0.0	0.0	88.0	117.9	8	8	228
296B	5.0	67.9	100.0	81.9	97.0	0.0	0.0	89.0	110.4	8	8	205
296B	5.0	72.7	85.8	58.1	96.0	0.0	0.0	91.0	106.3	8	8	238
296B	5.0	67.8	100.0	74.5	96.0	0.0	0.0	87.0	125.8	8	8	150
296B	5.0	63.3	95.5	76.1	96.0	0.0	0.0	91.0	98.3	8	8	109
296B	5.0	72.7	94.8	76.8	98.0	0.0	0.0	89.0	110.0	8	8	359
296B	5.0	82.3	96.9	74.8	94.0	0.0	0.0	90.0	98.3	9	8	80
296B	5.0	79.5	100.0	76.6	97.0	0.0	0.0	92.0	111.7	8	7	145
296B	5.0	58.6	95.7	77.3	97.0	0.0	0.0	90.0	112.5	8	8	233
296B	5.0	66.7	92.5	82.4	96.0	0.0	0.0	90.0	111.7	8	8	166
IS 18551	1.0	41.2	79.9	48.5	69.0	152.3	68.7	86.0	238.3	3	5	1017
IS 18551	1.0	38.6	76.6	49.6	64.0	117.3	49.3	88.0	237.5	3	5	1134
IS 18551	1.5	38.8	80.2	53.8	69.0	127.3	61.3	88.0	243.3	3	5	1029
IS 18551	1.0	46.4	77.5	50.6	70.0	146.7	77.7	87.0	240.8	3	5	763
IS 18551	1.0	46.8	82.9	51.2	67.0	152.0	82.0	87.0	243.8	3	6	1079
IS 18551	1.0	41.6	76.0	51.2	67.0	139.0	70.7	87.0	248.3	2	5	1238
IS 18551	1.0	53.5	76.2	52.5	70.0	131.3	57.3	88.0	248.3	2	5	1023
IS 18551	1.0	53.3	75.4	48.1	67.0	191.3	75.0	88.0	251.7	3	5	1161
IS 18551	1.0	53.6	82.0	55.3	72.0	217.3	124.0	87.0	255.8	3	5	987
IS 18551	1.0	34.8	78.6	48.7	65.0	126.7	62.3	89.0	226.7	3	5	1183
IS 18551	1.0	47.3	79.7	51.3	68.0	164.3	86.0	89.0	243.3	4	5	1040
IS 18551	1.0	51.5	80.3	52.1	68.0	151.3	67.3	89.0	237.1	3	5	1178
IS 18551	1.0	44.8	82.4	56.2	73.0	158.7	63.3	88.0	241.7	3	4	1089

RIL No.	Glossiness Score	Egg (%) I	Egg (%) II	DH (%) I	DH (%) II	Tri_u	Tri_l	Flower	plht	ORS	AS	GR-Yi
CSH 9	5.0	72.1	93.8	84.9	95.0	1.7	0.7	75.0	163.3	7	8	548
CSH 9	5.0	56.8	98.2	74.6	96.0	2.0	0.7	73.0	160.8	7	8	643
CSH 9	5.0	66.7	98.2	78.9	96.0	2.0	0.7	75.0	159.6	7	8	678
CSH 9	4.5	79.9	96.5	81.6	97.0	2.0	0.0	73.0	165.0	7	8	626
CSH 9	5.0	62.7	98.2	79.0	97.0	1.0	0.0	71.0	152.9	6	8	810
CSH 9	5.0	61.6	100.0	85.0	99.0	1.0	0.0	74.0	160.8	6	8	551
CSH 9	5.0	72.3	97.1	75.0	97.0	1.3	0.3	76.0	161.7	7	8	681
CSH 9	5.0	69.1	95.0	74.9	96.0	2.7	0.7	74.0	165.4	7	8	560
CSH 9	5.0	75.6	97.0	85.0	99.0	1.7	0.3	73.0	154.6	6	8	764
CSH 9	5.0	75.7	93.9	82.9	99.0	3.3	0.7	74.0	163.8	6	8	861
CSH 9	5.0	73.1	97.1	83.4	99.0	2.3	0.7	73.0	152.5	6	8	630
CSH 9	5.0	72.0	91.9	77.8	94.0	3.0	0.7	73.0	155.4	6	7	712
CSH 9	5.0	80.3	95.9	82.2	96.0	1.7	0.3	71.0	154.2	7	7	738
SE (±)	0.26	5.71	3.42	3.73	2.45	6.7	3.86	2.0	8.9	0.53	0.53	100.45
Mean	3.59	61.13	89.8	72.69	87.56	76.81	31.12	83.43	191.69	5.73	6.5	536.31
CV (%)	14.55	18.67	7.63	10.27	5.6	15.11	21.49	4.8	9.3	18.54	16.39	37.46
F Ratio	16.86	1.85	2.25	4.14	7.57	-	-	8.8	18.02	6.39	4.2	5.55
h21	0.8	0.17	0.24	0.44	0.62	0.96	0.93	0.66	0.81	0.57	0.44	0.53
h22	0.94	0.46	0.56	0.76	0.87	0.99	0.98	0.89	0.94	0.84	0.76	0.82

h21 = plot mean basis
h22 = entry mean basis

Glossy	Glossiness intensity (score)
Egg (%) I	Oviposition (%) I 14 DAE
Egg (%) II	Oviposition (%) II 21 DAE
DH (%) I	Deadhearts (%) I 21 DAE
DH (%) II	Deahearts (%) II 28 DAE
Tri_u	Trichome density upper leaf surface (no./microscopic field)
Tri_l	Trichome density lower leaf surface (no./microscopic field)
Flower	Time to 50 % flowering (Days)
plht (cm)	Plant height (cm)

ORS	Overall recovery score (scale)
AS	Ahpid damage score (scale)
GR-Yi	Grain yield (g.plot)

APPENDIX V. Mean performance of RILs (1-259) and parents (296B, and IS 18551) evaluated under the late *rabi* screening environment during 2004-05

Entry No	gls	SV I	Seht I	ovi(%) I	ovi(%) II	Db(%)I	Db(%)II	Tri_u	Tri_l	Pigmen	ORS	midge	AS	agrs	Flower	Pl_ht	gr_yi
1	5.0	4.7	7.5	38.6	60.7	39.5	52.0	2.0	0.0	3.0	5.7	6.7	5.7	3.3	72.0	153.9	381.7
2	1.3	3.0	11.3	23.9	48.6	20.5	41.5	107.0	58.0	1.0	4.0	7.3	4.7	3.3	65.0	180.0	438.3
3	2.7	3.3	11.1	16.7	46.0	27.4	35.3	144.0	68.0	1.7	3.7	5.7	6.0	3.0	71.0	147.2	525.0
4	3.3	3.0	9.6	24.8	57.4	36.7	54.8	72.0	40.0	1.0	2.3	5.7	4.3	3.3	76.0	196.1	490.7
5	2.0	2.7	13.2	13.2	37.7	19.1	32.3	181.0	80.0	2.7	4.0	9.0	5.0	2.0	69.0	155.0	367.7
6	2.5	3.0	9.1	30.7	56.0	18.9	34.0	64.0	37.0	4.3	4.0	6.7	5.7	3.0	66.0	170.0	503.3
7	3.5	3.8	8.5	26.0	41.7	28.7	36.2	52.0	34.0	2.0	3.0	5.0	4.7	1.7	66.0	145.0	239.0
8	1.8	2.3	10.2	33.8	53.8	28.5	45.0	4.0	0.0	1.0	5.7	8.7	5.7	2.3	68.0	185.0	519.0
9	4.0	2.8	9.3	17.5	47.8	24.9	44.4	57.0	26.0	1.0	4.7	7.7	4.7	3.3	75.0	145.0	552.3
10	2.8	3.0	10.5	39.6	59.5	40.6	49.7	81.0	40.0	1.0	5.0	8.0	5.0	3.0	70.0	184.5	412.7
11	2.7	4.3	8.0	16.1	48.3	21.8	36.8	132.0	65.0	2.3	2.0	5.0	4.0	2.3	76.0	171.1	386.7
12	2.7	3.0	8.8	43.8	69.1	46.2	58.9	134.0	63.0	3.3	5.7	5.3	5.7	2.0	69.0	144.4	491.0
13	3.0	5.0	8.5	11.7	47.3	16.8	49.3	114.0	59.0	1.0	4.0	7.7	4.0	2.3	76.0	153.3	253.0
14	2.0	4.3	8.9	28.1	56.9	28.2	45.8	75.0	31.0	1.0	5.3	6.7	6.7	3.0	76.0	173.9	395.0
15	2.8	3.7	10.1	21.8	46.2	29.9	41.8	22.0	9.0	1.7	4.0	9.0	4.0	3.7	72.0	202.2	371.7
16	2.7	3.7	8.5	23.5	56.5	26.9	36.6	113.0	57.0	2.0	3.3	4.0	6.0	2.0	74.0	127.8	251.7
17	5.0	1.7	9.9	65.7	74.6	56.8	66.9	39.0	23.0	1.0	5.0	9.0	5.3	4.0	69.0	150.0	387.0
18	5.0	4.7	8.0	43.8	90.7	64.1	81.4	71.0	43.0	1.0	5.0	9.0	5.3	3.7	84.0	155.0	287.7
19	3.8	2.7	9.8	42.4	70.2	44.7	62.1	176.0	95.0	2.0	3.0	9.0	5.3	2.7	70.0	134.5	336.7
20	2.5	3.0	11.2	24.7	52.9	25.5	45.1	13.0	5.0	1.0	4.0	9.0	5.3	2.0	67.0	159.4	468.7
21	4.2	4.3	8.1	28.9	62.1	31.7	44.9	106.0	41.0	2.3	4.0	9.0	3.7	2.7	73.0	170.0	237.0
22	5.0	3.0	13.6	57.7	65.2	42.6	56.2	101.0	46.0	1.0	4.3	9.0	4.0	3.7	77.0	173.4	1018.0
23	4.7	3.7	9.0	34.1	73.5	40.8	62.6	25.0	10.0	1.7	3.7	6.7	3.3	3.7	70.0	190.6	681.0
24	2.3	2.7	10.1	33.1	61.9	39.7	57.6	55.0	28.0	1.0	6.3	7.7	6.0	3.3	71.0	205.6	448.7
25	2.5	4.0	9.7	20.7	31.0	12.1	31.2	45.0	10.0	1.0	4.0	9.0	5.3	2.0	73.0	153.3	488.7
26	3.8	3.3	7.9	43.0	67.9	34.9	45.2	87.0	43.0	1.7	3.0	6.3	4.3	3.0	72.0	200.6	540.0
27	5.0	3.3	10.7	17.4	45.8	22.7	37.8	165.0	72.0	1.0	4.3	5.7	6.3	3.0	73.0	165.0	432.3
28	2.8	3.5	8.0	31.7	61.7	38.8	55.9	190.0	85.0	2.2	4.3	7.0	5.7	2.7	69.0	149.5	829.7
29	2.0	3.3	11.3	12.8	35.8	17.0	28.2	119.0	72.0	1.3	3.7	9.0	4.3	3.7	72.0	202.2	598.0
30	4.2	1.8	12.2	49.8	64.0	46.4	56.3	110.0	47.0	1.0	4.3	8.7	4.0	3.0	73.0	170.6	387.7
31	5.0	2.5	12.6	53.3	69.4	42.3	52.8	55.0	28.0	1.0	4.0	9.0	4.3	3.3	78.0	148.3	242.3
32	4.8	1.3	10.4	64.1	79.8	54.4	67.9	61.0	39.0	1.0	6.0	7.3	5.7	3.0	77.0	183.3	543.0
33	4.0	2.8	11.1	41.3	62.4	35.3	51.1	159.0	56.0	1.0	5.3	9.0	7.0	1.7	69.0	171.1	589.7
34	4.5	1.7	13.3	47.1	54.8	29.9	41.9	76.0	41.0	1.0	4.7	9.0	4.0	3.3	72.0	173.3	489.7
35	2.3	4.0	8.8	21.9	56.5	32.1	47.8	127.0	62.0	1.7	5.0	6.0	7.3	2.3	71.0	160.6	449.7
36	2.3	3.7	9.9	23.2	35.3	19.6	25.9	106.0	52.0	2.0	3.3	5.7	6.7	2.0	72.0	152.2	878.7
37	4.5	3.5	7.9	42.8	67.5	41.7	60.0	7.0	2.0	1.3	4.0	6.3	5.0	1.3	77.0	135.5	383.0
38	2.3	4.7	7.1	26.0	67.3	40.8	64.0	25.0	4.0	1.0	3.3	9.0	3.7	3.7	76.0	166.1	536.7
39	2.3	2.7	9.3	22.3	46.6	26.2	42.0	186.0	110.0	1.7	7.0	6.3	6.7	3.0	70.0	125.0	635.3
40	4.7	4.3	8.4	17.5	51.0	25.8	44.8	111.0	52.0	3.7	4.0	9.0	4.0	2.7	78.0	157.8	193.0
41	4.7	3.7	9.3	40.6	69.3	40.1	56.8	108.0	69.0	4.3	4.7	7.3	4.7	2.7	77.0	184.4	485.7
42	4.7	4.7	8.4	15.1	58.0	38.0	49.8	151.0	89.0	1.7	2.7	9.0	4.7	2.3	78.0	166.1	306.0
43	3.2	4.3	9.5	13.7	46.7	17.8	38.4	6.0	1.0	1.7	3.3	9.0	4.0	3.3	78.0	187.2	236.7
44	2.5	4.7	8.6	28.2	55.3	28.2	47.8	169.0	79.0	1.7	3.7	9.0	4.0	2.7	77.0	181.7	340.3
45	4.7	4.0	7.3	26.7	67.0	37.6	56.7	122.0	60.0	1.0	4.0	5.0	5.7	1.3	73.0	110.6	474.3
46	2.7	3.3	8.5	20.3	44.5	12.2	29.7	141.0	86.0	3.7	3.0	9.0	5.0	1.7	80.0	171.7	194.3

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Entry No	gls	SV I	Seht I	ovi(%) I	ovi(%) II	Dh(%)I	Dh(%)II	Tri_u	Tri_l	Pigmen	ORS	midge	AS	aggrs	Flower	Pl_ht	gr_yi
47	3.3	1.3	10.8	40.0	57.5	34.3	42.6	75.0	41.0	1.3	4.3	6.7	4.3	2.7	72.0	203.3	414.3
48	3.8	2.3	10.0	31.4	51.9	24.6	42.3	74.0	41.0	1.0	3.0	5.0	4.3	3.0	66.0	137.8	620.7
49	4.7	1.3	10.4	46.8	55.6	36.8	42.3	134.0	77.0	1.0	5.7	7.0	5.3	3.0	66.0	166.7	631.0
50	2.7	3.0	10.1	29.5	45.4	23.2	41.1	176.0	67.0	1.0	5.3	9.0	6.3	2.0	74.0	160.5	560.7
51	2.5	5.0	10.0	24.6	50.7	21.7	46.4	109.0	35.0	1.0	3.3	7.0	4.3	3.0	83.0	153.9	175.0
52	2.5	2.3	11.7	31.2	40.2	27.5	37.0	78.0	37.0	1.3	5.0	8.0	5.3	3.0	70.0	188.9	423.3
53	2.5	1.5	11.9	23.2	45.4	30.9	40.7	55.0	14.0	3.7	5.3	6.7	5.3	3.0	68.0	170.6	582.0
54	4.5	2.7	12.8	49.0	69.1	45.0	52.0	102.0	47.0	1.7	5.3	8.3	4.3	3.0	69.0	158.9	653.7
55	5.0	3.7	7.9	28.7	52.3	30.0	51.5	148.0	69.0	1.0	3.3	6.3	5.3	3.0	78.0	175.0	493.3
56	2.2	3.0	9.7	30.7	47.3	22.8	45.0	155.0	101.0	1.0	3.7	6.3	5.0	3.7	66.0	160.6	531.7
57	3.5	2.3	8.2	60.6	72.9	54.0	67.2	23.0	12.0	3.5	2.7	9.0	5.7	2.3	68.0	160.0	611.7
58	3.5	5.0	7.4	4.4	57.6	13.7	56.7	112.0	65.0	3.9	4.3	7.0	4.0	2.0	83.0	172.8	346.7
59	2.5	4.0	8.7	25.6	50.1	26.6	48.1	74.0	30.0	1.3	3.7	7.3	4.7	4.0	77.0	187.2	611.7
60	2.8	2.3	10.3	47.4	66.4	42.9	52.4	110.0	69.0	1.0	4.0	9.0	5.0	2.3	66.0	166.7	393.3
61	3.0	3.3	10.2	39.8	60.1	39.0	55.9	8.0	1.0	1.0	5.7	8.7	6.0	3.0	75.0	188.9	769.3
62	5.0	1.3	9.2	72.6	87.5	76.0	79.4	5.0	0.0	1.0	5.7	6.7	6.0	2.3	65.0	154.4	592.3
63	3.8	4.0	9.3	48.2	63.8	48.7	59.7	150.0	88.0	2.0	4.3	7.0	4.7	3.7	78.0	171.1	300.7
64	2.5	3.7	9.8	32.5	54.5	25.5	52.2	7.0	1.0	1.3	4.7	9.0	5.0	3.7	78.0	170.0	510.7
65	4.7	4.7	9.3	26.1	53.1	35.5	47.4	66.0	36.0	3.3	4.0	6.7	5.0	2.0	68.0	130.0	218.0
66	4.7	1.7	10.3	62.3	76.0	65.5	69.8	85.0	51.0	1.7	6.0	8.3	5.0	2.7	72.0	167.8	564.3
67	3.0	3.3	9.9	27.2	44.9	20.3	42.5	100.0	37.0	3.3	3.7	5.0	4.0	3.0	66.0	177.2	471.3
68	5.0	2.7	10.8	46.4	72.3	44.8	66.7	35.0	7.0	2.3	4.0	9.0	5.0	3.7	73.0	184.5	518.3
69	3.8	4.0	9.7	38.7	56.1	35.3	52.7	15.0	6.0	1.3	3.7	9.0	4.0	2.7	74.0	203.9	492.3
70	5.0	3.7	8.9	47.2	78.9	53.7	67.8	6.0	0.0	2.0	4.3	7.7	4.3	3.0	79.0	204.4	426.7
71	5.0	5.0	8.3	31.7	69.0	41.1	53.5	130.0	68.0	3.3	4.7	9.0	5.0	2.0	79.0	162.8	467.0
72	2.7	3.0	8.1	25.4	50.7	30.2	38.9	57.0	33.0	3.7	4.3	5.7	4.7	2.7	72.0	176.1	627.7
73	5.0	5.0	7.4	35.2	64.4	29.2	66.3	2.0	0.0	1.0	5.3	7.0	5.7	2.0	77.0	153.9	617.3
74	4.0	2.5	10.3	45.6	57.7	45.9	49.6	84.0	49.0	1.3	7.0	7.3	6.7	2.7	72.0	165.6	644.0
75	4.3	4.7	8.2	29.7	71.6	49.9	65.2	2.0	0.0	1.0	2.7	5.3	4.0	3.7	80.0	166.7	515.0
76	4.2	3.0	9.9	36.1	53.4	32.4	53.2	75.0	43.0	1.7	4.7	7.7	5.7	2.7	73.0	140.5	411.3
77	2.7	3.2	9.3	35.7	71.8	39.3	60.3	5.0	2.0	1.0	4.7	9.0	5.7	3.3	74.0	194.5	679.3
78	5.0	2.5	9.5	60.2	81.2	64.0	75.0	4.0	0.0	1.7	9.0	7.7	6.0	3.0	72.0	130.6	388.7
79	5.0	2.7	8.7	53.4	73.0	62.9	69.5	0.0	0.0	4.7	6.7	4.7	6.0	2.0	61.0	137.2	388.7
80	1.8	5.0	10.2	22.8	65.4	27.8	48.1	127.0	82.0	1.0	4.0	9.0	4.7	2.0	74.0	157.8	478.0
81	4.0	3.7	10.1	43.5	75.8	48.4	55.8	134.0	57.0	1.7	4.3	7.3	4.3	3.7	78.0	173.9	603.3
82	2.8	2.3	10.6	40.5	52.1	32.0	49.7	82.0	42.0	1.0	4.3	8.3	4.7	3.3	74.0	145.5	254.3
83	5.0	1.0	11.9	46.7	68.2	53.0	61.9	84.0	47.0	1.0	6.7	6.3	6.0	3.0	66.0	176.1	387.3
84	3.7	2.0	10.6	40.6	51.9	29.2	45.6	81.0	32.0	1.3	6.7	8.0	5.7	3.0	62.0	142.2	495.7
85	3.7	3.7	10.9	36.7	60.0	35.0	49.3	5.0	0.0	2.7	5.7	8.3	4.3	2.7	64.0	187.2	717.3
86	2.8	3.7	10.3	37.6	62.3	32.4	54.1	105.0	56.0	4.0	3.3	5.3	5.0	3.0	76.0	177.8	531.7
87	2.3	2.2	12.2	29.0	46.0	28.7	41.9	122.0	70.0	3.3	3.3	7.0	5.0	3.0	65.0	187.8	878.3
88	4.0	1.7	9.2	41.5	58.8	34.1	46.5	84.0	48.0	1.3	5.3	7.7	4.7	2.7	68.0	189.4	416.0
89	4.7	3.7	9.2	39.8	64.9	37.9	54.6	78.0	21.0	1.0	6.0	6.7	5.7	2.7	74.0	169.5	824.7
90	2.8	2.0	10.0	27.5	50.3	29.0	42.5	133.0	70.0	3.0	5.7	7.3	4.7	3.0	69.0	141.7	274.7
91	3.0	1.8	10.1	28.7	55.1	35.6	47.5	92.0	44.0	1.0	4.7	9.0	5.3	3.0	71.0	175.0	709.0
92	2.2	3.3	10.4	19.6	41.7	26.6	32.7	115.0	45.0	2.2	3.7	6.3	3.7	3.3	72.0	172.8	530.3

Cont...

ntry No	gls	SV I	Seht I	ovi(%) I	ovi(%) II	Dh(%)I	Dh(%)II	Tri_u	Tri_l	Pigmen	ORS	midge	AS	agrs	Flower	Pl_ht	gr_yi
93	3.2	3.0	10.2	31.8	57.7	40.6	46.5	116.0	66.0	2.0	4.0	6.7	4.3	3.3	75.0	161.7	246.0
94	3.2	3.3	9.9	32.2	46.8	30.9	37.6	135.0	74.0	1.0	3.7	7.3	4.7	3.0	72.0	171.1	753.3
95	2.8	3.3	8.6	28.5	49.0	33.0	39.1	38.0	20.0	3.7	3.3	5.3	4.7	2.0	73.0	166.1	395.3
96	1.8	4.3	8.9	38.6	57.6	37.5	47.6	73.0	41.0	1.0	3.7	5.7	5.0	3.3	76.0	170.6	391.0
97	2.5	2.0	11.9	23.8	44.2	32.8	45.9	139.0	64.0	1.0	5.7	8.3	5.0	3.3	69.0	184.5	655.3
98	4.3	4.3	10.0	46.4	68.1	40.4	64.6	58.0	35.0	2.3	4.0	7.3	4.3	3.7	74.0	169.4	814.3
99	3.2	4.7	8.6	27.6	44.2	16.9	38.0	15.0	5.0	2.0	4.0	6.3	3.7	3.0	78.0	173.4	343.7
100	2.5	4.3	8.5	13.1	47.3	22.8	46.6	3.0	0.0	1.8	4.3	7.0	3.7	3.3	79.0	216.1	572.0
101	5.0	2.8	9.8	51.2	70.2	50.2	65.6	74.0	40.0	1.0	4.7	9.0	4.0	3.7	71.0	175.5	496.0
102	3.0	3.7	8.9	24.6	45.2	26.4	41.5	10.0	5.0	2.3	4.0	6.3	4.0	2.7	76.0	162.8	529.7
103	2.8	4.3	9.8	18.3	45.3	19.9	40.0	138.0	75.0	2.3	4.7	6.7	4.3	3.0	68.0	121.6	515.3
104	4.8	3.0	8.9	43.5	66.7	54.7	64.0	102.0	42.0	2.7	5.0	6.3	5.7	1.7	72.0	157.8	346.3
105	2.8	3.3	12.2	20.4	39.5	19.1	33.9	57.0	34.0	5.0	5.3	9.0	5.0	2.3	62.0	170.6	176.3
106	5.0	3.3	9.0	29.2	49.0	28.3	45.8	76.0	32.0	2.7	5.0	7.3	5.3	2.7	69.0	166.1	328.7
107	4.7	4.0	9.5	45.8	69.8	50.1	62.1	75.0	34.0	2.0	6.3	7.3	6.0	2.0	69.0	197.2	468.3
108	3.5	4.5	7.9	40.8	62.6	39.8	53.3	87.0	52.0	1.3	4.7	6.3	4.3	3.3	79.0	189.4	476.7
109	2.3	2.7	11.5	38.0	53.8	34.7	46.3	92.0	53.0	1.7	3.7	6.0	4.3	3.0	77.0	160.0	436.0
110	3.7	4.0	8.1	23.7	77.8	34.5	73.6	157.0	100.0	1.0	4.3	7.0	5.3	3.7	79.0	172.2	614.7
111	2.8	4.0	10.4	25.4	55.9	29.7	40.4	98.0	56.0	1.0	5.0	9.0	4.7	3.0	77.0	185.6	422.7
112	4.2	3.0	9.6	23.0	51.8	30.5	40.6	38.0	19.0	1.0	3.3	9.0	3.3	4.3	70.0	179.4	438.3
113	2.5	3.7	8.8	17.6	47.8	27.2	45.4	109.0	48.0	1.0	2.7	5.7	6.3	3.3	75.0	162.2	404.0
114	1.7	4.3	7.8	18.3	43.3	9.4	34.9	44.0	11.0	2.3	2.7	4.0	4.3	2.3	73.0	158.9	484.7
115	2.7	2.5	10.9	31.4	56.0	20.4	46.1	79.0	41.0	1.0	5.0	6.0	5.7	3.3	64.0	152.8	460.0
116	5.0	4.5	5.7	60.4	83.1	57.9	83.5	46.0	29.0	2.8	2.9	5.0	4.5	1.0	83.0	162.8	315.0
117	5.0	4.7	8.4	37.9	83.1	61.5	76.1	139.0	71.0	2.3	3.7	8.7	5.0	3.7	79.0	157.2	374.0
118	4.3	3.7	9.1	40.1	78.0	36.1	71.0	170.0	92.0	4.0	3.7	6.7	4.7	4.0	66.0	170.0	640.7
119	2.3	4.3	8.3	23.4	57.0	25.9	51.1	104.0	35.0	1.5	5.0	6.7	6.3	3.0	78.0	182.2	640.0
120	2.3	3.0	9.8	25.6	49.6	19.0	33.7	154.0	64.0	1.3	3.0	8.0	3.7	3.0	78.0	128.3	545.0
121	3.5	3.3	8.9	37.6	47.6	26.1	44.4	184.0	62.0	2.3	3.7	7.0	4.7	2.0	69.0	160.6	449.3
122	3.3	1.7	10.9	34.1	49.4	31.7	44.9	84.0	38.0	1.0	4.0	7.3	4.0	3.0	72.0	127.8	365.7
123	2.5	3.7	10.9	17.1	34.2	20.3	26.9	108.0	66.0	5.0	3.7	4.3	5.0	3.0	74.0	142.8	587.0
124	2.5	2.7	8.6	37.8	55.5	27.8	40.5	16.0	5.0	2.3	3.7	4.7	5.7	1.7	74.0	162.8	605.0
125	3.3	4.0	8.4	26.0	58.2	30.1	38.1	99.0	44.0	1.3	3.0	5.7	3.7	3.0	77.0	151.7	527.3
126	3.7	3.7	8.0	40.0	58.9	35.8	56.2	114.0	84.0	1.0	4.3	6.7	5.7	2.0	68.0	158.9	325.0
127	2.5	2.2	10.3	44.8	59.2	47.2	55.8	105.0	35.0	2.3	3.7	7.7	5.0	2.0	66.0	203.3	547.0
128	5.0	2.5	9.9	65.8	83.4	64.9	77.3	115.0	53.0	1.0	4.3	9.0	5.3	3.7	79.0	178.9	636.3
129	2.3	2.5	9.6	14.3	38.0	19.1	35.5	137.0	84.0	1.3	4.7	8.7	5.3	3.7	77.0	157.2	485.0
130	3.7	3.3	12.1	37.2	50.6	33.7	45.1	54.0	24.0	1.3	4.3	8.7	5.7	3.3	77.0	133.9	271.7
131	4.0	1.3	12.4	44.5	52.8	35.8	45.9	74.0	36.0	1.0	4.3	8.3	5.3	3.0	76.0	202.8	315.7
132	5.0	4.0	7.3	43.2	68.9	44.9	62.7	45.0	21.0	1.3	5.3	6.3	5.0	3.0	80.0	184.4	427.7
133	5.0	3.7	8.6	28.7	76.9	46.4	70.7	181.0	108.0	1.8	4.0	9.0	5.0	3.0	79.0	199.4	190.0
134	2.8	4.0	9.7	28.5	54.6	31.3	47.2	104.0	56.0	1.0	2.7	9.0	5.3	3.3	72.0	151.1	275.3
135	4.8	2.0	8.0	64.8	73.8	62.3	69.9	61.0	41.0	3.7	7.7	9.0	6.0	3.3	66.0	155.6	237.0
136	5.0	3.0	9.9	56.2	73.5	48.0	57.5	94.0	57.0	1.7	3.3	6.0	3.7	3.0	71.0	181.7	591.7
137	5.0	3.0	9.9	40.3	63.7	36.0	54.8	61.0	25.0	3.0	3.3	6.7	4.3	2.7	69.0	164.4	460.7
138	2.7	2.5	9.4	31.3	50.1	29.7	41.9	110.0	53.0	1.3	2.3	9.0	4.3	2.0	69.0	113.9	471.0

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Entry No	gls	SV 1	Seht 1	ovi(%) I	ovi(%) II	Dh(%)I	Dh(%)II	Tri_u	Tri 1	Pigmen	ORS	midge	AS	agrs	Flower	Pl ht	gr yi
139	2.8	3.0	10.3	36.6	58.5	38.9	50.6	95.0	50.0	1.2	3.0	9.0	4.0	2.7	79.0	190.5	438.3
140	2.8	3.3	8.8	22.5	39.2	22.3	34.5	120.0	73.0	1.3	7.0	5.7	6.0	4.0	74.0	167.2	533.3
141	1.5	2.8	8.8	37.6	51.8	24.3	37.3	124.0	68.0	1.3	3.0	9.0	5.0	2.3	79.0	172.8	413.0
142	1.8	3.0	9.9	37.1	59.4	34.2	50.5	149.0	73.0	1.0	3.7	9.0	4.3	3.0	67.0	168.3	306.0
143	3.2	2.8	10.5	47.5	63.3	45.1	56.1	10.0	4.0	2.0	6.0	8.0	4.7	2.0	67.0	193.9	432.7
144	5.0	3.3	10.0	43.3	66.9	43.3	58.8	114.0	53.0	2.0	3.3	9.0	3.7	2.3	74.0	161.6	239.3
145	1.7	1.7	12.1	21.8	40.1	18.0	32.2	39.0	22.0	1.0	5.3	8.7	4.0	2.7	73.0	176.7	352.3
146	1.7	2.3	12.7	26.3	39.6	19.8	35.3	96.0	49.0	1.0	5.3	9.0	4.3	3.3	72.0	204.4	489.0
147	4.7	2.3	11.2	45.8	62.3	48.1	60.0	71.0	23.0	2.8	3.3	6.3	4.3	3.3	69.0	178.3	574.3
148	4.2	2.8	9.1	42.7	56.7	45.3	56.3	175.0	93.0	1.8	4.0	5.3	4.7	3.0	72.0	151.1	614.3
149	4.7	5.0	9.8	34.2	67.1	33.0	58.5	121.0	78.0	1.7	3.3	5.3	5.3	3.0	76.0	157.8	476.7
150	2.3	4.0	8.2	16.6	35.9	19.4	35.0	64.0	29.0	1.7	2.7	9.0	3.3	2.7	78.0	188.9	184.0
151	4.3	4.3	8.6	55.9	66.7	40.1	60.6	82.0	33.0	2.3	5.0	5.3	4.7	2.0	81.0	141.1	297.3
152	2.0	3.7	9.9	39.2	46.3	26.7	38.7	123.0	67.0	1.0	3.7	5.7	4.7	2.7	67.0	162.2	640.0
153	3.0	4.3	8.1	33.0	58.5	35.6	47.4	121.0	60.0	2.7	4.0	5.3	5.0	2.0	68.0	141.1	889.0
154	5.0	5.0	9.7	12.5	53.2	36.0	49.7	67.0	46.0	2.3	3.7	6.0	5.7	3.3	77.0	128.3	495.7
155	5.0	3.3	10.9	32.7	60.2	31.0	50.4	21.0	0.0	2.2	3.0	6.7	5.0	3.7	74.0	167.8	423.3
156	2.7	2.0	10.3	49.9	69.6	48.7	55.5	142.0	73.0	1.3	5.7	8.0	6.0	2.0	64.0	152.8	548.3
157	4.7	2.3	10.2	41.5	61.6	40.5	54.9	11.0	5.0	1.0	5.3	9.0	4.7	3.7	68.0	199.4	688.3
158	3.2	3.3	8.7	37.0	60.6	31.5	50.5	22.0	4.0	2.3	4.0	5.3	5.0	2.0	67.0	150.0	464.0
159	3.0	4.0	8.5	26.0	64.3	38.2	53.9	15.0	4.0	3.0	3.7	6.7	6.0	2.3	71.0	153.9	355.7
160	2.7	5.0	9.3	15.0	41.9	20.0	39.3	85.0	49.0	1.0	3.7	9.0	4.7	3.0	83.0	175.0	56.3
161	2.7	2.0	10.8	40.1	51.5	35.9	48.7	10.0	4.0	1.0	4.0	9.0	4.0	2.7	78.0	181.1	192.7
162	4.0	1.3	13.2	49.2	60.6	47.0	56.4	88.0	51.0	2.0	4.7	8.3	4.7	3.0	70.0	178.9	837.3
163	4.3	3.7	9.6	19.3	44.1	19.6	40.1	219.0	138.0	2.3	4.7	6.7	5.3	3.0	72.0	193.4	714.3
164	4.7	3.7	10.3	54.3	82.9	68.8	81.3	101.0	48.0	5.0	8.0	7.3	6.0	3.0	70.0	143.3	410.7
165	2.8	3.5	8.8	37.3	60.7	39.1	56.3	52.0	28.0	1.3	4.7	6.0	4.7	2.7	71.0	171.1	554.3
166	2.2	3.2	9.5	19.6	37.3	10.8	27.4	141.0	89.0	2.7	5.0	5.3	7.0	2.3	76.0	147.8	429.7
167	3.3	3.3	9.8	47.7	59.7	46.0	56.5	14.0	5.0	2.3	5.0	7.3	6.0	2.7	74.0	158.4	465.0
168	2.5	5.0	8.0	34.0	49.9	29.0	44.9	9.0	0.0	1.3	4.7	6.3	5.0	2.3	68.0	131.7	277.0
169	3.5	3.0	10.1	39.6	49.8	30.3	45.0	8.0	4.0	1.5	4.7	6.3	5.0	2.3	67.0	217.8	591.3
170	3.3	3.3	9.8	44.7	60.8	36.0	49.6	92.0	53.0	1.3	6.0	7.3	5.3	3.3	72.0	173.3	469.0
171	4.3	4.0	8.6	51.8	70.0	59.7	70.2	17.0	3.0	1.0	4.3	7.0	4.3	3.3	74.0	177.2	488.7
172	5.0	2.0	9.9	35.8	60.0	30.8	48.5	120.0	57.0	1.3	4.0	7.7	5.0	3.7	71.0	183.3	663.7
173	2.7	2.7	10.4	21.1	44.1	22.2	35.2	43.0	12.0	2.0	3.3	5.0	5.0	2.7	64.0	148.3	254.7
174	3.0	4.0	8.5	19.8	63.8	25.7	57.8	180.0	86.0	1.0	5.0	5.3	5.0	2.3	80.0	148.9	222.0
175	2.8	4.0	8.8	35.9	50.9	32.6	40.0	17.0	5.0	1.3	3.0	9.0	4.7	2.0	79.0	183.9	351.7
176	2.8	3.2	11.2	23.2	37.1	21.6	32.7	22.0	10.0	1.7	3.0	9.0	4.0	2.7	73.0	162.8	283.3
177	4.0	3.3	10.9	34.0	50.7	30.4	47.9	81.0	42.0	2.7	4.0	6.3	5.7	2.7	75.0	174.4	441.3
178	1.8	3.5	9.0	20.4	48.0	26.4	36.0	52.0	29.0	1.7	3.3	5.3	3.7	3.3	67.0	162.2	389.7
179	1.7	3.0	10.3	18.9	45.8	29.3	44.4	149.0	55.0	1.0	3.7	6.0	5.3	2.0	75.0	139.4	414.3
180	4.3	3.3	10.8	45.6	65.2	34.1	55.4	150.0	71.0	1.7	5.3	7.3	5.7	2.3	73.0	160.6	565.0
181	3.0	4.3	8.1	18.9	54.5	21.0	44.5	82.0	50.0	3.2	2.7	5.3	4.0	3.3	74.0	163.3	676.0
182	3.3	4.0	8.9	36.7	64.2	42.3	59.5	125.0	69.0	2.5	2.3	4.0	4.0	2.7	77.0	169.4	614.3
183	4.2	3.0	11.6	32.5	47.0	25.6	45.2	232.0	108.0	2.0	4.3	8.3	5.0	3.0	69.0	193.3	574.7
184	3.8	3.7	10.1	46.7	70.5	48.4	66.8	126.0	57.0	1.3	7.3	8.7	5.0	3.0	68.0	179.4	491.3

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Entry No	gls	SV I	Seht I	ovi(%) I	ovi(%) II	Dh(%)I	Dh(%)II	Tri_u	Tri_l	Pigmen	ORS	midge	AS	agrs	Flower	Pl_ht	gr_yi
185	2.8	2.8	10.1	24.3	61.1	42.6	47.1	12.0	6.0	1.7	4.3	9.0	4.0	2.3	75.0	202.8	410.3
186	3.3	2.8	9.5	41.4	59.2	32.9	48.6	24.0	0.0	1.7	5.0	9.0	5.3	2.7	75.0	184.5	456.3
187	2.7	2.7	9.9	40.0	58.6	37.8	54.9	135.0	54.0	1.3	3.0	6.0	5.0	4.3	67.0	162.8	725.0
188	2.8	2.7	10.3	37.8	48.4	24.7	39.5	102.0	48.0	1.7	2.3	9.0	4.0	2.0	70.0	167.8	361.7
189	2.8	2.2	13.1	34.1	45.4	23.7	35.0	70.0	35.0	1.0	4.3	8.3	4.3	4.0	71.0	177.8	581.7
190	2.5	2.7	11.5	54.9	75.2	49.7	58.7	65.0	41.0	1.3	7.7	9.0	5.3	3.3	67.0	206.7	695.7
191	4.0	2.2	10.6	43.9	65.0	43.2	57.6	34.0	7.0	1.3	5.3	9.0	5.0	3.0	73.0	166.1	496.3
192	4.0	3.8	9.9	29.3	53.5	35.4	47.9	20.0	9.0	1.3	4.7	9.0	4.3	2.7	69.0	179.4	620.7
193	3.5	3.5	4.0	0.0	16.7	5.6	55.6	126.0	85.0	1.8	4.0	6.7	4.0	3.3	81.0	166.1	340.3
194	4.7	5.0	7.9	31.5	64.6	32.0	50.7	72.0	41.0	2.0	3.0	6.7	3.7	3.3	76.0	163.3	328.7
195	3.5	5.0	6.8	28.0	68.8	35.1	62.8	78.0	29.0	3.3	3.3	6.0	5.0	1.0	82.0	132.8	367.3
196	3.5	4.7	10.4	22.0	43.5	16.9	33.2	64.0	27.0	3.7	3.0	7.3	4.3	3.0	77.0	167.2	661.0
197	3.3	3.0	9.7	34.1	71.5	52.3	61.4	71.0	32.0	2.0	3.0	6.0	3.7	3.7	80.0	185.6	570.3
198	5.0	4.0	8.4	41.2	69.4	50.2	64.0	69.0	24.0	2.7	4.0	9.0	3.7	2.7	78.0	152.2	393.3
199	3.0	3.7	8.7	35.7	53.8	24.5	46.2	87.0	41.0	4.0	4.7	5.3	5.0	3.3	68.0	175.6	395.3
200	3.2	2.2	10.7	36.8	51.3	30.7	45.7	105.0	62.0	3.7	3.3	5.0	4.7	3.3	67.0	163.9	443.3
201	4.5	5.0	5.7	18.0	74.8	37.1	73.0	5.0	0.0	3.3	5.7	8.3	5.0	2.7	76.0	176.1	745.3
202	5.0	4.3	9.2	22.2	57.5	29.4	45.0	10.0	0.0	3.7	4.3	8.3	4.7	2.7	66.0	142.2	560.7
203	4.7	2.5	8.8	43.2	59.0	38.4	48.7	69.0	30.0	1.3	4.0	6.0	5.3	2.3	64.0	147.2	542.7
204	4.0	4.0	8.7	35.3	57.3	31.7	54.8	124.0	39.0	2.0	4.3	6.0	4.7	2.3	75.0	141.1	586.0
205	4.7	3.3	9.6	39.7	62.9	34.3	56.7	144.0	62.0	3.7	2.0	9.0	4.0	3.0	74.0	163.4	226.0
206	4.8	4.5	8.9	31.3	60.8	37.0	61.0	156.0	74.0	3.0	3.0	9.0	3.7	2.7	77.0	191.7	433.3
207	2.8	3.3	7.6	25.5	49.4	32.1	45.9	224.0	112.0	2.5	4.3	4.3	5.3	3.0	75.0	182.2	794.0
208	4.2	3.0	8.6	49.3	63.8	50.7	55.0	181.0	86.0	3.3	5.0	6.7	7.0	3.0	75.0	172.8	396.0
209	2.3	3.2	8.3	34.0	54.3	30.6	44.6	108.0	51.0	1.0	3.0	9.0	4.0	2.0	78.0	195.0	346.7
210	1.8	4.3	8.8	19.7	40.2	22.6	32.5	102.0	46.0	1.0	3.3	9.0	3.7	2.3	78.0	192.2	360.7
211	4.2	3.8	8.7	28.8	44.3	30.6	43.1	126.0	48.0	1.0	3.0	6.3	4.7	3.0	64.0	142.8	718.0
212	4.3	3.3	11.4	46.0	60.6	39.3	55.5	168.0	99.0	1.0	4.3	9.0	4.7	2.7	69.0	164.5	693.7
213	5.0	1.5	12.1	58.9	74.5	57.5	70.4	44.0	29.0	5.0	5.7	6.3	6.7	1.7	66.0	160.6	459.7
214	5.0	2.0	8.3	59.4	85.3	66.7	74.9	3.0	0.0	1.0	7.3	6.0	6.7	2.3	63.0	155.6	443.7
215	4.2	3.8	8.4	50.7	68.5	53.1	67.8	80.0	46.0	1.0	4.3	9.0	4.7	3.0	78.0	174.4	427.7
216	4.3	4.3	10.0	52.7	80.3	49.3	75.7	2.0	0.0	1.0	2.7	6.0	4.7	3.3	75.0	177.8	423.7
217	4.7	4.7	8.8	70.2	82.9	61.3	74.8	3.0	0.0	1.0	3.3	6.3	4.0	3.0	75.0	157.8	385.3
218	5.0	3.0	10.4	51.8	79.8	59.1	72.8	46.0	24.0	1.7	4.7	7.7	5.3	3.0	72.0	158.3	568.7
219	4.5	4.5	9.1	34.7	56.7	38.2	54.5	14.0	3.0	2.0	4.7	7.0	4.3	3.3	76.0	166.7	391.7
220	4.7	3.8	9.6	49.8	60.7	39.7	51.8	32.0	15.0	1.5	4.7	7.0	5.0	3.0	76.0	168.9	497.3
221	3.2	3.0	9.3	39.1	55.4	41.3	54.0	126.0	53.0	2.7	4.3	5.0	5.0	2.7	64.0	152.2	412.3
222	2.3	2.7	8.8	24.7	54.0	26.9	48.3	66.0	36.0	3.0	5.0	7.7	6.0	3.3	75.0	134.4	377.0
223	2.0	3.2	9.8	18.0	45.0	18.0	38.2	72.0	32.0	1.7	3.7	7.3	6.0	3.3	76.0	155.0	346.7
224	2.2	4.0	10.2	27.3	37.5	25.7	32.0	75.0	28.0	1.3	5.3	9.0	5.3	3.3	75.0	175.0	346.7
225	5.0	2.5	9.5	56.9	71.0	60.3	65.8	149.0	68.0	1.7	3.7	6.3	5.3	3.0	79.0	160.6	187.7
226	3.7	5.0	9.1	29.9	51.5	25.3	45.5	22.0	12.0	1.3	4.0	9.0	5.0	2.3	77.0	150.0	289.3
227	2.2	2.7	11.7	20.4	40.4	20.2	29.1	111.0	44.0	1.8	3.0	9.0	5.3	3.3	70.0	202.2	729.7
228	5.0	2.5	11.5	21.9	49.8	23.6	44.8	114.0	53.0	1.3	5.0	5.7	4.7	4.0	71.0	190.0	616.0
229	2.0	1.7	12.6	45.0	51.0	28.4	41.3	82.0	42.0	2.8	5.0	9.0	4.0	3.7	65.0	187.2	635.3
230	3.0	3.0	7.2	40.6	50.9	35.8	50.2	118.0	69.0	2.7	4.3	5.0	5.0	2.7	76.0	191.1	962.7

Entry No	gls	SV I	Seht I	ovi(%) I	ovi(%) II	Dh(%)I	Dh(%)II	Tri_u	Tri 1	Pigmen	ORS	midge	AS	agrs	Flower	Pl_ht	gr_yi
231	2.8	2.7	10.1	43.8	62.2	43.6	52.2	145.0	68.0	2.3	2.0	9.0	4.0	1.7	78.0	181.1	669.3
232	1.8	4.0	8.0	27.1	41.2	22.5	37.1	83.0	29.0	2.3	2.7	6.7	5.7	2.0	76.0	157.2	321.3
233	4.0	3.2	9.5	25.6	63.2	38.8	51.1	18.0	6.0	2.3	4.7	6.0	4.7	3.0	63.0	123.3	516.0
234	4.7	3.0	9.2	51.4	67.4	50.7	60.0	65.0	34.0	4.0	4.7	7.0	5.0	3.0	69.0	170.0	426.3
235	3.7	3.3	8.9	31.7	54.8	26.2	50.5	240.0	135.0	1.0	3.0	5.7	5.0	2.7	77.0	100.0	420.7
236	2.5	3.3	9.7	22.0	41.1	14.7	33.6	122.0	53.0	1.7	3.3	7.3	4.3	2.7	67.0	126.7	732.7
237	5.0	3.7	10.3	48.7	60.6	48.0	60.8	113.0	52.0	2.2	4.7	9.0	5.7	3.0	75.0	168.3	348.3
238	4.2	4.7	8.5	28.2	57.7	29.4	48.5	51.0	26.0	1.0	4.0	9.0	5.3	3.3	73.0	152.8	335.0
239	4.0	4.3	8.0	21.4	46.4	26.2	37.7	188.0	107.0	3.7	4.3	4.0	5.7	2.3	70.0	143.3	635.0
240	4.3	4.0	8.9	44.5	67.0	32.5	53.1	130.0	64.0	3.5	6.0	4.3	5.7	2.0	75.0	126.7	467.0
241	3.3	2.8	10.1	33.4	56.2	23.5	41.9	98.0	56.0	1.3	3.3	6.0	5.3	3.0	68.0	172.2	559.0
242	3.3	2.7	8.8	30.0	64.1	41.3	55.0	158.0	64.0	1.8	2.7	9.0	4.3	3.0	75.0	184.5	316.0
243	3.8	3.3	10.0	34.5	59.5	24.6	55.2	113.0	78.0	1.0	4.3	5.3	4.7	2.7	77.0	167.8	483.0
244	4.2	4.2	11.1	24.4	59.6	26.1	53.1	118.0	70.0	1.0	3.3	6.0	4.3	2.7	78.0	174.5	801.7
245	5.0	5.0	7.4	46.1	77.6	57.0	71.8	14.0	6.0	3.3	4.3	7.3	5.0	2.0	73.0	174.4	601.7
246	3.0	4.7	9.0	56.7	67.1	43.3	58.2	9.0	1.0	3.5	3.0	5.7	4.7	3.0	77.0	180.6	399.7
247	3.0	2.7	12.5	21.7	43.3	19.9	37.7	186.0	94.0	3.7	5.3	7.0	6.3	3.0	69.0	183.9	424.7
248	3.3	4.0	9.0	36.2	53.4	37.8	49.4	7.0	0.0	2.2	4.7	6.7	4.3	3.3	75.0	147.2	485.7
249	4.2	4.3	8.3	65.4	82.8	55.1	69.7	124.0	46.0	1.7	2.7	5.0	4.0	2.3	75.0	160.0	577.0
250	5.0	3.0	9.5	43.9	64.0	39.7	55.8	76.0	47.0	1.7	3.3	5.7	4.3	2.3	67.0	127.8	326.7
251	4.3	3.0	9.2	52.2	61.8	45.4	59.0	97.0	62.0	1.8	3.3	4.7	4.0	3.0	69.0	138.9	772.0
252	2.8	2.7	9.0	32.6	57.6	43.0	47.9	126.0	68.0	1.0	3.0	6.7	4.0	3.0	72.0	150.0	595.0
253	2.3	3.3	8.8	45.1	61.8	37.2	48.7	125.0	55.0	1.0	2.7	6.0	4.7	3.0	65.0	138.3	500.0
254	4.7	2.3	12.9	29.9	44.9	29.2	43.7	201.0	106.0	1.0	3.3	7.3	3.7	2.7	78.0	202.8	612.7
255	5.0	4.0	8.8	49.9	74.0	41.9	62.7	5.0	0.0	1.3	3.7	6.0	4.7	3.0	76.0	162.8	564.0
256	4.0	2.5	10.1	55.2	69.8	47.0	65.4	110.0	46.0	1.7	6.0	6.7	6.0	3.0	70.0	157.2	471.0
257	4.0	3.0	10.0	33.7	57.1	41.7	52.7	113.0	66.0	2.0	3.3	5.2	5.0	2.7	74.0	161.1	468.0
258	2.2	2.8	11.8	35.6	39.2	21.1	35.4	179.0	107.0	3.7	4.7	5.3	5.7	2.3	75.0	165.0	541.0
259	2.0	4.0	9.3	30.7	51.3	37.6	47.7	3.0	0.0	4.0	4.0	7.3	5.3	2.3	69.0	132.8	320.3
260	2.5	4.0	8.8	22.0	52.6	27.0	40.5	131.0	71.0	1.7	5.0	7.0	4.7	3.0	75.0	100.5	317.7
296B	5.0	4.3	7.9	55.7	81.1	57.1	79.4	0.0	0.0	2.0	4.0	7.0	5.3	1.3	80.0	103.9	450.7
IS 18551	1.5	3.0	12.0	14.3	32.7	11.9	24.5	192.0	83.0	1.0	3.3	9.0	4.0	4.0	74.0	100.0	444.7
296B	5.0	5.0	8.1	50.0	75.0	57.5	73.0	0.0	0.0	2.3	4.3	6.3	5.3	1.0	81.0	102.8	209.7
296B	5.0	3.3	8.2	62.3	84.8	74.9	78.3	0.0	0.0	3.7	3.7	6.0	5.7	1.0	83.0	106.7	373.3
296B	5.0	4.7	7.3	53.1	84.1	50.4	78.7	0.0	0.0	3.3	3.7	6.0	5.0	1.7	84.0	106.7	540.3
IS 18551	1.0	3.2	10.7	10.3	35.9	21.2	27.7	193.0	75.0	1.3	4.0	8.7	4.7	3.7	78.0	196.1	600.0
IS 18551	1.0	3.0	11.9	21.9	30.3	17.9	25.1	184.0	91.0	1.0	3.0	8.0	4.0	3.7	72.0	188.3	572.0
CHS 9	5.0	1.8	10.0	67.3	81.9	67.6	73.9	0.0	0.0	2.3	4.7	6.0	5.3	1.7	63.0	189.4	829.7

Cont...

Entry No	gls	SV I	Seht I	ovi(%) I	ovi(%) II	Dh(%)I	Dh(%)II	Tri_u	Tri l	Pigmen	ORS	midge	AS	agrs	Flower	Pl ht	gr_yi
CHS 9	5.0	1.0	12.0	71.1	82.4	70.0	75.3	0.0	0.0	2.0	5.3	5.7	5.3	1.7	65.0	188.9	661.7
CHS 9	4.8	1.0	10.4	58.7	85.5	58.9	70.8	0.0	0.0	2.0	5.3	6.7	5.3	1.7	64.0	173.9	491.0
SE (±)	0.4	0.4	0.9	7.4	6.6	7.6	6.6	8.0	4.9	0.4	0.6	0.5	0.4	0.3	1.8	7.7	109.3
MEAN	3.5	3.3	9.6	35.4	58.2	35.6	51.0	86.5	43.0	1.9	4.3	7.2	4.9	2.8	72.8	165.1	480.4
CV(%)	17.1	22.1	15.6	36.1	19.7	36.9	22.4	16.0	19.8	34.3	25.3	11.2	15.0	18.5	4.4	8.1	39.4
FRATIO	9.5	5.0	2.7	3.3	3.7	3.0	3.6	48.8	37.7	6.6	3.4	9.7	3.4	4.5	7.1	8.3	2.1
h21	0.7	0.6	0.4	0.4	0.5	0.4	0.5	0.9	0.9	0.7	0.4	0.7	0.5	0.5	0.7	0.7	0.3
h22	0.9	0.8	0.6	0.7	0.7	0.7	0.7	1.0	1.0	0.9	0.7	0.9	0.7	0.8	0.9	0.9	0.5

h2 first on plot basis; second on mean basis

Glossy	Glossiness intensity (score)
SV I	Seedling vigor I score
Seth l	seedling height I (cm)
Egg (%) I	Oviposition (%) I 14 DAE
Egg (%) II	Oviposition (%) II 21 DAE
DH (%) I	Deadhearts (%) I 21 DAE
DH (%) II	Deahearts (%) II 28 DAE
Tri_u	Trichome density upper leaf surface (no./microscopic field)
Tri l	Trichome density lower leaf surface (no./microscopic field)

pigmen	pigmentation score (scale)
ORS	Overall recovery score (scale)
midge	Midge damage score (scale)
AS	Ahpid damage score (scale)
agrs	Agronomic score (scale)
Flower	Time to 50 % flowering (Days)
plht (cm)	Plant height (cm)
GR-Yi	Grain yield (g plot)