SSR MARKER-ASSISTED BACKCROSS INTROGRESSION OF QTLs FOR HOST PLANT RESISTANCE TO *ATHERIGONA SOCCATA* IN *SORGHUM BICOLOR*

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CERTIFICATE

This is to certify that the thesis entitled ***"SSR marker-assisted backcross introgression of QTL for host plant resistance to Atherigona soccata in sorghum bicolor"*** submitted for the award of degree of Doctor of Philosophy in Genetics, Osmania University, Hyderabad, is a record of bonafide research work carried out by Ms T. Jyothi, under my supervision and it had not previously formed the basis for the award of any degree or diploma or other similar title. The candidate's research work has been satisfactory and the thesis is an original research or observation.

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CERTIFICATE

This is to certify that the present dissertation entitled, ***"SSR marker-assisted backcross introgression of QTL for host plant resistance to Atherigona soccata in sorghum bicolor"*** is in part or in full doesn't constitute any part of thesis/dissertation/monograph. This work is original observation and has not been submitted by me or any others for any degree or diploma of any University.

Signature of the Candidate

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CONTENTS

List of Tables

List of Plates

List of Figures

List of Abbreviations and Symbols

CIAA	Chloroform:Isoamyl alcohol
CTAB	Cetyltrimethylammonium bromide
Cm	Centimorghan
DAE	Days after emergence
DH	Dead hearts
Dd 20	Double Distilled water
E/P	Eggs/plants
et al.	And other
EDTA	Ethylene diamine tetra acetic acid (disodium salt)
EtOH	Ethanol
Glo	Glossiness
gDNA	genomic DNA
IL	Introgression Library
LG	Linkage group
l	Litres
KCL	Potassium Chloride
Kg/ha	Kilograms per hectare
MDP	Marker data points
MAS	Marker assisted selection
M	Molar
m	Meter
min	Minutes
mg	Milligram(s)
ml	Millilitre(s)
mm	Millimetre(s)
mM	Millimolar
mol. Wt	Molecular weight
µL	Microliters
µg	Microgram(s)
NILs	Near Isogenic Lines
NaCl	Sodium Chloride
NaOAc	Sodium acetate
NH4Ac	Ammonium acetate
nm	Nanometer
No.	Number(s)
NS	Non-significant
ng	Nanogram
OV	Oviposition
OD	Optical density
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RIL	Recombinant Inbred Line
RP	Recurrent Parent
RPG	Recurrent Parent Genome
SSR	Simple sequence Repeats
SV	Seedling vigor
SE	Standard Error
Sec	Second(s)
TAE	Tris-acetate-EDTA
TBE	Tris-borate-EDTA
Tris	Tris(hydroxymethyl)amino methane
U	unit(s)
UV	Ultraviolet
V	Volts
v	Volume
W	Watts

INTRODUCTION

INTRODUCTION

OBJECTIVES

1. To introgress shootfly resistance QTLs from resistant source IS18551 into the genetic backgrounds of two shoot fly susceptible maintainer lines, *Viz.*,BTx623 and 296B.
2. Transfer of shoot fly resistant QTLs directly from four recombinant inbred line sources from the mapping population of BTx623 x IS18551, *Viz.*, RIL 153. RIL252, RIL 189 and RIL 166.
3. Field evaluation of the near isogenic pairs for shoot fly resistance in *kharif* and *rabi* seasons.

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop globally in terms of production and utilization (FAO, 2004), after wheat, rice, maize and barley. Sorghum grain is produced annually as dietary staple for millions of people in semi-arid areas of Asia (mainly India) and Africa where drought stress causes frequent failures of other crops. Global cultivation of sorghum covering an area of 42.6 million hectares (FAO, 2004) (more than 80 countries) with annual production of 58.5 million tones (FAO, 2004). India is a major producer of sorghum with the crop being grown in an area of 8.6 million ha with an annual production of 7.2 million t and 835 kg ha^{-1} (FAO, 2006). Sorghum grain yields on peasant farms generally are low, ranging from 500 to 800 kg ha^{-1}. Lower yields of sorghum have been attributed to a number of factors; among them the loss caused by the insect pests has been considered as one of the important factors. Nearly 150 insect species have been reported as pests on sorghum (Reddy and Davies, 1979; Jotwani *et al.*, 1980). The sorghum shoot fly, *Atherigona soccata* Rondani, is the most destructive one among the major different insect pests that attack sorghum. It causes maximum yield losses of 75.6% in grain and 68.6% in fodder as reported by Pawar *et al.* (1984). Sorghum shoot fly is a widespread pest in Asia, Africa, and Mediterranean Europe but is absent from the Americas and Australia.

Shoot fly females lay cigar-shaped eggs individually on the lower surfaces of seedling leaves at the 1-7 leaf stage, *i.e.* 5-25 days after seedling emergence. Eggs hatch in 1 or 2 days and the larvae move along the shoot to the apical meristem. The first instar larvae feed on the growing point, cutting the base of the central leaf whorl, which results in wilting and drying of the central leaf known as a "dead heart". The dead heart produces a bad smell and can be pulled out of

the whorl easily. Larval development is completed in 8 to 10 days and pupation usually takes place in the soil. The pupal period lasts for 8 days. The entire sorghum shoot fly life cycle is completed in 17 to 21 days. Normally shoot fly damage occurs one week to four weeks after seedling emergence. The damaged plants produce side tillers, which may also be attacked (Singh and Rana, 1986). Damage caused by the shoot fly ranged from 9.22 to 60.82 percent in CSH-5 and from 60 to 95.33 percent dead hearts in CSH-8R (Mote, 1989). In some instances seedlings are killed while some plants recover and yield normally (Dogget, 1970). The sorghum shoot fly population increases in July after early rainy season sowings of the sorghum crop, peaks in August to September and declines thereafter. Shoot fly infestations are normally high in the postrainy season crop that is sown from late September into October.

Cultural practices can greatly affect the level of attack by shoot fly. In particular, it is known that sowing date exerts a very great influence on the damage by the pest. Sowing immediately after the onset of rains, preferably within a period of two weeks in any area, is therefore recommended (Jotwani *et al.*, 1970). A commonly recommended age-old cultural practice is sowing sorghum with a high seed rate (10-15 kg ha^{-1}) and later thinning out the infested plants (Gahukar and Jotwani, 1980). Infested seedlings should be well buried or burned after they are removed. During the 1981-82 postrainy season at ICRISAT, plots of CSH 5 thinned 30 days after emergence, suffered less shoot fly infestation than plots thinned 10 days after emergence. Shoot fly damage is higher when plant populations are low (Davies and Reddy, 1981). Recently, it was found that early sowing with high seed rate @ 10 kg ha^{-1} and thinning at 28 DAE of seedling is a superior method to chemical treatment of seed with endosulfan (0.07%), neem leaf spray (5%) and whorl application of carbofuran 3G at 10DAE, and sowing with high seed rate (10 kg ha^{-1}) followed by whorl application of carbofuran 3G @ 18.5 kg ha^{-1} (Shekkarappa and Bhuti, 2007).

Vedamoorthy *et al.* (1965) carried out trials at different centers with seed and seed furrow applications of a number of insecticides including lindane, dieldrin, metasytox, phosphamidon, dimethoate, isolan and phorate. They found phorate 10% granules applied at 1.5 to 2.0 kg ha^{-1} in the seed furrow at sowing time, to be an outstanding treatment at most locations. In subsequent trials, disulfoton, another systemic organo-phosphate insecticide was also found to be equally effective (Anonymous, 1965-67, 1968-69; Sadhu, 1969). These two insecticides were also reported to give effective control of the shoot fly in Israel (Yathom, 1967). Unfortunately, due to high cost of these insecticides and the cumbersome process of mixing the granules with soil in the furrows, which is necessary to

avoid injury to seeds, cultivators in general, have not taken advantage of these findings.

Research was undertaken at the Indian Agricultural Research Institute in 1968 for the discovery of an effective, convenient and economical insecticide for sorghum shoot fly control. Four different insecticides were tested and one of these (carbofuran – a systemic carbamate insecticide gave very encouraging results (Jotwani and Sukhani, 1968). Subsequently a number of trials were carried out in the All India project at Delhi and other centers. Based on these results a recommendation was made for the use of carbofuran seed treatment at 5 parts A.I. carbofuran per 100 parts of sorghum seed. Carbofuran as seed treatment and granules applied in the seed furrow has also given effective control in Thailand (Boonson *et al.*, 1970). Seed soaking with endosulfan 35EC at 0.07% for 4 hours decreased shoot fly incidence (Hiremath *et al.*, 1992). The present recommendation of soil application of carbofuran 3G @ 30 kg ha^{-1} is an expensive proposition in view of high cost of insecticide (Anonymous, 1992). The effectiveness of different chemicals to this pest has been reviewed by Gahukar (1991) and Hiremath *et al.* (1995).

In general, management of sorghum pests exclusively by application of insecticides fails to ensure control (slow decline) and often increases pest incidence instead (Jotwani and sukhani, 1982). The chemical method of shoot fly control is also not cost effective and feasible to the poor sorghum-growing farmers of SAT (Sukhani and Jotwani, 1982). Therefore management of pests by other methods, limiting the use of insecticides, deserves particular attention. A number of genotypes with resistance to shoot fly have been identified, but the levels of resistance are low to moderate (Sharma *et al.*, 2003a). Plant resistance to sorghum shoot fly appears to be a complex trait (quantitative) and depends on the interplay of a number of component characters (Dhillon, 2004).

Non-preference is the predominant mechanism for resistance to shoot fly and it is quantitatively inherited, mostly through additive gene action (Rao *et al.*, 1974; Sharma *et al.*, 1977). Rana *et al.*, (1981) reported that the F_1 generation is almost intermediate between its two parents for shoot fly resistance. Resistance was found to be partially dominant under low to moderate shoot fly pressure but not under heavy levels of shoot fly infestation. Most resistant varieties have glossy leaves in the seedling stage (Maiti *et al.*, 1984). In addition, the majority of shoot fly resistant sorghum cultivars have a high density of leaf trichomes. Maiti and Bidinger (1979) noticed that trichomes on the abaxial surface of leaf deterred egg laying. Omori *et al.*, (1983) suggested that glossiness expression in

sorghum seedlings could be utilized as a simple and reliable selection criteria for shoot fly resistance.

Physico-chemical traits such as leaf glossiness, trichome density, and plumule and leaf sheath pigmentation were found to be associated with resistance, and chlorophyll content, leaf surface wetness, seedling vigour, and waxy bloom with susceptibility to shoot fly and explained 88.5% of the total variation in dead heart incidence (Dhillon, 2005). He further indicated through step-wise regression that 90.4% of the total variation in dead heart incidence was due to leaf glossiness and trichome density. The direct and indirect effects, correlation coefficients, multiple and step-wise regression analysis suggested that dead hearts, plants with eggs, leaf glossiness, trichomes on the abaxial surface of the leaf, and leaf sheath pigmentation can be used as marker traits to select for resistance to shoot fly, *A. soccata* in sorghum (Dhillon, 2005).

Host plant resistance is one of the most effective means of pest management in sorghum. It is compatible with other methods of pest control, does not involve extra cost for the farmers and is environmentally friendly. In spite of the importance of host plant resistance as a component of integrated pest management breeding, host plant resistance has not been rapidly accepted. This is because conventional host plant resistance to insects involves quantitative traits at several loci and as a result the breeding programs to incorporate this into high-yielding genetic backgrounds having high quality produce have been slow (as one needs to evaluate large numbers of progenies to be able to select the plants with appropriate trait combinations) and the goals of these breeding programs have been difficult to achieve. Thus the development of shoot fly resistant varieties via conventional breeding has been hampered by the complex, quantitative inheritance of resistance against shoot fly and the occurrence of strong genotype × environment (G×E) interactions for resistance. The application of DNA markers and mapping technology might facilitate breeding for shoot fly resistance. Recently, SSR markers have been found to be linked to shoot fly resistance traits [Ph.D. theses of Sajjanar (2003), Deshpande (2005) and Mehtre (2004)]. This project focused on backcrossing with marker-assisted selection of putative shoot fly resistance QTLs from donor source IS 18551 to move these into the genetic backgrounds of agronomically elite shoot fly sensitive seed parent maintainer lines BTx623 and 296B, and testing the resulting QTL introgression lines in order to validate these shoot fly resistance QTLs. The QTL introgression lines obtained were evaluated for shoot fly resistance in the BC_4F_3 generation.

To identify genes or QTLs for shoot fly resistance by their linkage to DNA markers, two lines differing widely for resistance reaction and marker genotype

must be crossed and the progeny selfed for one or more generations. Due to recombination and segregation of the various resistance genes, the resulting mapping population (F_2 or any further inbred generation of so called recombinant inbred lines or RILs) will reveal a wide range of resistance reaction, from very susceptible to highly resistant. The population is evaluated for shoot fly resistance and concurrently the DNA profile of each individual plant or line is examined in the laboratory for marker loci. Data are then analyzed for co-segregation, *i.e.*, linkage of the DNA markers with the resistant phenotype, in order to identify genomic regions that contribute significantly to variation in host plant resistance reaction.

The advantage of using DNA markers in resistance breeding depends on finding reasonably close linkage between markers and the resistance genes of interest. The presence of a resistance gene(s) can be inferred by identifying the resistance donor DNA marker allele that is linked to resistance instead of evaluating the segregating materials for resistance. Selection in early segregating generations can then be done in the absence of the insect, saving money and gaining time. Use of many markers evenly distributed within the genome, large mapping populations, large phenotypic distance between the parents, and high heritability of the trait of interest all contribute to accurate location of QTLs. Where target trait heritabilities are low, multilocational testing and many replications can partially compensate for low heritability.

QTL analysis has identified several regions of the genome that appear to influence the expression of shoot fly resistance. However this analysis provided only limited information concerning the expression of individual QTLs. Sets of Near-Isogenic Lines (NILs) that differ at specific QTLs but otherwise share a common genetic background can be used to carefully evaluate the phenotypic expression of individual QTLs, and to validate the presence of QTLs in specific genomic regions. In this study NILs have been developed in two different genetic backgrounds for four genomic regions putatively associated with shoot fly resistance. Initial evaluations have revealed significant phenotypic differences for shoot fly resistance between NILs contrasting at these QTLs. The evaluation of QTLs in NILs can be used to address several questions. First, marker linkage to a QTL can be confirmed by examining the phenotypes of NILs that differ for markers flanking individual putative QTLs. QTL analysis indicates regions of the genome that may contain QTLs but the phenotypic effects of these genomic regions need to be confirmed. Second, NILs can be used for fine-mapping of QTLs. Evaluation of a series of NILs that contrast at a specific locus can be used to narrow the

genetic interval known to contain the QTL (Paterson *et al.*, 1990). Third, NILs that differ at a QTL can be used to characterize the expression and function of a specific locus. NILs differing for QTLs associated with shoot fly resistance can be used to identify the specific mechanisms of shoot fly resistance controlled by each QTL.

The QTLs governing shoot fly resistance (SFR) are being transferred from IS 18551 (shoot fly- resistant donor) into the genetic background of two elite maintainer lines 296B and BTx623 (hybrid seed parents of several popular hybrids such as CSH 1, CSH 9, CSH 10, CSH 11, CSH 13, and CSH 13R). Parent IS 18551 was the donor of alleles conferring resistance to sorghum shoot fly. The recurrent parents BTx623 and 296B are more susceptible to shoot fly. The backcross seedlings from each of the backcross generations were used for tissue sampling for DNA isolation and marker analysis. The crossed seeds produced on selected individuals were advanced for further backcrossing. Full marker-assisted selection was used to select plants carrying IS 18551 alleles at markers flanking shoot fly resistance QTL target regions and BTx623 or 296B alleles at markers in the non-target regions in the BC_1F_1, BC_2F_1, BC_3F_1, BC_4F_1 and BC_4F_2 generations. In each generation up to BC_4F_1, progenies with the heterozygous condition in the target regions of linkage groups SBI-01, SBI-07, SBI-10, short and long arms of SBI-05 and homozygous for BTx623 or 296B alleles in the remaining linkage groups (non-target regions) were selected. BC_4F_2 plants with homozygous alleles for target region were selfed and selected BC_4F_1 plants backcrossed to produce BC_5F_1 generation. Five near-isogenic line (NIL) pairs were field screened in the rainy season of 2006, the postrainy season of 2006/07, and the rainy season of 2007 at ICRISAT-Patancheru.

Interlard fish meal technique was used for shoot fly resistance screening. For this, an adequate level of shoot fly infestation was achieved by manipulating the sowing date, using infester rows ("interlards") and spreading fish meal (which attracts the shoot flies) in the field. At ICRISAT-Patancheru, sowing the test material in mid-July in the rainy season and during October in the postrainy season allows effective screening for resistance to shoot fly. The interlard fish meal technique, is useful for increasing shoot fly abundance under field conditions, involves sowing sets of four rows of a susceptible sorghum cultivar (CSH 1 or CSH 5), approximately 20 days before the sowing of test material in sets of 12 to 20 rows between each interlard of the susceptible sorghum cultivars. Fishmeal is spread uniformly one week after seedling emergence of these susceptible "interlards" or kept in plastic bags in the interlards to attract shoot flies from the surrounding areas. One generation of the shoot fly is completed on

the interlards and the emerging flies then infested the test material (Taneja and Leuschner, 1985a; Sharma *et al.*, 1992). To evaluate the damage caused by sorghum shoot fly, the number of eggs, plants with eggs, plants with dead hearts, and the total number of plants at 14 and 21 days after seedling emergence are recorded. Glossiness and trichome densities were recorded as indirect traits contributing to the percentage of dead hearts caused. The total number of tillers, number of tillers with productive panicles at maturity, grain yield under protected and unprotected conditions can also be recorded as a measure of genotype recovery ability. The field evaluation of the introgression lines was performed in two seasons, Kharif (rainy season 2006) and Rabi (postrainy season 2006/07) and a confirmatory trial was conducted in Kharif 2007. Significant and substantial differences were observed between introgression lines and their near-isogenic recurrent parent for shoot fly deadhearts incidence, seedling glossiness score, and seedling leaf blade trichome density. Association of genomic regions with shoot fly resistance characters confirmed a role of the genomic regions between markers on linkage group SBI-05 – *Xisp*258 to *Xtxp*15; on linkage group SBI-10 – *Xtxp*141 to *Xgap*1; but failed to confirm the roles of genomic regions on linkage group SBI-01 – *Xtxp*37 to *Xtxp*75 and on linkage group SBI-07 – *Xtxp*159 to *Xtxp*40. The genomic region *Xisp*258 to *Xtxp*65 on the short arm of chromosome SBI-05 is linked with the expression of glossiness and *Xtxp*15 on the long arm of this chromosome is linked with seedling vigor. The QTLs on linkage group SBI-10 are associated with resistance factors seedling vigor, glossiness and ad-axial, ab-axial trichome densities. However introgression lines for QTLs on SBI-01 and SBI-07 have given unexpected results in kharif-*06* and Rabi-*06* seasons. When the experiment was repeated in Kharif 2007, this unexpected behavior in the SBI-01 and SBI-07 QTL introgression lines was not observed, suggesting that these QTLs have minor effects that are readily overcome under heavy levels of shoot fly infestation. Increasing the plot size and number of replications can compensate such QTL effects.

LITERATURE REVIEW

REVIEW OF LITERATURE

Sorghum is an important self-pollinated cereal crop in Asia, Africa, the Americas, and Australia. Sorghum grain yields on farmers' fields in Asia and Africa are generally low (500–800 kg ha^{-1}), mainly due to low soil fertility, insects (Dogget, 1988), disease, weeds, and drought. Nearly 150 insect species have been reported as pests on sorghum (Reddy and Davies, 1979a; Young and Teetes, 1977; Jotwani et al., 1980; Sharma, 1993). Sharma (1993) listed 43 insect and mite species as important arthropod pests of sorghum, and indicated the damage, economic importance and distribution of these pests. *Atherigona soccata* (Rondani) is one among the major sorghum insect pests (total eight).

Sorghum shoot fly was first reported and named by Rondani (1871), but its injury to sorghum seedlings was first recognized by Fletcher (1914) and Ballard and Ramachandra Rao (1924). Sorghum shoot fly completes its life cycle in 19 days (Swaine and Wyatt, 1954). Shoot fly infestation causes 'deadheart' symptoms in the seedling shoot, as well as in tillers, resulting in considerable damage to the crop. Sorghum shoot fly causes an average loss of 50% in India (Jotwani, 1982) but the infestations at times may be over 90% (Rao & Gowda, 1967). In addition to cultivated sorghum, shoot fly also attacks several wild graminaceous plants in various parts of Africa (Deeming, 1971). *Sorghum verticilliflorum* is reported as a common wild host of *A. soccata* in East Africa (Nye, 1960). Ogarvo (1978b) reported that *Sorghum bicolor* was markedly preferred in Kenya to other graminaceous plant species. In India, Davies and Seshu Reddy (1980) reared shoot flies on 21 (17 wild, 4 cultivated) species of graminaceous hosts and noticed that *Sorghum halepense* was most important alternative host followed by *S. verciliflorum, S. almum, S. vigatum* and *Echinocloa colonum* and to a lesser extent *S. sudanense*. Granados (1972) found that, in Thailand, the shoot fly could complete development on only *Brachiara reptans*, which is least preferred among *Digilaria asandens* and *Eleusine indica*. These observations suggest that sorghum shoot fly seems to require more than one host and that the wild hosts serve to maintain small populations of shoot fly, which do not build up until cultivated sorghum is available. However, Delobel and Unnithan (1981) observed that shoot fly populations are usually higher on wild sorghum.

Ogwaro (1978b) stated that before ovipositing, the female sorghum shoot fly moves from plant to plant and leaf to leaf, probing the leaf surface with her fore

legs and hind legs, as well as with her ovipositor. When finally the right plant and oviposition site is selected, she presses the ovipositor against the leaf surface and lays her eggs (white, cigar shaped), usually parallel to the midrib. As the eggs are extruded, the female moves upwards, vibrating the ovipositor while at the same time pressing it against the leaf surface. This ensures that the eggs are cemented firm on the surface. Unlike in field conditions, under controlled no-choice conditions where there is a shortage of oviposition substrate (2 or 3 plants per cage), many eggs may be laid in a line and sometimes close and parallel to each other on the same leaf. In contrast, the female has the opportunity to select several different plants and leaves for oviposition under field conditions. Field and laboratory observations of Delobel (1981) revealed that placement of eggs of the sorghum shoot fly on sorghum seedling leaves tended to be random or slightly aggregated rather than regular, which suggest that site of oviposition by a female is little or not affected by the presence of other eggs already laid.

According to Delobel (1982), females laid more eggs on sorghum plants measuring 4 to 8 cm in height (in the field) or 12 to 16 cm (under cages) than on plants of any other size. Newly hatched larvae survived only on plants measuring less than 24 cm in height. Survival of the first instar larvae depended on the size of the host plant. Larval survival was influenced by the resistance to penetration of the leaf sheaths and the distance between infestation site, in the case of artificial infestation and growing point. Larval survival therefore depends on the ability of the female sorghum shoot fly to select for oviposition a leaf of suitable position.

The choice of oviposition sites is different under field and insectary conditions. In the insectary, the second leaf was most preferred for oviposition followed by the third, first and fourth with 52.5, 28.6, 16.8 and 2.1% of the total number of eggs deposited, respectively. In the field, the third leaf was most preferred followed by the second, fourth, fifth, sixth, first and then seventh leaf, with 54.1, 28.5, 13.3, 3.2, 0.5, 0.4 and 0.1% of total eggs deposited (Ogwaro, 1978b). After egg hatch, the larvae crawl to the seedling leaf whorl and move downward between the folds of the young leaves till they reach the growing point. They cut the growing tip resulting in deadheart formation. It takes nearly 1 day for deadheart formation after egg hatching.

The incidence of sorghum shoot fly is highly seasonal. The populations are extremely low during the dry period and the beginning of the following rainy season and thus early-sown sorghums escape or are less severely injured than late-sown crops (Ponnaiya, 1951a; Rivnay, 1960; Davies and Jowett, 1966;

Usman, 1968; Starks, 1970; Wongtong and Patanakamjorn, 1975; Clearwater and Othieno, 1977). Jotwani et al. (1970) observed that sorghum shoot fly populations had two peaks in India — one during March and April and the other during the months of August to October. It was indicated that shootfly activity was adversely affected mainly by extremes of temperatures, the maximum ranging between 30°C and 44°C (May and June) and the minimum between 2°C and 14°C (from November to middle of February). From July 4 to August 14 there was an increase in the incidence of shootfly (Ram et al., 1976). According to Kundu et al., (1971) there was only one peak period of infestation of shootfly during the months of August to October. Activity of the shoot fly was adversely affected mainly by extremes of temperature, the maximum ranging between 36°C and 40.6°C and the minimum between 3.5°C and 18°C. Based on the preliminary observations it is believed that hybrid Jowar CSH 1, when sown during March - June, would suffer least from shoot fly attack where as July - February sowings would subject the crop to varying degrees of shoot fly infestation.

The adoption of chemical methods for shoot fly control is not economically feasible for most sorghum-growing farmers. Therefore, utilization of host plant resistance combined with early sowing in the rainy season and late in the postrainy season are the most realistic alternative methods for reducing the losses caused by sorghum insect pests. Resistance of plants to insects is the consequence of heritable plant characters that result in a plant being relatively less damaged than plants without these characters (Sharma, 1997). Even though the genetic variability for shoot fly resistance is available in the sorghum germplasm, the level of resistance is not high and those cultivars with the highest levels of resistance have poor agronomic features. Genetic manipulation by conventional breeding procedures, to increase the resistance in agronomically superior backgrounds, is hindered by the complexity of inheritance of the resistance character due to its quantitative inheritance, and environmental interaction. Previous studies have revealed a number of component traits that are associated with shoot fly resistance.

Genetic manipulation of complexly inherited, environmentally-sensitive characters like shoot fly resistance by conventional breeding methods faces many additional difficulties including crossing barriers and transfer of undesired traits along with genes of interest. This difficulty in conventional breeding for such traits has been overcome by recent advances in molecular marker technology, which has been demonstrated in several crops. This readily feasible approach involves first

tagging genomic regions conferring resistance, then using molecular markers linked to such traits as selection aides during the segregating generations of crosses involving derivatives of the mapped resistance source. Quantitative trait locus (QTL) analysis, or the dissection of quantitative traits into Mendelian factors of inheritance, provides a powerful tool for identifying genomic regions with both major and minor effects, as well as characterizing the environmental interactions associated with each of them. It also enables the potential transfer of linkage blocks (set of genes) important for resistance to desirable lines. Thus selection for markers linked to chromosomal regions associated with favorable alleles conferring enhanced resistance has the potential to improve the efficiency of manipulation of quantitatively inherited insect resistance in plant breeding programs.

1.1 Shoot fly Resistance and its Genetic analysis

1.1.1 Shoot fly Control

In general control of sorghum shoot fly can be achieved by early and or timely sowing, increased seed rate, thinning and destroying the seedlings with dead hearts, crop rotations and fallowing, and other methods like use of insecticides (Singh and Sharma, 2002).

1.1.1.1 Chemical control

Vedamoothy et al. (1965) found phorate 10% granules applied at 1.5 to 2.0 kg ha^{-1} in the seed furrow at sowing time, to be an outstanding treatment at most locations and disulfoton, another systemic organo-phosphate insecticide was also found to be equally effective (Anonymous 1965-1976, 1968-1969; Sandhu, 1969; Sandhu and Young, 1974). Unfortunately, due to high cost of these insecticides and the cumbersome process of mixing the granules with soil in the furrows, which is necessary to avoid injury to seeds, cultivators in general, have not taken advantage of these findings. Research was undertaken at the Indian Agricultural Research Institute in 1968 for the discovery of an effective, convenient and economical insecticide for sorghum shoot fly control. Four different insecticides were tested and one of these (carbofuran — a systemic carbamate insecticide) gave very encouraging results (Jotwani and Sukhani, 1968). Subsequently a number of trials were carried out in the All India project at Delhi and other centers (Jotwani et al., 1971). Based on these results a recommendation was

made for the use of carbofuran seed treatment at 5 parts A.I. carbofuran per 100 parts of sorghum seed.

The efficacy of the insecticides decreased after third week following germination and the dosage required for effective control and consequently the cost involved is so high (Sukhani and Jotwani, 1982). The use of insecticides as seed treatment was recommended by Balasubramanian et al., (1987), but was later withdrawn considering the hazards associated with it (Patil et al., 1992). Patil et al., 1992 found seed soaking with endosulfan — 35EC at 0.07 percent concentration for 4 h effective in decreasing the deadheart percentage. Effect of seed treatment on sorghum with some new insecticides for control of shoot fly was studied (Manorama Sharma et al., 1996) with imidachlorprid, carbofuran, curacron and carbosulfan, and indicated that imidachloprid (Hiremath et al., 1995) is the most effective to control shoot fly attack.

1.1.1.2 Cultural control

1.1.1.2.1 Fertilizer and Nutrient balance

During the 1983 rainy season, unfertilized plots of CSH 1 at ICRISAT Center suffered heavy shoot fly damage compared with fertilized plots. Nitrogenous fertilizers are reported to decrease *A. soccata* Rond. incidence in sorghum (Reddy and Narasimharao, 1979; Chand et al., 1979) possibly by increasing plant vigor. However, Kundu et al. (1978) observed no effect of nitrogenous fertilizers on shoot fly damage. Channabasavanna et al. (1969) reported a decrease in shoot fly damage after the application of phosphatic fertilizers, but Rajashekhara et al. (1973) found no such evidence. These reported differences in fertilizer response may be due to genotypic variation or sowing date variation across the experiments.

1.1.1.2.2 Plant density: The traditional practice of using a high seeding rate helps to maintain optimum plant stands and reduce *A. soccata* damage. During the 1981-82 postrainy season at ICRISAT, plots of CSH 5 thinned 30 days after emergence, suffered less shoot fly infestation than plots thinned 10 days after emergence. Shoot fly damage is higher when plant populations are low (Davies and Reddy, 1981 unpublished).

1.1.1.2.3 Time of sowing: Sowing time considerably influences the extent of insect damage. From previous studies (Jotwani et al., 1970), it has been

established that in rainy season shoot fly incidence and damage increases with delay in sowing date. Early sowing may help to reduce its menace. However, in some areas and under certain situations early sowing is not feasible. Water logging hampers sorghum seedling growth, increasing vulnerability to sorghum shoot fly and resulting in an increase in deadhearts (Men et al., 1986). Sorghum hybrids do well under irrigation. However, shoot fly oviposition and deadhearts incidence are higher in treatments with full irrigation than in treatments to which less water was applied during the first 3 weeks after seedling emergence (Nwanze et al., 1996).

1.1.1.3 Biological control

It was found that there was emergence of parasites from sorghum shoot fly larval material collected in the months of August, September and October. The predominant parasite on *Atherigona varia soccata* Rond. was *Aprostocetus* sp. and a few specimens of *Callitula* (Kundu et al., 1971). Predators and parasites reduce egg survival, but their effectiveness is usually limited (Taley and Thakare, 1979). The sorghum shootfly has a wide range of natural enemies (Zongo et al., 1993a) including *Neotrichoporoides nyemitawus,* which is a widespread effective endo-larval parasitoid. Weekly inundation of sorghum sowings with egg-parasite *Trichogramma chilonis* gave encouraging results in effectively reducing the deadhearts percentage caused by shoot fly (23%) compared to the untreated control (95%). However, being an ectoparasite, its effective periodical release requires constant monitoring for inundative releases during stages of the crop susceptible to shoot fly and stem borer (Singh and Rana, 1996). Although 15 species of predators have been recorded, their predation potential has not been assessed under field conditions. Several species of spiders are important predators on eggs (Singh and Sharma, 2002). Among the parasitoids, *Trichogramma chilonis* Ishii and *Trichogrammatoidea simmondsi* Nagaraja on the eggs, and *Neotrichoporoides nyemitawus* Rohwer on the larvae are most important.

Effects of neem kernel extracts on egg and larval survival of *A. soccata* were studied by Zongo et al. (1993b), who observed significantly fewer eggs and dead hearts in plots treated with neem extracts. Kareen (1974) pointed out that neem kernel extracts at a rate of 10 and 5 kg kernels/ha, respectively, caused 20 to 27% less shoot fly damage than an unsprayed control. The damage to sorghum could be reduced if the shoot fly could be diverted into laying eggs on maize or other suitable non-host plants grown with the sorghum. The shoot fly doesn't

normally lay eggs on maize seedlings and even if it does, the larvae cannot survive or develop on this crop (Raina and Kibuka, 1983). A shoot fly ovipositional stimulant, previously reported (Unnithan et al., 1987) in the acetone extract of the seedlings of the susceptible sorghum CSH 1 extracts, elicited significantly greater oviposition (67-91%) on treated maize seedlings than (9-33%) on untreated controls (Unnithan and Saxena, 1990).

Recently, it was found that early sowing with high seed rate at 10 kg ha^{-1} and thinning seedlings (removing and destroying those damages by sorghum shoot fly) at 28 DAE is superior to 1) sowing with normal seed rate 7.5 kg ha^{-1} combined with soaking in endosulfan solution (0.07%) for 8 hours followed by seed treatment with calcium chloride (2%), 2) sowing with normal seed rate followed by 5% spray of neem leaf at 10 DAE, 3) sowing with normal seed rate followed by whorl application of Carbofuran 3G at 10 DAE, 4) sowing with high seed rate (10 kg ha^{-1}) followed by whorl application of carbofuran 3G at 18.5 kg ha^{-1} and 5) untreated check (Shekkarappa and Bhuti, 2007).

Table 1. Natural enemies on *Atherigona soccata* Rond. (Sharma, 1985)

Scientific name	Stage attacked	Reference
Abrolophus sp.	Preys on larvae	Reddy and Davies (1979)
Aprostocetus sp.	Larval parasite	Pradhan (1971), Kundu et al. (1971a), Kishore et al. (1976, 1977b), Jotwani (1978)
Callitula bipartitus Frq.	Larval parasite	Kundu et al. (1971b)
Callitula sp.	Larval parasite	Pradhan (1971), Kishore et al. (1977b), Jotwani (1978)
Crataepiella sp.	Pupal parasite	Feddy and Davies (1979)
Diaulinopsis sp.	Larval parasite	Kishore et al.(1977b), Jotwani(1978)
Ganaspis sp.	Larval parasite	Kishore et al. (1977b), Shivpuje (1977), Jotwani (1978)
Hemiptarsensus sp.	Larval parasite	Kishore et al. (1977b), Jotwani (1978)
Monelta sp.	Larval parasite	Taley and Thakare (1979), Taley (1978)
Odonteucoila sp.	Larval parasite	Shivpuje (1977)
Psilus sp.	Larval parasite	Kishore et al. (1977b)
Rhodtromeris sp.	Larval parasite	Taley and Thakare(1979), Taley (1978)
Spalangia indicus Walk.	Larval parasite	Taley and Thakare(1979), Taley (1978)
Tetrastichus nyemitawus Roh.	Larval parasite	Reddy and Davies (1979), Taley (1978)
Tetrastichus sp.	Larval parasite	Raodeo et al. (1972), Kishore et al. (1976)
Trichogramma austracicum Gir.	Egg parasite	Taley (1978)
Trichogramma japonicum Ashm.	Egg parasite	Anonymous (1981)
Trichogrammatoidea sp.	Egg parasite	Anonymous (1981)
Trichopria sp.	Larval parasite	Taley and Thakare (1979), Taley(1978)

1.1.1.4 Host plant resistance

The use of resistant varieties may offer the best (and perhaps only) economical method of control of certain pests like sorghum shoot fly because the control of insects on a crop of low value per unit area precludes the use of insecticides (Dahms, 1943). In rainfed agriculture, the sowing date cannot be manipulated to avoid pest damage, so sowing pest resistant cultivars is especially useful under subsistence farming conditions of the semi-arid tropics (Davies, 1981). 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 from insect populations that would cause greater damage to other plants of the same species under similar environmental conditions. Painter (1951) defined resistance in plants to insect attack as the relative amount of heritable qualities possessed by the plant that influence the ultimate degree of damage done by the insect or reduce the probability of successful utilization of the plant by the insect (Beck, 1965). Over the past five decades, a large proportion of the world sorghum germplasm collection has been evaluated for resistance to insect pests, and a number of lines with resistance to major insect pests have been identified (Sharma et al., 1992, 2003). Large-scale screening of the sorghum germplasm at ICRISAT has resulted in identification of several lines with reasonable levels of resistance to shoot fly and other pests (Table 2).

1.1.1.4.1 Resistance sources

It was established first by Ponnaiya (1951a) that genetic differences exist for resistance to sorghum shoot fly, permitting identification of resistant cultivars; most shoot fly resistant sorghums identified from peninsular India. Several sorghum lines with resistance to shoot fly have since been reported (Rao and Rao, 1956; Blum, 1967; Singh et al., 1968; Young, 1972; Jotwani, 1978; ICRISAT, 1978), although the levels of resistance available are not sufficient to prevent considerable loss in crop stands when infestation levels are high. The world germplasm of sorghum has since been screened for reaction to sorghum shoot by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India (Sharma et al., 1992b, 2003).

1.1.1.4.2 Phases of Resistance: Painter (1951) distinguishes two 'mechanisms' of resistance in the strict sense; *non preference*, where insect behavior is influenced to lead away from plant utilization and *antibiosis* where plant utilization has adverse effects on the insect life history. Saxena (1969) regarded these terms as imprecise and suggested that resistance should be considered at successive phases of the insect/plant relationship.

Table 2. Germplasm accessions identified to be resistant to sorghum shoot fly in sorghum.

Insect Pest	Germplasm accessions (IS numbers)
Shoot fly	923, 1032, 1034, 1037, 1044, 1054,1071, 1096, 1104, 1119, 2122, 2123, 2146, 2162, 2168, 2195,2205, 2265, 2269, 2291, 2309, 2312, 2394, 2681, 3461, 3962, 4224, 4273, 4646, 4663, 4664, 4835, 4881, 4981, 5075, 5076, 5078, 5210, 5429, 5469, 5470, 5480, 5484, 5490, 5511, 5538, 5566, 5571, 5604, 5613, 5619, 5622, 5636, 5648, 8064, 8100, 8320, 8571, 8721, 8811, 8887, 8891, 8918, 8922, 8988, 9009, 9692, 6566, 10711, 10795, 12150, 13674, 14108, 15437, 15896, 16235, 16357, 7726, 17742, 17745, 17747, 17750, 17948, 17966, 18274, 18325, 18366, 18368, 18369, 18371, 18476, 18551, 18580, 18635, 18662, 18700, 18733, 19485, 19569, 19706, 20064, 21871, 21877, 21969, 22039, 22114, 22121, 22144, 22145, 22148, 22149, 22196, 25744, and 26789.
	Improved lines with resistance to insects (ICSV numbers)
Shoot fly	700, 701, 702, 705, 707, 708, 711, 712, 713, 714, 717, 726, 89013, 89018, 89025, 93093, and 25001-25055.
Agarwal, B.L., H.C. Sharma, S.L. Taneja, B.V.S. Reddy, and J.H. Stenhouse (Unpublished)	

1.1.2 Mechanism of Resistance

The major mechanisms of resistance to sorghum shoot fly that are so far known are ovipositional non-preference or oviposition antixenosis, antibiosis, and tolerance. Thus all three resistance mechanisms suggested by Painter (1951) are known to exist in sorghum for shoot fly resistance. The primary mechanism of

resistance to sorghum shoot fly has been observed to be non-preference for oviposition and perhaps a low level of antibiosis to the larvae (Soto, 1971). Blum (1971) attributed resistance to a cumulative effect of non-preference, antibiosis and some morphological factors.

1.1.2.1 Non-preference for Oviposition (Oviposition antixenosis)

The term antixenosis (Kogan and Ortman, 1978) was proposed to replace the term non-preference proposed by Painter (1951). It describes the inability of plant to serve as host to an insect herbivore. It may be due to morphological or chemical plant characters that affect the insect behavior adversely, resulting in selection of alternative host plants. Jain and Bhatnagar (1962) screened 196 sorghum varieties from the world collection and reported significantly less oviposition on resistant varieties than on susceptible ones. Later, Blum (1967) also found that a susceptible sorghum variety was preferred for oviposition so non-preference for oviposition is probably an important mechanism of resistance.

The female sorghum shoot fly starts to lay eggs from the appearance of the first seedling leaf onwards. Very rarely eggs can be seen on cotyledonary leaves also. The flies prefer usually the third and fourth leaves most, and egg laying is reduced beyond the fifth leaf (Krishnananda et al., 1970). Their observations of oviposition on 19 varieties suggested that the fly was capable of discriminating between the varieties for oviposition. Raina (1982) also observed the middle region of the abaxial surface of the 4^{th} leaf of a 5-leaf stage plant was preferred for oviposition. Soto (1974) screened exotic and Indian sorghum varieties and found that the female fly showed strong preference for ovipositing on exotic varieties. On the more resistant varieties, the average number of eggs deposited per plant was less than 1, whereas on susceptible genotypes, up to 2.33 eggs per plant were found (Narayana, 1975). Total numbers of eggs laid on resistant lines varied from 0.76 to 1.03 eggs per plant in contrast to susceptible genotype Swarna, where 5.13 to 5.73 eggs per plant were observed (Jotwani et al., 1971). The mean daily rate of oviposition per female was 13.5 eggs with maximum of 41 eggs (Ogwaro, 1978a).

Oviposition was more on highly susceptible varieties than on susceptible and moderately resistant groups and an increase in the age of the plants at the time of exposure to the pest decreased oviposition and formation of deadhearts (Singh and Narayana, 1978). Singh and Jotwani, 1980a, observed that oviposition by shoot fly was significantly lower on the 15 selected resistant varieties as

compared to susceptible checks, CSH 1 and Swarna. The efficacy of this mechanism is not stable and breaks down under no choice conditions in a cage or under heavy shoot fly pressure in the field (Singh and Jotwani, 1980a; Borikar et al., 1982; Sharma et al., 1997; Taneja and Leuchner, 1984; Dillon, 2005).

Raina et al. (1984) reported that in a single choice test, to study the behavioral resistance it was observed that females exhibited a highly significant non-preference for oviposition on IS 2146, IS 3962 and IS 5613. The first landing by a female was always random, but time spent on these cultivars was very brief and did not result in oviposition. Female flies laid eggs on the non-preferred cultivars only after laying several eggs on alternative CSH 1 plants. None of the test cultivars expressed immunity to shoot fly infestation in either single choice or no choice tests. Thus non-preference resistance appears to be a relative term since none of the known resistant cultivars were completely non-preferred for egg laying (Sharma and Rana, 1983). While one egg can potentially kill the plant of a susceptible variety; the probability of deadheart formation in resistant varieties is relatively lower than that in susceptible ones (Sharma and Rana, 1983).

Dark green colour (Jadhav et al., 1986; Mote et al., 1986) and rough surface (with ridges) were important factors in selection of the oviposition substrate by female flies (Raina, 1982). Cultivars possessing dark green leaf colour with high quantity of HCN (Bapat et al., 1975) and high transpiration rates were preferred for oviposition (Mate at al., 1979). Narrowness and erectness of leaves reduced egg laying and deadhearts incidence as shoot fly had less area for egg laying compared to broad-leaved plants (Mote et al., 1986). Bapat and Mote (1982) reported leaf colour and hairiness (trichomes) as non-preference mechanism. The presence of trichomes on the leaf surface was related to a lesser frequency both of oviposition by the shoot fly and subsequent larval damage (Maiti et al., 1980). Under heavy shoot fly infestation; the density of trichomes appears to make the difference between preference and non-preference for a cultivar (Raina, 1984).

1.1.2.2 Antibiosis

Blum (1967) defined antibiosis, as an 'additional factor of resistance that blocks the path of the larva from the hatching site to the growing apex'. He observed most larvae on resistant plants did not reach the seedling's growing apex. Live larvae found on resistant plants were smaller and reduced in vigor, than those on susceptible plants. Even after the non-preference mechanism was eliminated, some of the varieties when infested artificially exhibited moderate levels of

resistance to shoot fly (Jotwani and Srivatsta, 1970). Blum (1971) made observations in susceptible (6674) and resistant (221) varieties. He checked the seedlings carefully 10 days after oviposition and found significantly fewer larvae in an infested resistant variety (60% plants without larvae) than in an infested susceptible variety (26% plants without larvae). Resistant and susceptible varieties also differed clearly with respect to the site of larval development within the plant.

Soto (1974) observed 90% larval survival on susceptible control variety Swarna compared with 77% and 80% survival on more resistant varieties IS 2123 and M 35-1, respectively. The level of adult emergence on M 35-1 was 54% as compared with 71% and 66% on Swarna and IS 2123, respectively. Though egg deposition was low on varieties IS 1004 and IS 4651, there was a moderated infestation of 50.4% and 55.5%, respectively (Narayana, 1975). The fecundity of shoot flies was higher when reared on susceptible cultivars like Swarna and CSH 1, compared to those reared on moderately resistant ones like IS 2123 and IS 5604 (Singh and Narayana, 1978). These authors also concluded that susceptible varieties are more suitable for growth of larvae and pupae, resulting in reduced lengths of larval and pupal periods. Growth and development were retarded, and the larval and pupal periods were extended by 8-15 days on resistant varieties (Singh and Jotwani, 1980b).

Singh and Jotwani (1980a) observed a high percentage (83%) of the oviposited plants exhibited deadhearts in susceptible variety Swarna but a much lower percentage (45% to 71%) of oviposited plants were similarly affected in resistant varieties. Thus is seems that these resistant varieties possess an inherent resistance mechanism of antibiosis. High mortality of the first instar larvae on IS 2146 and IS 2312 accompanied by a reduced growth among the survivors is a clear indication of post-oviposition factor(s) contributing to resistance (Raina et al., 1981) and larvae in these cultivars were confined to the upper region of the central shoot. Stability parameters for IS 8315 and IS 2123 revealed that the level of oviposition will differ on these two resistant lines under different infestation pressures, but there will be relatively less mortality. This is probably an indirect evidence of antibiosis in these two genotypes (Borikar and Chopde, 1982). Thus sorghum shoot fly resistance appears to be governed, at least in part, by antibiosis as the probability of deadheart formation in resistant varieties is lower than in susceptible genotypes in spite of egg laying on both (Sharma and Rana, 1983).

Unnithan and Seshu Reddy (1985) found under two choice situation, IS 2291 was least preferred for oviposition (0.65 eggs/plant). However relatively low incidence of deadhearts (60%) and the recovery of a few infested plants (7%) after artificial infestation indicate that antibiosis is another (secondary) mechanism of resistance to shoot fly in this cultivar. The larval and pupal weights, lengths and periods were significantly different on resistant and susceptible varieties and the percentage pupation on resistant lines was significantly lower compared with that on susceptible lines (Dhavan et al., 1993). Some cultivars are preferred for oviposition, however percent infestation as measured by deadheart production is low mainly due to antibiosis (Mate et al., 1996).

There is a report that trichomeless cultivars of pearl millet accumulate more dew and stay wet longer (Burton et al., 1977). A similar situation in sorghum would facilitate the movement of freshly hatched larvae to the base of the central shoot (Raina, 1981). On the other hand, trichomed cultivars would tend to dry faster, making the downward journey of the larvae more difficult (Raina et al., 1981). The earliest work that referred to antibiosis as a possible mechanism of resistance to shoot fly in sorghum was that of Ponnaiya (1951a, 1951b). He attributed to this an early deposition of irregular shaped silica crystals in the resistant cultivar M 47-3. Blum (1968) confirmed Ponnaiya's observation that plants of resistant cultivars possess a high density of silica bodies in the abaxial epidermis at the base of the first, second and third leaf sheaths (increasing from leaf sheaths one to three). He also reported a distinct lignification and thickening of walls of cells enclosing the vascular bundle sheaths within the central whorl of young leaves. These observations of Ponnaiya and Blum were confirmed when various treatments with sodium silicate to a susceptible variety caused a significant reduction in infestation for 10 days (Blum, 1971)

Raina (1985) proposed that three different factors individually or in combination, may contribute to the expression of antibiosis to shoot fly in sorghum: 1) trichomed cultivars hinder the movement of newly hatched larvae towards the base of the shoot; 2) resistant cultivars have greater silica deposits and lignification of cells, which may restrict larval penetration to the base of the central shoot, and 3) biochemical deficiencies or the presence of chemical factors in resistant cultivars may adversely affect the development and survival of larvae and reduce the fecundity of the resulting adults.

1.1.2.3 Tolerance or Recovery resistance

Blum (1967) referred to tiller survival as the ability of the resistant selections to produce a greater number of shoot fly free tillers that might be ascribed to faster growth rate of tillers or a larger number of tillers. Five shoot fly resistant and two shoot fly susceptible sorghum varieties were studied in order to evaluate the association between several plant traits and tiller survival both under field and simulated conditions (Blum, 1969). Tillers of resistant varieties showed lignification of the walls of cells that enclose the vascular bundles in the central whorl of young leaves, grew faster than those of the susceptible ones and the infestation of shoot fly was delayed by two days in resistant varieties as compared with susceptible ones (Blum, 1969). Recovery resistance comes into the picture when there is little seedling resistance and when infestation levels exceed 90% (Doggett et al., (1970). Doggett et al. (1970) reported good recovery resistance was shown by the cultivars 'Serena' and 'Namatare' and more than 70% of infested plants recovered and yielded normally. Heritability of this trait is high.

Blum (1971) quoted from his experiments that the total number of tillers formed by a variety was directly related to rate of infestation of that variety. Doggett (1971) pointed out that synchronous tillers of resistant varieties are few but most of these are productive. Tiller development consequent to deadheart formation in the main shoot and subsequent survival and recovery of the plant depends upon the level of primary resistance (Sharma et al., 1977; Dhillon, 2004). The tillers of susceptible varieties were repeatedly attacked and significant differences between resistance and susceptible varieties for tiller survival were maintained (Dhillon, 2005).

1.1.3 Components of Shoot fly Resistance

It was established that some morphological characters (Blum, 1968; Maiti and Bidinger, 1979; Raina, 1985; Maiti et al., 1984), and biochemical factors (Singh and Jotwani, 1980c), of sorghum seedlings are associated with shoot fly resistance. Resistant cultivars are usually tall with thin stems having long internodes and a short peduncle. They have narrow, glossy and yellowish-green leaves (Maiti and Bidinger, 1979). These leaves possess trichomes on the abaxial leaf surface, which act as physical barriers to penetration of young maggots into the whorl (Kishore et al., 1985). Color, glossiness and trichomes of leaves are

prominent attributes that contribute to resistance to shoot fly in sorghum (Jadhav et al., 1986). These factors had been studied in detail and hence are required to be reviewed individually.

1.1.3.1 Glossiness

Soto, 1974 first recognized the differences in leaf shape, color and texture exist between Indian sorghums (leaves usually elongated, pale green non waxy) and exotic varieties (Leaves broad, dark green, waxy) but it was not known to him whether these differences influence oviposition by the shootfly. Later glossiness is identified as a characteristic trait of most of the winter (rabi) sorghum varieties of India (Ponnaiya, 1951a; Rao et al., 1978). It was reported to be associated with shoot fly resistance (Bapat et al., 1975; Maiti and Bidinger, 1979; Maiti, 1980; Taneja and Leuschner, 1984; Omori et al., 1983; Kamatar and Shalimath, 2003). Tarumoto (1980) reported a simple screening technique for identification of glossy cultivars among large germplasm by observing whether or not sprayed water adheres on leaf blades.

Sorghum seedlings can be glossy or non-glossy. Seedlings with dark green leaves (normal) are non-glossy; and seedlings with light yellow green and shining leaf surfaces are glossy (Bapat and Mote, 1982). The intensity of glossiness of the leaves at seedling stage is positively associated with resistance to shoot fly (Sharma et al., 1997). Most resistant lines exhibit the glossy leaf characteristic during the seedling stage. Expression of glossiness in seedlings is an important trait for identifying shootfly resistance in sorghum and it is easily identifiable (Maiti et al., 1984). Glossiness of leaves affects the quality of light reflected from leaves and influences the orientation of shoot flies towards their host plants. Glossiness may also influence the host selection by means of chemicals present in the surface waxes and or leaves (Sharma, 1993).

A systematic survey of world germplasm collection indicated a low frequency of accessions with the glossy trait (only 495 of 17536 accessions) and 84% of these lines were of Indian origin. While glossiness is clearly manifested in the seedling stage, it gradually disappears as the seedling grows (Maiti et al., 1984). Taneja and Leuschner (1984) 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 post-rainy seasons revealed that shoot fly incidence was higher on non-glossy lines than glossy lines in the post-rainy season. However, glossiness contributed less to shoot fly resistance during the

rainy season. Thus, most of the less susceptible lines have glossy seedling leaves (narrow, pointed and pale green), but all the glossy lines are not necessarily less susceptible to shoot fly (Maiti and Bidinger, 1979). Omori et al., 1983 concluded that the shootfly resistance componential characters trichome density and glossiness intensity showed significant associations with shootfly resistance but do not play any direct role in contributing to the total variability in shoot fly resistance. The major portion of shootfly resistance (measured in terms of deadhearts incidence) is contributed by the number of eggs/plant. Agarwal and Abraham (1984) reported that glossiness is highly correlated with shoot fly resistance. Jadhav et al. (1986) reported negative and highly significant correlation (r=-0.77) between dead hearts and glossiness. Vijayalakshmi(1993) also reported that glossiness was negatively correlated in general with percentage of plants with eggs, number of eggs/100 plants and deadheart percentage in tall as well as dwarf genotypes. Glossy sorghums show multiple resistances to shoot fly, stem borer and several other insects and tolerance to abiotic stresses like drought, salinity, high temperature and low nutrient availability. Therefore, this trait can be used to identify shoot fly and seedling drought tolerance in preliminary screening of large germplasm sets and breeding populations (Maiti et al., 1984).

1.1.3.2 Trichomes

Earlier mention of 'prickle hairs' on the abaxial surface of leaf blades of shoot fly resistant sorghum varieties was made (Blum, 1968; Langham, 1968) but it was not clear if trichomes were described. Levin (1973) described the role of trichomes in plant defense and pointed out that in numerous species there was negative correlation between trichome density, insect feeding, ovipositon responses, and nutrition of larvae. The association between trichomes and pest resistance was reviewed for numerous plant species by Webster (1975) and Norris and Kogan (1980). Later observations at ICRISAT indicated that many sorghum lines having field resistance to shootfly had trichomes on the abaxial leaf surface (ICRISAT Annual Report 1977-1978). Maiti and Bidinger (1979) identified 32 lines (from 8000 germplasm lines) with trichomes on the abaxial surface of thier leaf blades, which showed lower egg count and fewer deadhearts than 35 lines without trichomes. Lines possessing both trichomes and the glossy seedling character were more resistant than lines with only one of these traits. Trichomes are of infrequent occurrence in sorghum; of approximately 5504 entries selected from the germplasm to represent all taxonomic groups in the collection only 16 were found to have trichomes (Maiti et al., 1980). Trichomes are found on both

surfaces of the leaf but tend to be more numberous on the adaxial surface Maiti et al. (1980). Maiti et al (1980) observed that the presence of trichomes on the leaf surface resulted in a lower frequency both of oviposition by shoot fly and subsequent seedling damage by larvae. Three wild species (Sorghum versicolor, S. purpureosericeum and an unidentified wild type) were found to be immune to shoot fly amongst 57 different species since neither eggs nor deadhearts were noticed on them (Bapat and Mote, 1982). It was observed that these immune entries had high densities of trichomes on the lower surface of seedling leaf blades that contribute to their shootfly resistance.

Density of trichomes per unit area of leaf lamina surface is genetically controlled, but the presence of trichomes probably is more important for increasing resistance to shoot fly than is density (Maiti and Gibson, 1983). 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 and early postrainy season plantings (Maiti and Gibson, 1983). Birader et al. (1986) reported that the intensity of trichomes on the adaxial leaf blade surface was 2–6 times greater than abaxial leaf blade surface. Presence of trichomes on the lower surfaces of seedling leaf blades and unknown antibiotic factors are likely to create hindrance for egg laying by shoot flies. Kharanjkar et al. (1992) opined that although there is a highly significant and negative correlation between the trichome density and shoot fly infestation, it seems that trichomes do not have any role in reducing deadhearts incidence, but help indirectly in reducing oviposition. Peter et al. (1995) reviewed the role of plant trichomes in insect resistance and suggested that trichomes can act as an insect resistance mechanism in one of three ways: including acting as a physical barrier limiting an insect's contact or movement on plant surfaces; which is precisely the mechanism acting in case of sorghum shoot fly. Jayanthi et al. (1999) observed that the expression of trichomes in hybrids depended on the type of parents involved. If postrainy season adapted resistant male sterile lines were involved, trichome expression in hybrids was lower in the rainy season than in the postrainy season.

1.1.3.3 Interaction of Glossiness and Trichomes

Most glossy lines also show the presence of trichomes. Approximately 8000 lines were screened (Maiti and Bidinger, 1979), only 70 from this were glossy and 85% of these had trichomes on their leaves confirming the association of these traits. A study of four combinations–glossy leaf and trichomes, glossy leaf only,

trichomes only and neither revealed that the mean dead hearts 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 than trichomes alone (84% dead hearts) in reducing deadhearts incidence. The combination of both the characters, however (61% deadhearts), was significantly superior to the mean of the two traits taken singly. Similarly, Maiti et al., (1984) also reported that the level of resistance was greater when both the glossy and trichome traits occur together.

1.1.3.4 Leaf surface wetness

Rivnay (1960) suggested the importance of dew for the movement of the shootflly larvae. Blum (1963) also reported that freshly hatched shootfly larvae when placed on sorghum leaves in the laboratory, repeatedly fell down unless the plants were moistened with a fine spray of water. The time of egg hatching coincides with the presence of moisture on the leaf (Raina, 1981; Nwanze et al., 1992b). Leaf moisture is important for larval movement and deadheart formation (Raina et al., 1981). Cultivars with high transpiration rates are preferred for oviposition (Mate et al., 1988) and shootfly abundance is affected by temperature and relative humidity (Taneja et al., 1986). There are genotypic differences between resistant and susceptible genotypes in surface wetness of the central shoot leaf (Nwanze et al., 1990) and LSW of the central shoot leaf was higher in 10-day old seedlings than in seedlings of other ages. The highest amount of LSW (6.29mg of water) was recorded in August in the shoot fly susceptible sorghum genotype CSH1 while the lowest (0.07mg) was recorded in November in the resistant genotype IS18551 and was highest between 2.00 and 4.00 h. (Nwanze et al., 1992b).

Nwanze et al., (1990) concluded that the leaf surface wetness of the central shoot leaf is a more reliable predictor of resistance than the glossy leaf trait or trichome density. Leaf blade cuticles of resistant and moderately resistant genotypes are characterized by a smooth amorphous wax layer, and sparse wax crystals. Susceptible genotypes possess a dense meshwork of crystalline epicuticular wax (Nwanze et al., 1992a). The LSW of the central whorl leaf originates from the plant and is not due to atmospheric condensation (P.S. Sree et al., 1994). Although the ability of resistant and susceptible genotypes to move water from the soil into the leaf doesn't differ, the mechanism for transfer of water to the leaf surface is reduced in resistant genotypes (Soman et al., 1994) and is genetically controlled. The physical and physiological evidence of the origin

of leaf surface wetness from the plant has been confirmed by radiolabelling methods using tritium and carbon^{-14}. There were significant differences in the amount of tritiated water collected from susceptible (CSH5) and resistant (IS 18551) genotypes, while there was similar amount of radioactivity in the leaf tissues of both genotypes. The presence of (small amounts of) solutes in the surface water may affect larval movement and survival (Sivaramakrishnan et al., 1994).

1.1.3.5 Seedling Vigor

Blum (1972) found that shoot fly resistant sorghum lines grew faster as compared to susceptible ones. Greater seedling height and faster growth rate of resistant plants may also result in reduced fecundity of the insect. Singh and Jotwani (1980d) indicated that longer and narrow leaves and faster seedling growth as indicated by length of leaf sheath (8.36 cm in CSH1 compared to 12.36 cm in IS 5469) and seedling height (29.13 cm in CSH1 compared to 39.33 cm) coupled with greater hardness of the leaf sheaths may be contributing towards the resistance to shoot fly (relative force required for the penetration of the leaf sheaths in resistant varieties ranged from 29.6 to 35.4 g as compared to 26.5 g in the susceptible control variety).

Khurana and Verma (1985) studied plant characters of nine sorghum lines (6 resistance and 3 susceptible) and concluded that faster growing resistant plants may remain in the favourable height for oviposition for a relatively lesser period as compared to the slow growing susceptible plants. Taneja and Leuschner (1984) observed that in postrainy season, shoot fly incidence was higher in sorghum lines that were less vigorous at the 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 larvae from reaching the 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 shoot fly interaction measured in terms of deadhearts and concluded that apart from the glossy seedling trait, seedling height, trichomes density, and initial faster plant growth rate contribute to resistance to shoot fly in sorghum. Based on the correlation studies, the negative correlation of seedling height and stem length with oviposition and deadhearts by shootfly, suggested that pest resistant plants grew faster and might therefore escape damage and therefore, long and thin stem with longer internodes should be sought for selecting shoot fly

resistant genotypes (Patel and Sukhani, 1990b). Kharanjkar et al. (1992) observed positive relationship between vigor of the plant and its escape from shoot fly attack, and concluded that the trichome density and seedling vigor can be used as selection criteria for shoot fly resistance.

Rapid growth of seedlings may retard the first instar larvae from reaching the growing tip of seedling shoot. In contrast slow growth due to poor seedling vigour, low fertility or environmental stress increases shoot fly damage (Taneja and leuschner, 1984). Shoot fly resistant lines have rapid initial plant growth (Narayana 1975, Jotwani 1978; Mate et al., 1979, Singh and Jotwani 1980d; Mote et al., 1986), greater seedling height and leaf sheath hardness (Singh and Jotwani, 1980c), and have longer stems and internodes and short peduncles (Patel and Sukhani, 1990b). Faster growing taller varieties are less susceptible. Seedling vigor was significantly and negatively associated with deadheart formation, oviposition percentage and egg count (Kamatar and Shalimath, 2003)

1.1.3.6 Biochemical Factors that influence Shoot fly Resistance

Earlier workers stated that biochemical constituents of host plants affect the growth, survival and reproduction of insects in various ways (Painter, 1951, 1958; Beck, 1965; Schoonhoven, 1968). Shoot flies emerged from 10 day old seedlings laid more eggs than those emerged from seedlings aged 15 and 20 days. Thus larval food appeared to have a definite effect on the oviposition of adult shoot flies Singh and Narayan, 1978. Females required a proteinaceous food before they can develop atleast the first batch of eggs (Ogwaro, K. 1978). The presence of olfactory chemoreceptors on the ovipositor suggests that *A. soccata* females detect volatile cues emanating from sorghum seedlings (Ogwaro and Kokwaro, 1981). A significantly higher response to white strips soaked in plant juice compared to the untreated strips suggests the importance of chemical signals from the sorghum plant (A.K. Raina, 1982).

Although morphological sources of resistance to shoot fly have been identified (Singh and Rana, 1986), the resistance to shootfly is also associated with biochemical components. Singh and Jotwani, 1980c showed higher percentage of nitrogen, reducing sugars, total sugars, moisture and chlorophyll of leaf in the susceptible hybrid CSH1 than in resistant varieties. Similarly leaf sheaths of susceptible hybrid had higher nitrogen, reducing sugars, starch and moisture. They also found lysine (essential amino acid) in the leaf sheath of susceptible hybrid CSH1 but was absent in all the three resistant varieties viz, IS1054,

IS5469 and IS5490. Susceptibility of sorghum to shootfly was found to be positively correlated with phosphorus and negatively with total phenol content (Kurana and Verma, 1983). High amino acid content associated with high HCN may create imbalance between amino acids and sugars, which may be responsible for inhibited larval feeding (Mote et al., 1979). (Khurana and Verma, (1982) observed total amino acid contents in the insect resistant sorghums were more than in susceptible ones. Bhise et al. (1996) observed highest protein content in CSH1 amongst the susceptible hybrids at all three stages of crop growth (10^{th}, 17th and 24^{th} days after emergence) whereas the resistant variety IS 5490 had the lowest protein content. He observed a positive correlation between percent infestation by shoot fly and crude protein content of sorghum and additionally, he observed the resistant lines had significantly higher activities of polyphenoloxidase and peroxidase followed by moderately susceptible and the more susceptible hybrids. Positive significant correlation was observed between shootfly infestation and cholorophyl, HCN, nitrogen, moisture and protein content (Mate et al., 1996). Low concentrations of reducing sugars, total sugars, nitrogen, phosphorus and potassium in sorghum seedlings greatly enhanced the degree of antixenosis for oviposition/feeding and deadheart formation and can be used as selection criteria for resistance to shoot fly (Singh et al., 2004).

1.1.4 Inheritance of Resistance

Resistance to *Atherigona soccata* is quantitatively inherited (Agrawal and Abraham, 1984) and polygenically controlled (Goud et al., 1983; Halalli et al., 1983). Sharma et al. (1977) observed continuous variation in different generations and indicated that shootfly resistance is due to gradual accumulation of genes contributing to resistance. They also observed intermediate nature of F1's, which confirms to the quantitative nature of inheritance. Both additive and non-additive gene actions were involved in shoot fly resistance (Borikar and Chopde, 1981; Halalli et al., 1982; Nimbalkar and Bapat, 1987). Both additive and nonadditive gene actions was recorded by Halalli (1982) for egg laying and deadhearts percentage, whereas recovery resistance was additively controlled. Broad-sense heritability for shootfly resistance was reported to be around 30% indicating the greater influence of environment (Halalli et al., 1983).

1.1.4.1 Inheritance of glossiness

Glossiness is simply inherited being controlled by a single recessive gene (Agarwal and House, 1982; Tarumoto, 1980) and highly heritable. Intensity of glossiness is quantitatively governed and is controlled by both additive and non-additive genes (Agrawal and Abraham, 1984). Inheritance of glossiness was studied by Tarumoto (2004) in the F2 populations of crosses among non-glossy (Gl), glossy (gl) and true glossy (tgl) genotypes. The segregation analysis revealed that the genes controlling the phenotypes of Gl (non-glossy), gl (glossy) and tgl(true glossy) plants are multiple alleles on the same locus. gl^+ (non glossy) is a simple dominant gene to gl^2 (true glossy) or gl^1 (glossy) and gl^1 (glossy) was a simple dominant gene to gl^2. The gl^1 gene was found to produce pleiotropic effects not only on the cellular structures but on digestibility and possibly the sorghum shoot fly resistance of the leaves also (Tarumoto, 2004). Similarly, The glossy seedling was reported in corn (*Zea mays* L.), in which a series of genes, gl1 to gl10, each of which causes younger leaves to have glossy surfaces (Emerson at al., 1935). Series of glossy mutants with gly1 to gly9 in Brassica oleracea were also inherited quantitatively (Amasino and Osborn, 2002).

1.1.4.2 Inheritance of trichome density

Studies on trichome inheritance have been conducted in several grain crops. Ringlund and Everson (1968) reported that offspring from matings between densely and sparsely pubescent wheats (*Triticum aestivum* L.) ranged from moderately to densely pubescent and that the inheritance of density was complex with greater density being partially dominant. The inheritance of sorghum trichome density has been studied (Gibson and Maiti, 1983; Maiti and Gibson, 1983) and reported that presence of trichomes is recessively inherited and controlled by a single locus (tr) with Tr being trichomeless and trtr genotypes being trichomed. Heritability for the character between F_3 and F_4 generations was observed to be 75% and thus shows much of the variability for trichome density is genetically controlled (Gibson and Maiti, 1983). Trichome density is controlled by both additive and non-additive gene effects (Halalli et al., 1982). Jayanthi et al (1996) observed season specificity for trichome density reflected in the hybrid groups depending upon the type of parents involved in the crossing and accordingly the gene action differed for the different sets of the hybrids.

The mean trichome density of sorghum seedling leaf blade on adaxial surface was lower on F_1s than the average of the parents, indicating the role of partial

dominance in respect to trichome density (Biradar et al., 1986). It was observed that R x S and S x R F_1s exceeded the parental limits. Backcrosses involving 168 (susceptible) as the recurrent parent exhibited higher trichome density on seedling leaf blade adaxial surfaces. Complementary type of epistasis coupled with significant heterosis was observed for trichome density on the seedling leaf blade abaxial surfaces in crosses SF 863 x 168 and SF 863 x IS 923. These results indicate the possibility of developing sorghum hybrids with higher density of trichomes on their lower leaf blade surfaces (Biradar et al., 1986).

1.2 Molecular marker analysis

One of the main objectives of plant breeding is to improve existing cultivars, which are deficient in one or more traits by crossing such cultivars with lines, which possess the desired trait. Conventional breeding procedures are laborious and time consuming, involving several crosses, several generations, and careful phenotypic selection. Moreover, polygenic traits are difficult to manipulate by conventional breeding procedures. With DNA marker technology, it is possible to overcome many of the problems faced using conventional breeding (Kumar, 1999).

The use of molecular markers in breeding programs is increasing rapidly as they greatly improve the efficiency of breeding programs for traits for which conventional phenotypic selection is difficult, expensive or time-consuming (Jones et al., 1997; Mohan et al., 1997; Prioul et al., 1997). The ability to score genotypes at the molecular level is the advantage of molecular markers. This technology is capable of handling large numbers of samples. PCR-based molecular markers have the potential to reduce the time, effort and expense often associated with phenotypic screening.

1.2.1 Isozymes (bochemical markers)

The first molecular markers used were isozymes, which are protein variants detected by differences in migration on starch gels in an electric field (Stuber and Goodman, 1983). These biochemical markers have been particularly useful both in breeding practice (Ainsworth and Gale, 1987) and further development of marker-aided selection technology (Stuber et al., 1987). The major weakness of isozyme markers is that each of the proteins that are being scored may not be expressed in the same tissue and at the same time in development (Winter and Kahl, 1995)

Table 3. Factors/traits associated with sorghum resistance to shoot fly and stem borers.

Factors/Traits	Selected Reference	Shoot Pests
Seedling vigor	Maiti et al., 1994	SF,SB
Internode elongation	Taneja and Woodhead, 1989	SF,SB
Leaf glossiness	Maiti & Budinger, 1979	SF
Leaf surface wetness	Nwanze et al., 1990	SF
Epicuticular wax	Nwanze et al., 1990; Bernays et al., 1983	SF,SB
Trichomes	Blum, 1968	SF
Silica bodies	Blum, 1968	SF

SF=Shoot fly

SB=Stem borer

Plate. 1 Difference between shapes of droplets adhering to non-glossy leaves (left two) and glossy leaves (right two) when sprayed with water (Tarumaoto, 1980).

Therefore several samplings of the genetic population need to be made. Since the late 1960s this class of markers has been extensively applied to a variety of population genetic problems. Another limitation with protein markers lies with insufficient protein variation for high-resolution mapping (Burrow and Blake, 1998). However as methods for evaluating variation directly at the DNA level became widely available during the mid 1980's, DNA-based markers replaced isozymes in mapping studies. A significant breakthrough in genetic analysis came when the first genetic map using restriction fragment length polymorphisms was constructed (Botstein et al., 1980). Since then molecular biology has ushered in a new era with techniques that directly assayed DNA and overcame many of the problems that have previously limited the applied use of biochemical markers.

1.2.2 DNA markers

DNA markers may be broadly divided into three classes based on the method of their detection: (1) hybridization-based (Southern, 1975) (2) polymerase chain reaction (PCR)-based (Mullis, 1986) and (3) DNA sequence-based (Gupta et al., 1999; Jones et al., 1997; Joshi et al., 1999; Winter and Kahl, 1995). DNA markers reveal genetic differences that can be visualized by using a combination of gel electrophoresis and staining with chemicals (ethidium bromide or silver) or detection with radioactive or colorimetric probes. Recently, Mohan et al. (1997), Gupta and Varshney (2000) and Kumar (1999) extensively reviewed the details for these markers systems. A wide array of DNA-based markers is now available including RFLPs (Tanksley et al., 1989), RAPDs, AFLPs, SSRs (Staub et al., 1996; Gupta and Varshney, 2000) and SNPs (Casa et al., 2008). These polymorphic markers provide the framework maps around which QTLs can be located. The advantages and disadvantages of the most commonly used markers are presented by Collard et al. (2005).

1.2.3 SSR markers

SSRs are known by many acronyms, including simple tandem repeats (STRs), variable number tandem repeats (VNTRs), sequence tagged microsatellite sites (STMS), microsatellites, and simple sequence repeats (SSRs). SSRs have received considerable attention and are probably the current marker system of choice for marker-based genetic analysis and marker-assisted plant breeding (Akkaya et al., 1992; Chin et al., 1996). Simple sequence repeats are generally among the most reliable and highly reproducible of molecular markers, forming the foundation for many framework linkage maps. VNTR loci are, in principle, co-

dominant markers, but in RFLP analysis they often behave as dominant markers (Arens et al., 1995). They also display high levels of polymorphism even among closely related accessions (Akkaya et al., 1992; Sanghai-Maroof et al., 1994) and are amenable to simple and inexpensive PCR-based assays (Brown et al., 1996). In some instances, particularly in plant genomes, it has been shown that two different SSR units located adjacent to each other give rise to compound microsatellites (Vogel and Scolnick, 1998). These have structures like, $(GATA)_nGT(CAC)_n$, $(CT)_n(GT)_n$ and $(ATA)_nGCC(TAT)_n$.

SSRs are abundant and uniformly dispersed in both human (Weber, 1990) and plant genomes (Lagercrantz et al., 1993; Wang et al., 1994; Akkaya et al., 1995). The repeat regions are generally composed of di-, tri-, tetra-, [e.g. (CA)n, (CAA)n, (GATA)n] and sometimes greater length perfectly repeated, nucleotide sequences (Taut and Ranz, 1984) that exhibit a high degree of polymorphism (Weber and May, 1989). This variation often results from changes in the number of copies of the basic repeat, referred to as Variable Number of Tandem Repeats (VNTRs). SSRs are highly mutable loci. The variability in the number of repeat units is the typically basis of observed polymorphism. The high degree of polymorphism is thought to be the result of increased rates of sequence mutation affecting the number of repeat motifs present at an SSR locus with observed variation likely due to replication slippage or unequal crossing over (Edwards et al., 1992) insertions and deletions (Charlesworth et al., 1994). Since the flanking sequences at each SSR may be unique, if SSR loci are cloned and sequenced, primers to the flanking regions can be designed to define a sequence-tagged micro satellite (STMS) (Beckman and Soller, 1990).

There are well-established methods of finding microsatellites by screening phage libraries with oligonucleotide probes (Condit and Hubbell, 1991; Thomas and Scott, 1993; Lavi et al., 1994). But a quicker, if limited, approach is to examine the sequence data banks for their presence (Burr, 2001). SSR-based primers representing tri-, tetra-, and penta-nucleotide repeats have been used successfully to generate distinct banding patterns that are resolvable on low resolution agarose gels using ethidium bromide staining (Gupta et al., 1994; Weising et al., 1995) on high resolution polyacrylamide gels by silver staining (Buscot et al., 1996), through primer radiolabelling followed by auto radiography (Gupta et al., 1994), or through primer labeling with fluorescent dyes and automated high resolution visualization of PCR products separated by PAGE or capillary electrophoresis. As would be predicted, the best product size

discrimination is obtained with polyacrylmide-based gel analysis although agarose gel is sufficient for many applications (Vogel and Scolink, 1998). SSRs have played a critical role even in merging disparate linkage maps (Bell and Ecker, 1994; Akkaya et al., 1995) since they define specific locations in the genome unambiguously (Young, 2001). There are several other important advantages of sequence-tagged microsatellites. A single locus, because of the high mutation rate, is often multi-allelic (Saghai-Maroof *et al.* 1994). They can be detected by a PCR (non-hybridization based) assay. They are very robust tools that can be exchanged between laboratories and their data are highly informative (Morgante and Oliveri, 1993). Although some changes can be resolved on agarose gels, it is common to distinguish STMS on polyacrylamide sequencing gels where single repeat differences can be resolved and all possible alleles detected. The assay is relatively quick and throughput can be increased by selecting a small number of different STMS with alleles of non-overlapping size ranges and multiplexing either the PCR reactions, or, more easily, the products of the separate reactions, so that all the alleles of the different loci can be run in a single lane on the gel.

Simple sequence repeat (SSR) or microsatellites are highly polymorphic and allele-specific markers but are limited in number for high density map construction (Gupta and Varshney, 2000). Although SSRs have the advantage of providing mostly co-dominant markers, the technique can require considerable investment to generate the necessary primer sequences, since this requires sequence information from more conserved flanking regions, which is expensive and time-consuming to generate. The large start-up costs for this technique should be justifiable for crops where large-scale mapping and MAS are a practical necessity (Hash and Bramel-Cox, 2000). Among different classes of available molecular markers, SSRs are useful for a variety of applications in plant genetics and breeding because of their reproducibility, multiallelic nature, co-dominant inheritance, relative abundance and good genome coverage. SSR markers have been useful for integrating the genetic, physical and sequence-based physical maps in plant species, and simultaneously have provided breeders and geneticists with an efficient tool to link phenotypic and genotypic variation.

1.2.3.1 Sorghum SSR markers

Ten of 13 sorghum SSR loci characterized by Taramino et al. (1997) were isolated from an AG-enriched gDNA library and three from database searches; seven of the eight loci mapped by Tao et al., 1998, were isolated from a size fractionated gDNA library and one from a database search (see Brown et al., 1996). And all of

the 38 sorghum SSR loci characterized by Kong et al. (2000) were isolated from a size fractionated gDNA library. SSR-containing clones isolated from both two bacterial and artificial chromosome (BAC) and three enriched genomic-DNA (gDNA) libraries and DNA sequences present in public databases were the sources of the sorghum SSRs mapped by Bhattramakki 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 the segments. 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 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), who reported that sorghum fragments can be amplified using at least some maize SSR primers (Brown et al., 1996).

Map location of 46 SSR loci (Taramino et al., 1997(7 SSR); Tao et al., 1998a (8 SSR); Kong et al., 2000(31 SSR) and 113 novel SSR loci (including four SSR containing gene loci)(Bhattramaki et al., 2000) were reported through 2000. SSR markers have been incorporated into the existing RFLP-based maps of Kong et al. (2000) and into the map of Peng et al. (1999) (Bhattramakki et al., 2000). The number of SSR loci per sorghum linkage group ranges from 8 to 30. Eight SSR loci, reputed to have high degree of homology to known genes, were found to be monomorphic among the 18 survey accessions (Bhattramakki et al., 2000) and so could not be mapped. The average number of alleles detected per locus at the polymoprphic loci was 3.88. $(AG/TC)_n$ and $(AC/TG)_n$ repeats comprised 91% of the di-nucleotide SSRs and 52% of all the SSRs at polymorphic loci; where as four types of trinucleotide repeats $(AAG/TTC)_n$, $(AGG/TCC)_n$, $(AAC/TTG)_n$ and $(ATG/TAC)_n$, comprised 66% of the trinucleotide SSRs (Bhattramakki et al., 2000). The number of repeats and the number of alleles at SSR loci in the 18 survey accessions are positively correlated. However, some SSRs with low numbers of repeats are highly polymorphic (Bhattramakki et al., 2000).

It was found that as much as 57% of SSRs containing triplets rich in G-C base pairs were located in gene coding regions of the total genomic DNA (Wang et al., 1994). The estimated average probability that two accessions (selected at random) in a working group will have different alleles at a locus ranges 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 is positively correlated

(r=0.68, which is significant at 1% level) 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 most *Sorghum bicolor* SSR loci are sufficiently polymorphic to be useful in marker-assisted selection programs (Kong et al., 2000).

Table 4. Different microsatellite-based markers (Gupta and Varshney, 2000)

Abbreviation	Expanded	Reference
SSR	Simple sequence repeat	Hearne et al., 1992
STR	Short tandem repeat	Edwards et al., 1991
STMS	Sequence tagged microsatellite	Beckmann and Soller, 1990
SSLP	Simple sequence length polymorphism	Tautz, 1989
MP-PCR	Microsatellite primed PCR	Meyer et al., 1993
SPAR	Single primer amplification reaction	Gupta et al., 1994
AMP-PCR	Anchored microsatellite primed PCR	Wolff et al., 1995
ISA/ISSR	Inter SSR amplification / Inter simple sequence repeats	Zietkiewicz et al., 1994
ASSR	Anchored simple sequence repeat	Wu et al., 1994
RAMP	Random amplified microsatellite polymorphism	Wu et al., 1994
RAMPO	Random amplified microsatellite polymorphism	Richardson et al., 1995
RAHM	Randomly amplified hybridization microsatellites	Cifarelli et al, 1995
RAMS	Randomly amplified microsatellites	Ender et al., 1996
SAMPL	Selective amplification of microsatellite polymorphic loci	Morgante and Vogel, 1994
REMAP	Retrotransposon microsatellite amplified polymorphism	Kalendar et al., 1999

1.3 Sorghum Linkage Maps

A fully integrated sorghum molecular genetic map would be the basis for gene mapping, marker-assisted selection, candidate gene cloning and sequencing of the full sorghum genome. The first complete molecular genetic linkage map containing 10 LGs corresponding to 10 pairs of sorghum chromosomes was built by Chittenden et al. (1994), who used 56 F_2 plants derived from the cross of BTx623 x *S. propinquum*. The map was 1445 cM long, consisted of 276 endogenous and exogenous RFLP markers with an average distance of 5.2 cM between markers. An F_2 segregating population was mostly used in the early genetic linkage mapping. Most of the maps constructed after 1997 used recombinant inbred lines (RIL).

RFLP was the most commonly used marker type in the initially reported DNA-marker based genetic linkage maps because of its stable and co-dominant characteristics. However, the technique is expensive, time-consuming and has low polymorphism. After the development of the SSR and AFLP techniques, these polymorphic, more repeatable and stable markers were gradually added to the previous and newly built maps (Tao et al., 1998; Bhattramakki et al., 2000; Subudhi and Nguyen, 2000; Tao et al., 2000; Klein et al., 2001; Xu et al., 2001; Haussmann et al., 2002; Menz et al., 2002). A few randomly amplified polymorphic DNA (RAPD) markers are also included in some maps (Subudhi and Nguyen, 2000; Xu et al., 2001; Haussmann et al., 2002), but because of their low stability and repeatability, they are scarcely used in recent maps. In maps published in recent years, marker loci numbers have increased and average distances between markers have decreased.

For the first time, Taramino et al. (1997) mapped seven SSR loci using an existing sorghum RFLP map (Pereira et al., 1994). Segregation analysis was performed on a F_2 population (Pereira et al., 1994) from the cross CK60 x PI 229828. Next, a genetic map was constructed using 120 F_5 sorghum RILs, developed from a cross between 2 Australian elite sorghum Inbred lines, QL39 x QL41 (Tao et al., 1998). A variety of DNA probes, including sorghum genomic DNA, maize genomic DNA and cDNA, sugarcane genomic DNA and cDNA and cereal anchor probes were screened to identify DNA polymorphism between the parental lines. A total of 155 RFLP loci and 8 SSR loci (from 17 SSR primer pairs identified by Brown et al., 1996 as detecting polymorphisms between sorghum lines) were mapped onto 21 linkage groups, covering a map distance of approximately 1400 cM. Later, 31 SSRs were added into the framework map of Peng et al. (1999) by Kong et al. (2000). Linkage mapping was performed in a population of 137 F_6-F_8 Recombinant Inbred Lines developed by Dr K. F. Schertz from a cross between BTx623 x IS3620C (Peng et al., 1999). Segregating data for these loci were placed on the framework RFLP map composed of a subset of the RFLP loci. Then, Bhattramakki et al. (2000) constructed a linkage map composed of 147 SSR loci and 323 RFLP loci by integrating the 113 novel SSR loci (including four SSR containing gene loci) and 31 of Kong et al. (2000) and into the peng et al. (1999).

Among the recent maps, two highly dense ones with nearly saturated markers are most effective for usage. Menz et al. (2002), constructed a map with 2926 markers, 2454 of which are AFLP markers, 136 are SSRs and 336 are RFLPs from

rice, barley, oat and maize cDNA and genomic clones using a RIL population derived from the sorghum cross of BTx623 x IS3620C. Ten linkage groups have total map length of 1713 cM, with an average distance of 0.5 cM between markers. This map was constructed from the same RIL population used by Peng et al. (1999); Kong et al. (2000) and Battramakki et al. (2000) (combining the RFLP and SSR data). Bowers et al. (2003) reported a map built with all RFLP products. The 1059 cM map includes 2512 RFLP loci from 2050 endogenous and exogenous probes, of which 1189 were from sorghum cDNA and gDNA clones, others from maize, sugarcane, wheat, barley, rice, millet, oat, rye and Arabidopsis genomes. The average length between markers is 0.4 cM, c. 300 kb, with the biggest gap being 7.8 cM, and only seven gaps are over 5 cM.

Using markers across the 10 sorghum linkage groups (Menz et al., 2002) with a multi-probe cocktail FISH (Kim et al., 2002) and mitotic metaphase chromosomes of root tip cells from the sorghum elite line BTx623, Kim et al. (2005b) developed the first sorghum chromosomal karyotypic map based on molecular marker FISH, an integrated sorghum cytogenomic map. The centromere positions of each chromosome were determined by the centromere specific probe pCEN38 (Zwick et al., 2000). Chromosomes were ordered and designated according to their lengths at metaphase, namely SBI-01 (longest) to SBI-10 (shortest) in which the acronym SBI designates the genus and species. The linkage groups are aligned as LG-01 (longest) to LG-10 (shortest) corresponding to LG A, B, C, D, J, I, E, H, F and G in the map of Menz et al. (2002).

1.3.1 QTL mapping

The first attempt for identification of an individual QTL was made by Sax (1923) in *Phaseolus vulgaris*. The term quantitative loci refer to genomic regions or loci, having effects on characteristics of the organism that can be expressed as continuous variables (Harshbarger and Reynolds, 1993). Most traits of agronomic importance, including yield, nutritional quality and stress tolerance are quantitatively inherited (Allard, 1960; Hallauer and Miranda, 1988). QTL mapping is the procedure of finding and locating QTLs and includes the construction of genomic maps and looking for association between traits and polymorphic markers. This association might be evidence for a QTL linked to the marker (Hui Liu, 1998). A number of methods for mapping QTLs and estimating effects have been suggested and investigated (Darvasi et al., 1993; Gimelfarb and Lande, 1995; Knapp et al., 1990; Knott and Haley, 1992, Paterson et al., 1990 & 1988). Improvement of quantitative traits is often a difficult and time consuming task.

Table 5. Linkage group data from various sources of sorghum maps

Source	Cross	Population type	Population size	Marker type	Probe source	Map length/cM	No of marker sites	Mean distance between markers/ cM	No. of linkage groups
Hulbert *et al.*, 1990	Shangui Red x M91051	F2	55	RFLP(37)	Maize	283	36		8
Binelli *et al.*, 1992	IS18729 x IS24756	F2	149	RFLP(21)	Maize	439	35		5
Whitkus *et al.*,1992	IS2482C x IS24756	F2	81	RFLP(85), Isozymes(7)	Maize	949	98	11.2	13
Melake-Berhan *et al.*,1993	Shangui Red x M91051	F2	55	RFLP(96)	Maize	709	96		15
Ragab *et al.*,1994	Bsc35 x BTx631	F2	93	RFLP(201)	Sorghum, maize	633	71	8.9	15
Xu *et al.*,1994	IS3620C x BTx623	F2	50	RFLP(190)	Sorghum, maize	1789	190	9.3	14
Chittenen *et al.*,1994	BTx623 x *S. propinquum*	F2	56	RFLP(276)	Sorghum, maize, rice and oat	1445	276	5.2	10
Pereira and Lee., 1995	CK60 x P1229828	F2	78	RFLP(201)	Sorghum, maize	1530	201	8	10
Dufour *et al.*, 1997	IS2807 x 379	RIL	110	RFLP(199)	Sorghum, maize and other cereals	1095	199	5.4	13
Tao *et al.*, 1998	QL39 x QL41	RIL	120	RFLP(155), SSR(8)	Sorghum, maize, sugarcane and other cereals	1400	163	10	21
Boivin *et al.*, 1999	IS2807 x 379	RIL	110	RFLP(298), AFLP(137)	Sorghum, maize, rice,oats, barley, pearl millet and wheat	1889	443		11
Peng *et al.*, 1999	BTx623 x IS3620C	RIL	137	RFLP(321)	Sorgum, maize, rice, barley and oat	1347	323		10
Subudhi and Nguyen., 2000	B35 x Tx7000	RIL	98	RFLP(50), SSR(3), RAPD(7)	Sorghum and maize	1200	214		10
Tao *et al.*, 2000	QL39 x QL41	RIL	152	RFLP(281), SSR(25)	Sorghum, maize, sugar cane, and other cereals	>2750	311		14
Kong *et al.*, 2000	BTx623 x IS3620C	RIL	137	RFLP(114), SSRs(31)	Sorghum, Cereals	1287			10
Bhattramakki *et al.*, 2000	BTx623 x IS3620C	RIL	137	RFLP(323), SSR(147)	Sorghum, maize, barley, oat and rice	1406	470	3.1	10
Kebede *et al.*, 2001	SC56xT x 7000	RIL	125	RFLP	Sorghum and maize	1355	144	9.4	10
Klein *et al.*, 2001	RTx420 x Sureno	RIL	125	AFLP(85), SSR(44) and 1 morphological marker		970	130	7	10
Xu *et al.*, 2001	Shagui Red x SRN39	RIL	94	RFLP, SSR, RAPD	Maize	1779	251	7.1	10
Haussmann *et al.*, 2002	IS98030 x E36-1	RIL	225	AFLP, SSR,RFLP, RAPD	Sorghum, maize, and other cereals	1424	339		11
Menz *et al.*, 2002	BTx623 x IS3620C	RIL	137	AFLP(2454), SSR(136), RFLP(336)	Sorghum, maize, rice, barley and oats	1713	2926	0.59	10
Bowers *et al.*, 2003	BTx623 x *S. propinquum*	F2	65	RFLP(2512)	Sorghum, rice, maize, sugar cane, oats, wheat, barley, rye, buffelgrass, millet, and Arabidopsis	1059	2512	0.4	10

However, marker-based QTL analysis will make it easer and faster for breeders to manipulate these traits (Soller and Beckman, 1983; Tanksley, 1983).

Molecular markers have been used to identify and characterize QTL associated with several different traits in sorghum including plant height and maturity (Pereira and Lee, 1995), characters concerned with plant domestication (Patterson et al., 1995), disease resistance (Gowda et al., 1995) and drought tolerance (Tuinstra et al., 1996, 1997, 1998). In addition several sorghum linkage maps have been generated (Yi Zhi-Ben et al., 2006). For MAS to be effective, reliable estimates of QTL positions and effects are required. An adequate precision of QTL analysis can only be expected from large mapping populations using a marker set with good genome coverage, and phenotypic values based on multi-environment field trials (Van Ooijen, 1992; Utz and Melchinger, 1994; Beavis, 1998). Sometimes the number of QTL is considerably underestimated and the percentage of variation explained by markers is highly erratic (Kearsey and Farquhar, 1998, Lynch and Walsh, 1998). Such uncertainties of QTL analysis seriously reduce the efficiency of MAS. Verification of individual QTL by re-estimation in advanced generations or by evaluating near-isogenic backcross lines (NILs) contrasting in the genome segments of interest (Romagosa et al., 1998) is therefore imperative. Close linkage between marker loci and QTL is required not only for minimizing the bias of estimated QTL effects but also for maximizing the frequency of desired QTL genotypes under MAS.

1.4 Transformation and Limitations

Efficient genetic engineering relies on being able to generate a specific gene product at the desired level of expression in the appropriate tissues, at the right time. This can be accomplished by creating gene constructs that include promoters and/or transcription regulation elements that control the level, location and timing of gene expression. A major constraint in the development of effective transgenic products has been the lack of promoters that can offer a high level of gene expression at this degree of specificity in the crop species of interest (Sharma et al., 2004).

1.5 Marker-assisted Backcross

The main advantage of using DNA markers is to accelerate the fixation of recipient alleles in non-target regions and to identify the genotypes containing crossovers close to target genes (Tanksley et al. 1989; Ribaut and Hoisington,

1998). According to Frisch et al. (1999a), molecular markers are used in backcross breeding for two purposes: (1) to trace the presence of a target allele when direct selection is difficult or impossible, such as the case of recessive alleles expressed late in plant development or quantitative trait loci. The use of markers as a diagnostic tool was first proposed by Tanksley (1983) and reviewed by Melchinger et al. (1990). The term 'foreground selection' was suggested by Hospital and Charcosset (1997). And (2) to identify individuals with a low proportion of undesirable genome from the donor parent, this approach is called 'background selection' and was first proposed by Tanskley et al. (1989) and then by Hillel et al. (1990) and was further investigated by Hospital et al. (1992) and later reviewed by Viescher et al. (1996).

Hospital et al. (1992) investigated the use of markers for the recovery of the recipient genome during an introgression breeding program and showed that marker-assisted introgression should be performed in three generations. These authors also recommended the use of markers with known map position and a density of two or three markers per 100 cM, because increasing this density results in only small benefits. Jarboe et al. (1994) have used the maize genome as a model for simulation and reported that three backcross generations and 80 markers were needed to recover 99% of the recurrent parent genotype.

Performing simulations with the published maize map of 80 markers and phenotypic selection, Frisch et al. (1999b) also found that increasing the population size from the first backcross (BC1) generation to the third backcross (BC3) generation reduced the number of marker data points by as much as 50% without affecting the recurrent parent genotype proportion. Ragot et al. (1994) demonstrated that MAB could be efficiently used for introgressing a transgene construct containing the *Bt*-gene (the *Bacillus thuringiensis* toxin gene) of a transformed parent in an elite maize inbred, these workers reaching the same level of parent genotype recovery in BC_3 as that expected for the sixth backcross (BC6) generation. Stuber (1994) using previously mapped favorable quantitative trait *loci* (QTLs) from two inbred lines, successfully transferred them to other inbred lines lacking these QTLs. Single genes with large effects conferring resistance to bacterial blight in rice have also been transferred using marker-assisted selection (Huang et al., 1997; Sanchez et al., 2000).

1.5.1 Classical Backcross Breeding

For the introgression of qualitative traits such as pathotype-specific disease resistances, which are typically controlled by single dominant genes, backcross breeding has been used for a long time (Allard, 1960). It allows the transfer of one or few genes from a mostly agronomically inferior donor genotype in to an

elite recipient genotype, the recurrent parent (RP). For the transfer of a single dominant gene, 6 BC generations would normally be conducted to recover 99% of the RP genome. In the BC1 generation, the proportion of the RP genome would be distributed normally around a mean of 75%, but given a sufficient sample size, it would contain also plants with more than 85% RP genome. These plants can be identified with molecular markers to accelerate a breeding process (Tanksley et al., 1989). Without molecular markers, it often impossible to remove the linkage drag coming as 'baggage' with the introgressed segment. This has been confirmed experimentally by Murray et al., (1988), who found using DNA markers, a recovery of only 90% RP genome in two phenotypically selected BC10 equivalent conversions of the maize inbred line A632, introgressed with resistance genes Ht1 and Rp1, respectively.

1.5.2 Marker-assisted Selection

In foreground selection flanking markers around a target gene are used for selection whereas in background selection, markers dispersed throughout the genome are used to recover the RP genotype. Marker-assisted foreground selection would be effective for the transfer of recessive genes since their classical transfer requires additional recurrent selfing generations. An example of foreground selection from the work of Sanchez et al. (2000) who introgressed three different bacterial leaf blight resistance alleles (each at a different chromosomal location) into elite 'new plant type' (NPT) rice breeding lines using marker-assisted selection (MAS). Donor parent IRBB59, has all three resistance alleles: *Xa21*, *xa13*, and *xa5* but is not a NPT line. Recurrent parents, IR65598-112, IR65600-42, and IR65600-96 are NPT lines. They were able to use markers to introgress desirable alleles at three different loci into NPT breeding lines; two of the three were recessive alleles and some of the loci overlapped in race specificity. A fine example of marker-assisted foreground and background selection was performed by Chen et al. (2000). They backcrossed the *Xa21* gene, which confers resistance to a wide spectrum of bacterial blight races, into the most popular rice line in China. During three backcross generations they selected

for donor alleles at two markers tightly linked to *Xa21* and for recurrent parent alleles at flanking markers outside of the gene region to reduce linkage drag. In the third backcross generation, they used background selection on 128 RFLP loci to recover a line essentially identical to the recurrent parent cultivar, but possessing the *Xa21* allele.

1.5.2.1 Recurrent Parent Genome Restoration

Markers can be of advantageous for foreground and background selection in backcross breeding (Hospital and Charcosset, 1997). In the first approach, the presence of a target allele in an individual is diagnosed by monitoring the genotype at flanking markers for alleles of the donor parent. The second approach devised by Tanksley et al. (1989), accelerates the recovery of recurrent parent genome (RPG). Openshaw et al. (1994) determined the population size and marker density required in background selection. They recommended the use of four markers per chromosome (of 200-cM length). An average marker density of about 20 cM is sufficient to warrant a good coverage of the genome in marker-assisted selection programs (Openshaw et al., 1994; Visscher et al., 1996; Frisch et al., 1998). In general terms a chromosome carrying the target locus is referred to as carrier chromosome and further chromosomes as the non-carrier chromosomes. For the selected individual in each generation, the percentage of the RPG was determined by dividing the number of loci (marker and background loci) homozygous for the recurrent parent allele by the total number of loci monitored. Background selection has two goals: 1) reduction of the proportion of the donor genome on the carrier chromosome of the target allele; and 2) reduction of the donor genome on the non-carrier chromosomes. The length of the chromosome segment from the donor that is linked to the target allele (linkage drag) is reduced by selecting individuals that carry the target allele and are homozygous for the recurrent parent alleles at tightly linked marker loci (Frisch et al., 1999).

Tanksley et al. (1989) demonstrated with computer simulations, use of molecular markers for background selection can accelerate recovery of the RPG by two or three generations. Frisch et al. (1999b) used software named PLABSIM to simulate the recombination process during meiosis. They found out from this software the Q10 value (measure of PRG) of 96.7% was reached only after six generations of backcrossing. This value was subsequently used as a threshold to

determine the termination of marker-assisted backcrossing program. From BC7-BC10, Q10 value increased only 2.0% with marginal gains in advanced generations. Their efforts culminated in conclusions that using different selection strategies, which differ only in the selection pressure applied to carrier versus non-carrier chromosomes, it is easy to save two-three backcross generations and reduce the total required MDP (marker data points). Their findings achieved a Q10 value amounted to 97.8% with n_t = 20 in BC4 and 97.1% with n_t = 60 in BC3. The first parameter setting resulted in saving two-backcross generation and required a total of 1180 MDP, while the second parameter setting saved three generations and required 3340 MDP. Thus in comparison to a constant population size across all generations, increasing population sizes from generation BC1 to BC3 reduced the number of required MDP by as much as 50% without affecting the proportion of the RPG.

1.5.2.2 The challenges in marker-assisted breeding

DNA markers are highly reliable selection tools as they are stable, not influenced by environmental conditions and relatively easy to score in an experienced laboratory. Compared to phenotypic assays, DNA markers offer great advantages to accelerate the variety development. Peleman and van der Voort (2003) presented the following views on advantages of marker usage: 1) Increased reliability: Errors on the measurement of phenotypes tend to be significantly larger than those of genotyping scores based on DNA markers. 2. Increased efficiency: DNA markers can be scored at the seedling stage for the traits which are expressed only at later stages of development, such as flower, fruit and seed characteristics. By selecting at the seedling stage, considerable amounts of time and space can be saved. 3. Reducing cost: there are many traits where the determination of the phenotype costs more than the performance of a PCR assay. The use of DNA markers for indirect selection offers greatest benefits for quantitative traits with low heritability, as these are the most difficult characters to assess in field experiments.

1.5.2.2.1 Removal of linkage drag

In the mid nineties, a novel lettuce variety resistant to the aphid *Nasonovia ribisnigri* (Jansen, 1996) was developed by a marker-assisted breeding approach. This aphid caused abnormal growth in addition to spread of viral diseases.

Resistance to this aphid could be introgressed from a wild relative of lettuce, *Lactuca virosa*, by repeated backcrossing. However, despite many rounds of backcrossing the new product was of extremely poor quality, bearing yellow leaves and a greatly reduced head. This could either have been caused by a pleiotropic effect of the resistance gene or by 'linkage drag', a negative trait closely linked to the positive trait of interest. The linkage drag was recessive, only visible in the homozygous state, thereby seriously increasing the difficulty to select for recombinations based on the phenotype. More than thousand F2 screened, leading to the selection of some 100 individuals bearing a recombination or even double recombinations in the vicinity of the gene. Only those individuals needed to be phenotyped for both the resistance and, at the F3 level, for the absence of the negative characteristics. This approach eventually led to the selection of an individual bearing recombination events very close to each side of the gene thereby removing the linkage drag. The results demonstrated that the (recessive) linkage drag was located on both sides of the resistance gene on top of being tightly linked. This result would have been very hard to obtain by classical selection methods.

1.5.2.2.2 Pyramiding resistance genes

The genes controlling different agronomic traits can be brought together in an existing variety. Genes responsible for resistance to different races or biotypes of a disease or insect pest can be pyramided together to make a line having multi-race or multi-biotype resistance, which are more durable than single race or single biotype resistance. Successful pyramiding of four genes, *Xa4, xa5, xa13 and Xa21* conferring resistance to four different races of bacterial leaf blight pathogen has been achieved in rice (Huang et al., 1997). Gene pyramiding has been used for the backcross transfer of QTL for downy mildew resistance in pearl millet (Witcombe and Hash, 2000). Here a limited number of RFLP markers have been used for marker-assisted selection to improve disease resistance in both parent lines of a popular hybrid variety. Thus, gene pyramiding has been successfully applied in several crop-breeding programs, and many varieties and lines possessing multiple attributes have been produced (Huang et al. 1997; Wang et al., 2001; Samis et al., 2002).

Interesting alleles of different resistance genes may be located in tandem, but present in different accessions. In such case, it is important to precisely fine map the alleles of the different genes. Subsequently, the linked markers can be utilized to select for the rare recombinants that combine the favorable alleles in

tandem (Peleman and van der Voort, 2003). Hash et al. (1997, 1999), Witcombe and Hash (2000); and Hash and Witcombe (2002), described how multiple resistance gene pyramids can be used practically to strategically deploy resistance genes in a potentially more durable manner than has been previously practiced. The frequency of genotypes having resistance alleles at several loci increases greatly in both seed parent and hybrid when the overall frequency of resistance alleles in maintainer lines increases.

1.5.2.2.3 Advanced backcross QTL analysis (AB-breeding)

Theoreticaly marker-aided selection can lead to the accumulation of valuable QTLs into new varieties within elite germplasm. However, in reality there are several practical problems with this strategy. 1. Frequently elite germplasm (especially in self-pollinated crops) has reduced levels of genetic variation making it difficult to find the necessary polymoprphism with the molecular markers required for QTL analysis (Helentjaris et al., 1985; Miller and Tanksley, 1990; Wang et al., 1992; Anderson et al., 1993b). 2. Tanksley (1996) proposed the advanced backcross QTL analysis strategy to reduce the frequency of donor alleles from unadapted germplasm. This is a combination of QTL analysis with variety development. Following this strategy QTL analysis is delayed until the BC2 or BC3 generation.

1.5.2.2.4 Marker-assisted breeding of polygenic traits

In simulation studies, marker-assisted approaches remain efficient for QTL with even very low heritabilities (Moreau et al., 2000). DNA markers help to understand the genetic basis of traits expressing continuous phenotypic variation. The simplification of these complex analyses is important in mapping the loci involved in these traits that can be obtained at several levels, 1. Simplification of the phenotype: division of a complex phenotype into its separate genetic components. For example, yield, is determined by a vast array of component characters, such as root size, plant size, number of fruit, size of fruit, fruit contents, etc. Mapping the genes involved in these separate components provides a better understanding of the complex trait and a higher chance of success. 2. Simplification of the mapping: separating the effect of each QTL by generating Near Isogenic Lines (NILs), using the technique of Introgression Line Libraries (Eshed and Zamir, 1995) and Reverse QTL Mapping (Wye et al., 2000; Peleman and Vander Voort, 2003), enables the more precise measurement of the effect of

the QTL and thereby the fine mapping of the QTL. Fine mapping of a QTL is an essential step in exploiting the QTL by marker-assisted selection.

MATERIALS AND METHODS

MATERIALS AND METHODS

1.6 Backcross introgression of shoot fly resistance QTLs

A cross between a hybrid derived from cross BTx623 x IS 18551 (or 296B x IS 18551) and one of its elite parents (BTx623 or 296B) is a backcross. In this project, the hybrid and the progeny of subsequent generations (starting from BC_1F_1) were repeatedly backcrossed (until BC_4F_1 generation) to their recurrent parents BTx623 and 296B (Table 6). As a result, the genotypes of the backcross progenies became increasingly similar to that of the parent to which it was backcrossed (Fig. 1). With each generation of backcrossing, the genetic contribution of the donor is reduced by a factor of $(^1/_2)^n$.

In conventional backcrossing, at the end of 6-8 backcrosses the progeny would be almost identical with the parent used for backcrossing. Selection for recurrent parent genotype at "background" markers mapping to positions other than the target locus of the backcrossing program can reduce the number of backcross generations required by 3-4 compared to conventional backcross breeding. Such background selection was performed in the BC_2F_1 and BC_3F_1 generations among plants previously identified (on the basis of "foreground selection" for heterozygosity at markers flanking particular shoot fly resistance QTL target regions on linkage groups A (SBI-01), E (SBI-07), G (SBI-10) and J (SBI-05).

Among the fourth generation backcross progenies, individual plants heterozygous for particular shoot fly resistance QTL introgressions (singly or in combination) were selected and selfed for the generation of homozygous shoot fly resistance QTL isoline families. Where segregation permitted, pairs of BC_4F_2 individuals homozygous for either the donor parent alleles at markers flanking the target QTL (*i.e.*, +QTL individuals) or the recurrent parent alleles at these markers (*i.e.*, -QTL individuals) were selected and selfed. Thus the selfed seed harvested from an individual plant in the BC_4F_2 generation represents a homozygous progeny that is near-isogenic to its recurrent parent (and to other +QTL or –QTL segregants derived from the same BC_4F_1 family). Performance of individual shoot fly resistance introgression isolines was field tested in *Kharif* and *Rabi* seasons in the year 2006 and *Kharif* 2007 at ICRISAT-Patancheru.

Figure 1. Flow chart of marker-assisted backcross introgression

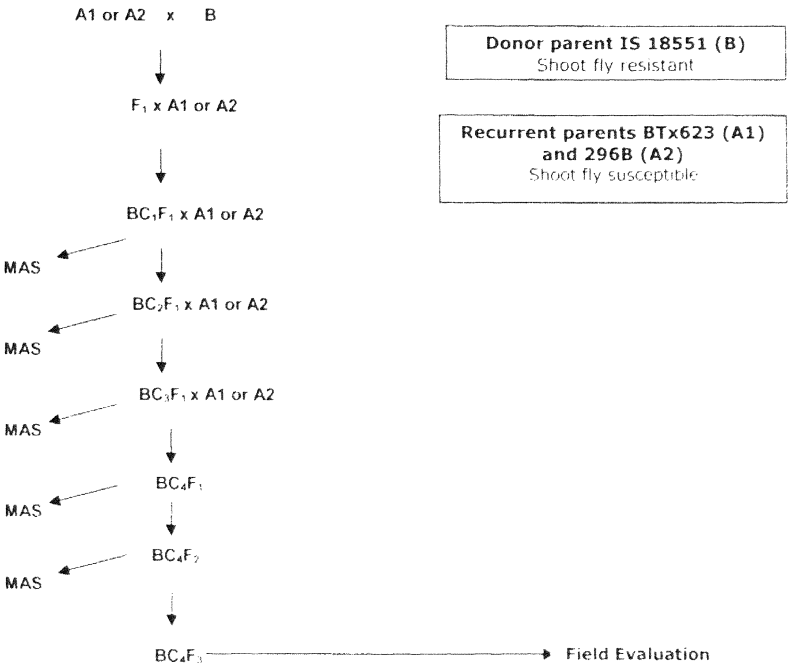


Figure 3. Standard graph expressing the correlation between RFU and DNA concentration

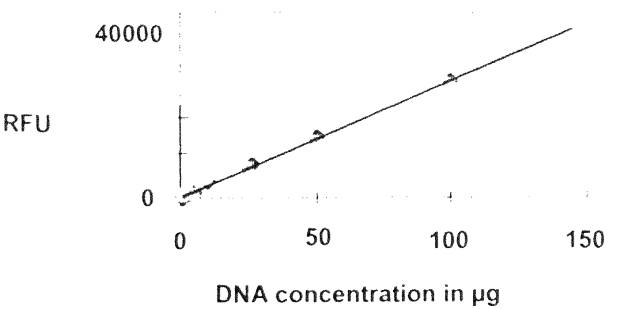


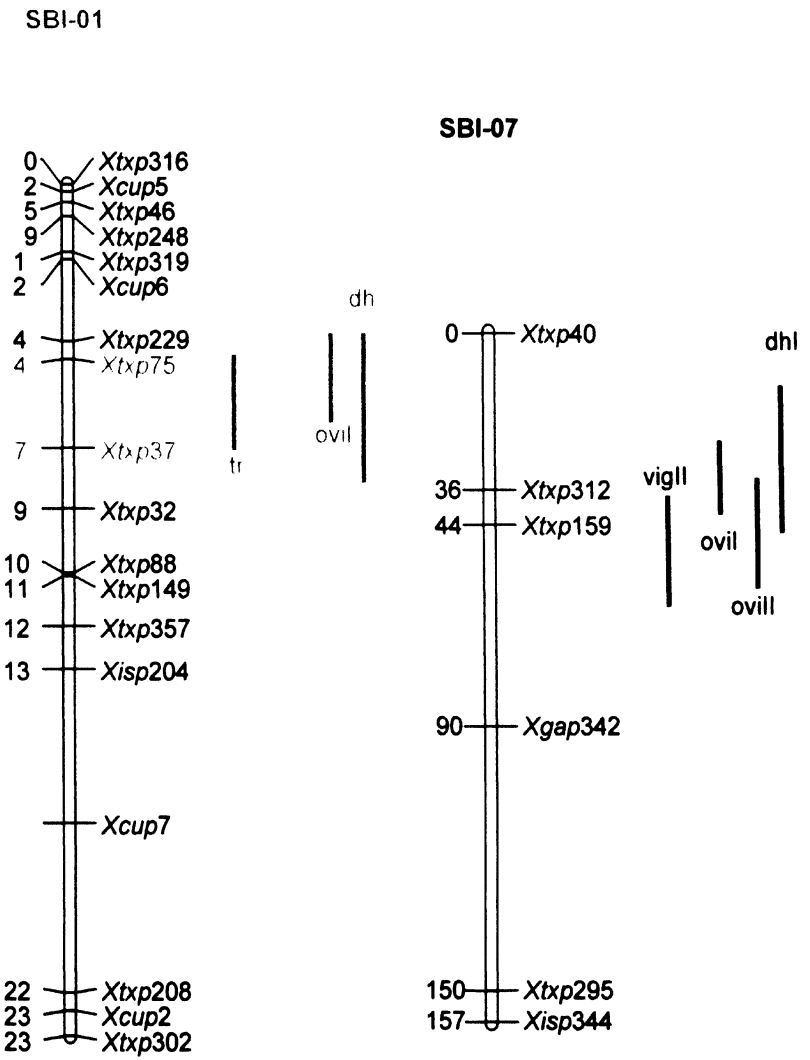
Table 6. Salient features of parental lines of backcross introgression program

Parental lines	Salient features
BTx623 (Recurrent parent)	Derived from cross between IS 40583 (kafir) and IS 21807 (caudatum). Grains are white (thick white mesocarp and reddish-purple spotted white pericarp) and glumes are reddish brown. Leaves of seedlings are dark green (non-tan), dull, broad and drooping with no trichomes. Highly susceptible to shoot fly. High yielding with medium (tall 3-dwarf) plant height and maturity.
296B (Recurrent parent)	Derived from landrace Aispuri. Semi-compact panicle, white grain (thin mesocarp and clean white pericarp), tan colored foliage. Leaves of seedlings are non-glossy with no trichomes. Susceptible to shoot fly. Medium (tall 3-dwarf) plant height and late maturity.
IS 18551 (Donor parent)	Origin from Ethiopia, race durra. Panicle with straw colored grain and large glumes. Leaves of seedlings are light green, shining, narrow and pointed upward with dense trichomes. Resistant to shoot fly. Very tall at maturity.

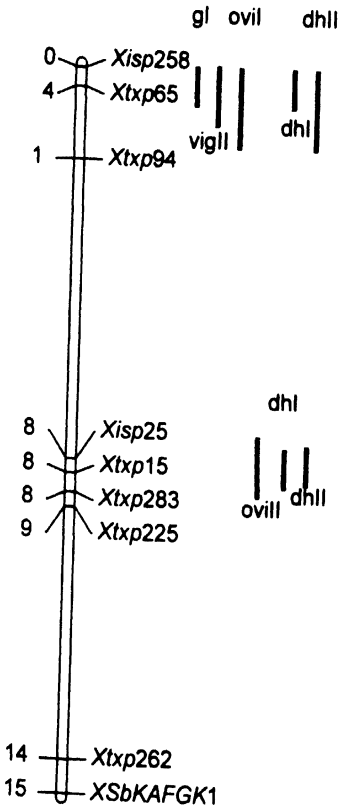
1.6.1 RILs used in backcross breeding

A set of 252 recombinant inbred lines based on the cross of BTx623 and IS 18551 were developed at ICRISAT-Patancheru by 6-7 generations of modified single-seed descent with selfing (*i.e.*, each randomly chosen selfed plant/panicle contributes a single row of off-spring to the next generation). Segregating generations of the cross were rapidly advanced with no intentional selection; each line being continued by harvesting a single selfed plant/panicle in each generation. A single representative plant of BTx623 was used as female parent and pollinated with a single representative plant from IS 18551. The F_1 seeds were space planted and individual vigorous F_1 plants were selfed. Selfed seed from a single vigorous F_1 individual were space planted and all F2 plants selfed, without selection. Seeds of a single panicle from each of the selfed F_2 plants were harvested separately and grown in progeny rows in the next generation. Individual plants were chosen randomly within each progeny row in F_3 and were selfed. The process of random selection and selfing individuals continued up to the F_{6-7} generation. Bulk selfed seed was harvested from random plants in each F_5 family to produce 252 F_6 recombinant inbred lines (RILs). Each F_6 line represented the individual F_2 plant from which it was derived. The RIL numbers

Figure 2. Molecular (SSR) mapping of components of resistance to shoot fly (*Atherigona soccata* Rond), (Folkertsma et al., 2005)



SBI-05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ovil
dhll
0
Xisp258
4
Xtxp65
vigll
dhl
1
Xtxp94
dhl
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Xisp25
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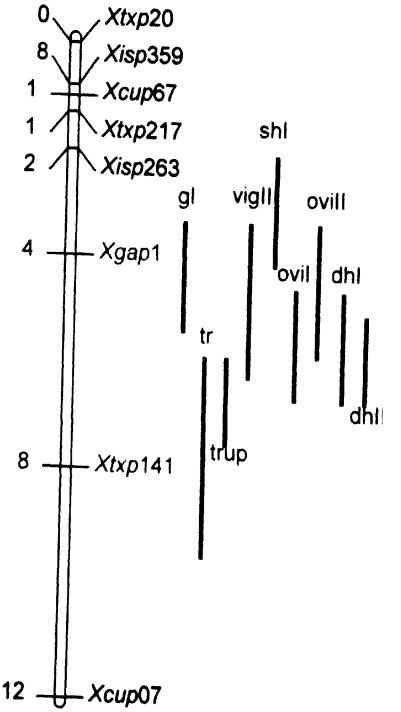
Xtxp15
8
Xtxp283
ovill
dhll
9
Xtxp225
14
Xtxp262
15
XSbKAFGK1

SBI-10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8
Xisp359
1
Xcup67
1
Xtxp217
shl
2
Xisp263
gl
vigll
ovill
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ovil
dhl
tr
dhll
trup
8
Xtxp141
12
Xcup07

153, 166, 189, 252 were found to have maximum shoot fly resistance *viz.*, RIL 153 (homozygous for favorable alleles from IS 18551 at shoot fly resistance QTLs on linkage groups A, E, G and J), RIL 166 (homozygous for favorable alleles at shoot fly resistance QTLs on linkage groups A, G and J), RIL 189 (homozygous for favorable alleles at shoot fly resistance QTLs on linkage groups A, E and J) and RIL 252 (homozygous for favorable alleles at shoot fly resistance QTLs on linkage groups A, G and J) after QTL mapping (Sajjanar, 2002; Folkertsma, et al., 2005) (Fig. 2). The selected RILs with maximum shoot fly resistance were also backcrossed to recurrent parent BTx623.

1.6.2 Selection of Markers

SSR markers linked to QTLs for shoot fly resistance traits were used for foreground selection to select the individuals presumably having the donor allele (foreground selection). Foreground markers indicate the presence or absence of a particular shoot fly resistance QTL. However, the tighter the markers are linked to the QTL the greater the chance that the QTL mapping between the flanking markers has indeed been transferred. At the same time selected markers unlinked to any shoot fly resistance QTLs (*i.e.*, background markers) have been used to select those individuals with minimal drag of non-target genomic regions from the shoot fly resistance donor parent.

Table 7. Characteristics of SSRs used in foreground screening

Linkage group	Locus name	Repeat type	Forward primer (5'-3')	Reverse primer(5'-3')	Annealing Temperature	
					F(°C)	R(°C)
SBI-01 = A	*Xtxp37*	(TC)23	AACCTAACAGGC CTATTTAACC	ACGGCGACTATGTAACTCATAG	56.5	58.4
SBI-01 = A	*Xtxp75*	(TG)10	CGATGCCTCGAAAAAAAAACG	CCGATCAGAGCGTGGCAGG	55.9	63.1
SBI-07 = E	*Xtxp159*	(CT)21	ACCCAAAGCCCAAATCAG	GGGGGAGAAACGGTGAG	53.7	57.6
SBI-07 = E	*Xtxp40*	(CCA)7	CAGCAACTTGCACTTGTC	GGGAGCAATTTGGCACTAG	53.7	56.7
SBI-10 = G	*Xtxp141*	(GA)23	TGTATGGCCTAGCTTATCT	CAACAAGCCAACCTAAA	52.4	47.9
SBI-10 = G	*Xgap1*	(ACC)4 +(CCA) 3CG	TCCTGTTTGACAAGCGCTTATA	AAACATCATACGAGCTCATCAATG	*60	*60
SBI-05 = J	*Xtxp65*	(cT)8	CACGTCGTCACCAACCAA	GTTAAACGAAAGGGAAATGGC	56	55.9
SBI-05 = J	*Xtxp94*	(TC)16	TTTCACAGTCTGCTCTCTG	AGGAGAGTTGTTCGTTA	54.5	47.9
SBI-05 = J	*Xtxp15*	(TC)16	CACAAACACTAGTGCCTTATC	CATAGACACCTAGGCCATC	55.9	56.7

1.7 DNA Extraction

Seed of backcross progenies were sown individually in small pots. At the same time the recurrent parent lines (BTx623, 296B) were sown in three intervals with a gap of one week. This staggered sowing method was employed to ensure co-

flowering of the recurrent parent and backcross progenies. DNA from the BC_1F_1, BC_2F_1, BC_3F_1, BC_4F_1, BC_4F_2, BC_4F_3 generations of BTx623 and 296B crosses with IS 18551 and the RIL-F_1, RIL-BC_1F_1, RIL-BC_2F_1, and RIL-BC_3F_1 populations was extracted from individual one week-old seedlings (leaf tips) using a modified CTAB method (Mace et al., 2003) in a 96-well format.

1.7.1 CTAB Mini-Prep DNA Extraction Procedure

1.7.1.1 Preparation: DNA extraction tubes were chilled in freezer for 30 min at -21°C with 2 steel balls per tube before sample collection. Samples were collected from one-week-old seedlings; leaf tips were cut into small pieces to a final weight of 20–30 mg/tube. During sample collection, CTAB buffer was heated to 65°C in a water bath for 1–1.5 hrs.

1.7.1.2 Grinding: 450 µl of freshly prepared and pre-heated 3% CTAB buffer (3% w/v CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris HCl, 0.17% mercaptoethanol, pH 8.0) was added to each sample. Samples were ground in the Sigma Genogrinder (2 min per round, at 500 strokes/min for 2–3 rounds) until leaf strips were sufficiently macerated. After this, the tube box was fitted into a locking device and incubated at 65°C for 10 min with occasional manual shaking.

1.7.1.3 Solvent extraction: 450 µl of chloroform:isoamyl alcohol (24:1 v/v) was added to the heated sample and the tubes were inverted to mix well and centrifuged at 6200 rpm for 10 min. The aqueous layer (approximately 300 µl) was transferred to a fresh tube.

1.7.1.4 Initial DNA precipitation: 0.7 volume (210 µl) of cold (-21°C) isopropanol was added to each tube containing the aqueous layer and centrifuged at 6200 rpm for 15 min. The supernatant was decanted under a fume hood and the pellet formed was allowed to air dry for a minimum of 20 min.

1.7.1.5 RNase treatment: 200 µl of low salt TE (10 mM Tris, 1 mM EDTA, pH 8) and 3 µl of RNase (10 mg/µl) (total 203 µl) were mixed in a tray and added to each tube contained with the pellet, tapped well and the solution was incubated at 37°C for 30 min. Alternatively the samples can be incubated overnight at room temperature.

1.7.1.6 Solvent extraction: After incubation 200 µl of henol:chloroform:isoamyl alcohol (25:24:1 v/v) was added, the samples were mixed well and the mixture was centrifuged at 5000 rpm for 5 min at 24°C. The aqueous layer was transferred to fresh tubes and the step was repeated with chloroform:isoamyl alcohol (24:1 v/v).

1.7.1.7 DNA precipitation: To the aqueous layer 15 µl of 3 M sodium acetate and 300 µl of 100% ethanol per well/tube was added, subsequently placed in freezer (at −30°C) for 5 min and centrifuged at 6200 rpm for 15 min.

1.7.1.8 Ethanol wash: Supernatant was carefully decanted and 200 µl of 70% ethanol was added to the pellet, which was re-suspended before being centrifuged at 6200 rpm for 5 min.

1.7.1.9 Final suspension: Supernatant was decanted and the pellet was air-dried for approximately 1 hour, the pellet was resuspended in 100 µl $T_{10}E_1$ buffer for 1 hr at room temperature to dissolve completely and finally transferred to 4°C or left at room temperature.

1.7.2 DNA Analysis

1.7.2.1 Agarose gel method

Agarose gel electrophoresis is the easiest and most common way of separating and analyzing DNA. The purpose of the gel might be to check quality of the DNA, to quantify it or to isolate a particular band. Ethidium bromide binds strongly to DNA by intercalating between the bases and is fluorescent meaning that it absorbs invisible UV light and transmits the energy as visible orange light. Most agarose gels are made between 0.7% and 2% agarose. A 0.7% gel will show good separation (resolution) of large DNA fragments (5–10 kb) and a 2% gel will show good resolution for small fragments (0.2–1 kb). Small 8x10 cm gels (minigels) are very popular and give good photographs. The volume of agarose solution required for a minigel is around 30–50 mL, for a larger gel it may be 250 mL. Typically, a band is easily visible if it contains about 20 ng of DNA. This study utilized 0.8% agarose gels for checking DNA quantity (and 1.2% gels for PCR product). To produce an 0.8% agarose gel, 0.4 g agarose was added to 50 ml 1x TBE buffer and dissolved by gentle shaking in the microwave oven (2 min) and 2 µl ethidium bromide was added. Samples to be separated on the gel were prepared from 1 µl diluted/original DNA and 1 µl loading buffer. Estimate of DNA concentration was made from standards (50-200 ng).

1.7.2.1 1.2% Ready made agarose gels (Amersham Biosciences)

The presence and quality of DNA in extracted DNA samples was also examined on ready to run agarose gels. This is the quickest and easiest method of determining the quality of DNA. In this method, loading samples were prepared by mixing 1 µl DNA sample, 8 µl DD water and 1 µl loading buffer in each well of a DNA quantification plate. The samples were then subjected to vortex, on vortex machine and then centrifuged to mix the dye well. About 10 µl sample was loaded on to the agarose gel with standard markers of known concentration (50–200 ng range). The gel was then run for 10 min. After the run the gel was developed in DD water for 20–30 min, after which the DNA quality was checked under UV. A smear of DNA indicated poor quality where as a clear band indicated good quality DNA. Samples of poor quality were re-extracted.

1.7.2.3 Spectrafluor plus Spectrophotometer

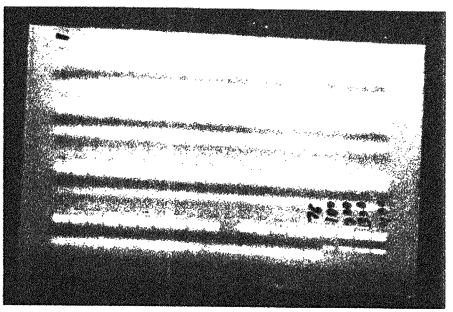
DNA quantity was also assessed using a fluorescence spectrophotometer (Spectrafluor Plus, Tecan, Switzerland) by staining DNA with Pico green™ ($\frac{1}{200}$ dilution) (Juro Supply Gmbh, Switzerland). Based on the Relative Fluorescence Unit (RFU) values and using a calibration graph (Fig. 3), DNA concentrations were calculated (*DNA concentration = -2.78273+0.002019*RFU*). Pico green™ binds to DNA, but it fails to bind with RNA and protein. Thus the machine readout estimates the exact amount of DNA present in the test sample. The DNA concentrations were normalized at 2.5 ng/µl to be used in PCR reactions.

1.7.3 PCR Material Up

1.7.3.1 PCR amplification of parental DNA

The purpose of a PCR reaction is to make a huge number of copies of a specific DNA sequence located between 2 flanking primer sequences. The target regions of BTx623, 296B and IS 18551 were amplified for different foreground and background SSR marker primer pairs according to the optimal conditions where they could be amplified. PCR reactions were conducted in 384-well plates in a PE 9700 Perkin Elmer (Norwalk, Conn., USA) DNA thermocycler. The reactions were performed in volumes of 5 µl using four different PCR protocols and a touch down program. The foreground markers were optimized for these protocols: *Xtxp37* (Protocol 7), *Xtxp75* (Protocol 5), *Xtxp159* (Protocol 7), *Xtxp40* (Protocol 5), *Xtxp141* (Protocol 5), *Xgap1* (Protocol 5), *Xtxp65* (Protocol 5), *Xtxp94* (Protocol 5) and *Xtxp159* (Protocol 5). In the following table are given PCR constituents for single PCR reactions for the four optimized protocols used in this study.

Plate. 2 BC_4F_2 set-2 296B x IS 18551 mother plate, 4 on 0.8% agarose



Lanes 1 to 76: samples J3107-J3182 followed by 100 ng, 200 ng, 300 ng, and 400 ng lambda DNA

Plate. 3 Spectrafluor spectrophotometer

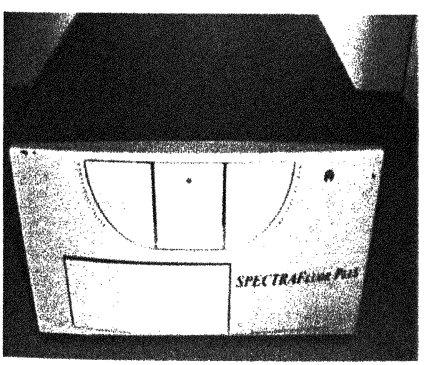


Plate. 4 PE9700 Thermocycler

Table 8. PCR reaction mix for the amplification of SSR alleles for parental and progeny screening

Protocol	Primer (2 pM/ul) in µl	$MgCl_2$ (10 mM) in µl	dNTPs (2 mM) in µl	DNA (2.5 ng) in µl	*Taq* polymerase (0.5 U/µl) in µl	Buffer (10x) in µl	Distilled water in µl
5*	0.500	1.000	0.250	1.000	0.200	0.500	1.550
7*	1.000	1.000	0.375	0.500	0.200	0.500	1.425
4*	0.500	0.750	0.500	0.500	0.250	0.500	2.000
8*	1.000	0.670	0.670	1.000	0.340	0.670	1.340

1.7.3.2 The Touch Down PCR program

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

Step 1: 94°C - 15 min hold (to activate the *Taq* polymerase)

Step 2: 10 cycles:

94°C - 15 sec (denaturation)

*61°C - 20 sec (primer annealing) 1°C drop per cycle for 10 cycles

72°C - 30 sec (primer extension)

Step 3: 31 or 35 cycles (depending on separation of fragments on ABI or PAGE, respectively)

94°C - 10 sec (denaturation)

54°C - 20 sec (primer annealing)

72°C - 30 sec (primer extension)

Step 4: 72°C - 20 min (final extension, to ensure amplification to equal length of both strands)

Step 5: 4°C - hold.

If the parents showed polymorphism more than 5 bp for a particular marker, then PCR products were separated on 6% non-denaturing PAGE gels and silver stained using the modified procedure developed by Kolodny (1984). If the polymorphism between the parents was less than 5 bp, then PCR products were separated by capillary electrophoresis using the ABI prism 3700 (Perkin Elmer) automated DNA sequencer. For this purpose fluorescent dye-labeled primers were used. For the BTx623-derived backcross generations alleles for *Xtxp40*, *Xtxp65 and Xgap1* were separated on the ABI, while for the 296B-derived backcross generations alleles for *Xtxp15*, *Xtxp40*, and *Xgap1* were separated on the ABI.

1.7.4 PAGE Electrophoresis

For separation and visualization of PCR products showing polymorphism greater than 5 bp, 6% polyacrylamide gels were used. The details on gel preparation and visualization of DNA bands are given below.

1.7.4.1 Gel casting

Non-denaturing polyacrylamide gels allow high resolution of amplified PCR products. Before preparation of the gel solution, glass plates were cleaned thoroughly with soap, DD water and ethanol. A few drops of Repel-Silane-ES were applied to the back plate and rubbed over the surface. This makes it easier to separate the plate from the gel after the electrophoresis run. To the front glass plate, a few drops of Bind Silane were applied and rubbed over the entire surface. This prevents the gel from dislodging during staining. The base plate and front plates were assembled and tightened with clamps. Gel solution was poured into the gap between the glass plates using a syringe and a comb (49, 68, or 100 well) was inserted at the top of the gel to create loading wells. The gel was allowed to polymerize for 30–45 min. Gels can be stored overnight as long as the plate ends are wrapped in pre-wetted tissue paper (1x TBE) and covered with plastic film. Cautions: 1. If plates are not thoroughly washed air bubbles can get trapped while pouring the gel. 2. Silane is carcinogenic so gloves and a facemask should be worn when applying the solution to the glass plates.

1.7.4.2 Gel composition and preparation

For a 6% gel [plate size 38 cm x 30 cm (Bio-Rad)] 75 ml of gel solution (in 200 ml Erlenmeyer flask) was prepared by mixing 7.5 ml 10x TBE buffer (109 g Tris base, 55 g boric acid, 40 ml 0.5M EDTA, pH 8, made up to 1000 ml) 15 ml 29:1 (v/v) acrylamide:bisacrylamide and 52.5 ml distilled water. Caution: Acrylamide is a neurotoxin. Always wear gloves, goggles and facemask. The gel solution was mixed vigorously (beware of the formation of air bubbles). TEMED (100 µl) was added and mixed by swirling the flask. 400-450 µl 10% APS was added and mixed. The acrylamide solution was poured into a syringe immediately following the addition of 10% APS. The syringe was connected to the glass plates, and a comb was inserted. The gel solution is slowly poured in between both glass plates. Care is taken to prevent the formation of air bubbles. Note: Polymerization is catalyzed by the addition of freshly prepared APS, so be quick in pouring the solution between the glass plates.

1.7.4.3 Gel Run

After polymerization the gel was prepared for electrophoresis. The comb was removed. The lower tank container connected to the back of the plate and the upper reservoir were filled with approximately 650–700 ml TBE (0.5x). Care was taken to ensure that the top of the gel was covered with buffer. The top of the gel was cleaned by aspirating and dispensing TBE buffer using a Pasteur pipette to remove small fragments of gel and tiny bubbles. The comb was placed on top of the gel [(at most <1 mm deep into the gel (don't force)]. The gel was pre-run to warm it for at least 10 min at 5 V/cm (approximately 400 V, 9 W).

1.7.4.4 Sample preparation and loading

The samples were prepared for loading by mixing 4 μl PCR mix with 1 μl 5x loading buffer (0.5 M EDTA 10 ml, pH 8, 5 M NaCl 1 ml, glycerol 50 ml, and double distilled water 39 ml). Between 2 and 5 μl was loaded on the gel per sample. Depending on the size of the comb, 50 or 100 samples were loaded on a gel. Lamda size marker (2 μl with a concentration 50 ng/μl) was loaded at either end of the gel. The gel was run at approximately 5 V/cm (400 V, 9 W). Higher voltages cause the gel to overheat and will cause the samples to run un-evenly. The gel was run until the desired resolution was reached. This was determined by the dye front; when the marker band reaches three fourths or half of the gel, the electrophoresis run was stopped. After the run the plates were carefully pulled apart so that the gel remained attached to the front plate.

1.7.4.5 Visualization of DNA bands

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

1.7.4.6. Gel Staining

Following are the steps used for the modified Tegelstrom (1992) silver staining procedure.

1. Gel wash: The gel was rinsed in distilled water for 3–5 min and soaked in 2 litres of 0.1% CTAB (2 g in 2 litres of water) for 20 min with smooth shaking.

2. Incubation in ammonia solution: The gel was incubated in 0.3% ammonia (26 ml in 2 litres) for 15 min with shaking.

3. Incubation in silver nitrate solution: Silver nitrate solution was prepared (2 g silver nitrate, 8 ml of 1 M NaOH/2 litres) and titrated with ammonia until the solution became clear (6-8 ml). The gel was placed in the silver nitrate solution for 15 min and was gently agitated.

4. Gel wash: The gel was then rinsed in water for 1 min.

5. Band development: The gel was placed in developer (30 g sodium carbonate, 400 µl formaldehyde, and 2 litres water) until the bands became visible. Cautions: 1. Developer must be made fresh each time. 2. As solution becomes cloudy, replace with new solution.

6. Gel wash: The plate was rinsed in water for 1 min to stop staining.

7. Final step: The gel was placed in fixer (30 ml glycerol in 2 litres water).

1.7.4.7 Gel Scanning

The gel was kept for air-drying for overnight and was scanned. The DNA polymorphism between the parents was observed based on length of amplified fragments in terms of number of base pairs by comparing with 100 base pair ladder (100-1000 bp) (50 ng/µl). Among the different bands observed in each lane, the least base pair size of a band was considered for scoring. Note: To remove the dried gel from plate, the plate was soaked in concentrated sodium hydroxide solution (40 g flakes in 1 litre of water) for a few hours and the gel was then gently scraped off the glass plate.

1.7.4.8 Data collection and analysis

The bands in the gel were scored as A, B, H, OFF and "-" based on their pattern compared with those of the parents. "A" was defined as the homozygous presence of allele from the recurrent parent (BTx623), "B" was defined as the homozygous presence of allele from donor parent IS 18551, "H" was defined as the heterozygote (presence of both recurrent and donor parent alleles), "OFF" was defined as an allele from neither parent, and "-" was a missing data point.

1.7.5 Capillary Electrophoresis

When the polymorphism between BTx623 or 296B and IS 18551 was found to be less than 5 bp, the PCR products produced were separated using capillary electrophoresis (ABI 3700 automated DNA sequencer, Applied Biosystems). For this purpose, forward primers were labeled with 4,7,2',4',5',7'-hexachloro-6-carboxyfluorescein (HEX), 6-carboxyfluorescein (6-FAM) or 7',8''-benzo, 5'-fluoro-

2', 4,7 trichloro-3-carboxyflourescein (NED) (Applied Biosystems). PCR products were pooled post-PCR, where 0.5 μl of the 6-FAM-labeled product, 0.5 μl of 6-HEX-labeled product and 1 μl of the 6-NED-labeled product were mixed with 0.05 μl of the ROX-labeled 500 HD size standard (Applied Biosystems) and formamide (Applied Biosystems) in a total volume of 12 μl. DNA fragments were denatured for 5 min at 94°C (Perkin Elmer 9700, Applied Biosystems) and size fractioned using capillary electrophoresis. The Genescan 3.1 software (Applied Biosystems) was applied to size the peaks patterns (Fig. 4), using the internal ROX 500 HD size standard and Genotyper 3.1 software (Applied Biosystems) was used for allele definition.

1.8.1 Evaluation of Near-isogenic lines for phenotypic characters: Locations, Seasons and Experimental Designs

1.8.1.1 *Kharif* Field Evaluation (2006)

The experiment was conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India in the *Kharif* season of 2006. The experimental material consisted of 29 simple sequence repeat-assisted backcross introgression near-isogenic lines that were carrying different individual shoot fly resistance QTLs (in homozygous condition) in the elite genetic background of BTx623. Two of these 29 test-entry genotypes were derived from RIL-derived backcross introgression homozygotes (for QTL A) after two backcrosses with BTx623 ($RILBC_2F_3$). Parental genotypes BTx623 (4 entries), IS 18551 (4 entries) and 296B (3 entries) were used as control entries; three RIL parents carrying different shoot fly resistance QTLs in multiple combinations *viz*, 4 entries each of RIL 153 (AEGJ), RIL 189 (AEJ), and RIL 252 (AGJ); and standard control entries IS 2312 (highly resistant; 4 entries), IS 1054 (moderately susceptible; 4 entries) and Swarna (highly susceptible; 4 entries) were also included in the field experiment. Shoot fly infestation was optimized in the test plot through use of the interlard fishmeal technique (Nwanze, 1997). Interlards of four rows of susceptible cultivar Swarna were sown 20 days before sowing of the test material, and open polyethylene bags of moistened fishmeal were distributed at regular intervals throughout these interlards. The susceptible cultivar served to multiply shoot fly attracted by the fish meal, hence providing a uniform sorghum shoot fly density in the later-sown test materials. The test materials were sown during the second week of July 2006. Each genotype was sown in single-row plots of 2-m length with inter-row spacing of 75 cm and inter-plot spacing of one meter within the row. There were six replications laid out in an 8x8 alpha lattice design.

Plate 5: BC_4F_2 background introgression lines screened for allelic composition at SSR marker locus Xtxp94 (50 samples) and RIL parents RIL 166, RIL 189, RIL 252 and RIL 153 (10 samples each).

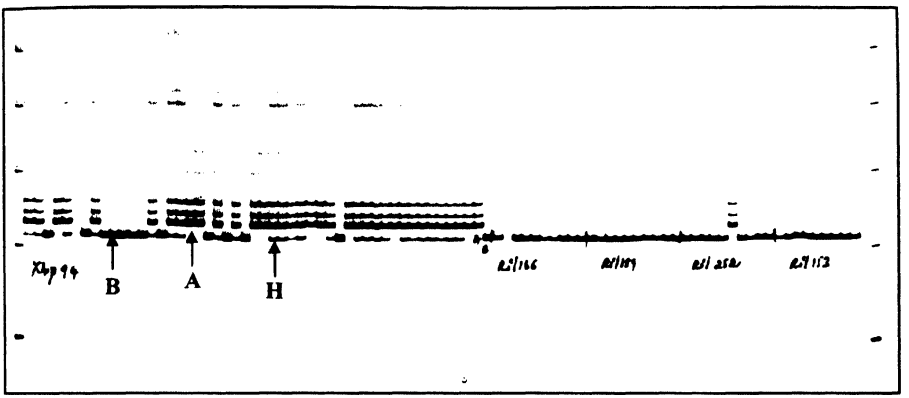
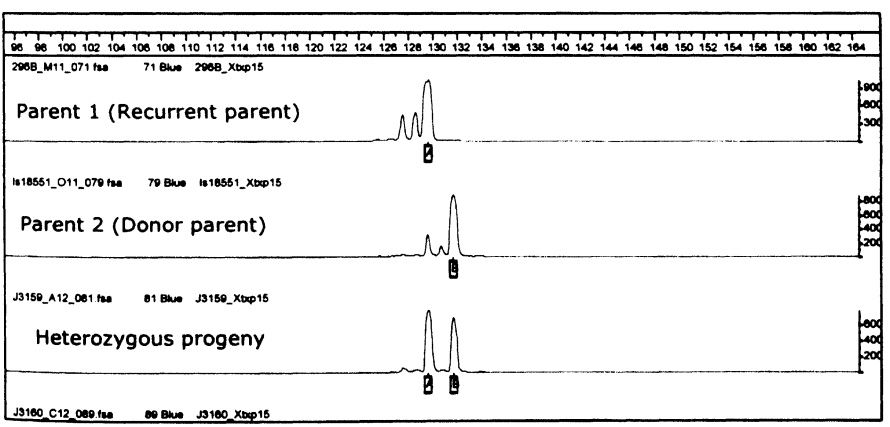


Figure 4. ABI chromatogram



The seed was sown with a four-cone planter at a depth of 5 cm below the soil surface. The field was irrigated immediately after sowing. Ten days after seedling emergence, thinning was carried out to maintain a spacing of 10 cm between plants within each experimental plot. Normal agronomic practices were followed for raising the sorghum crop and no insecticide was applied in the experimental plots. The interlard infester rows were chopped off 30 days after emergence in the main plots to avoid shading effects in the test plots.

Data were recorded on number of eggs and numbers of plants with eggs at 14 and 21 days after seedling emergence (DAE), and plants with deadhearts at 14 and 21 DAE on all plots. The data on number of eggs was expressed as number of eggs per 100 plants, and plants with eggs and deadhearts in terms of percentage of the total number of plants. Data were also recorded on plant traits such as leaf glossiness, trichome density on abaxial (lower) and adaxial (upper) surfaces of the leaf blade, and seedling vigor. Leaf glossiness was evaluated on a 1-5 scale at 9 DAE in the early morning hours when there was maximum reflection of light from the leaf surfaces (1 = highly glossy, light green, shining, narrow and erect leaves; and 5 = non-glossy, dark green, dull, broad and drooping leaves). To record data on trichome density, the central portion of the third leaf from the base was taken from seedlings selected at random for all six replications. The leaf pieces (approximately 2 cm^2) were placed in acetic acid and alcohol solution (2:1) in a stoppered glass vial (10 ml capacity). The leaf pieces were kept in this solution for 24 h. and thereafter transferred to 90% lactic acid. Leaf segments thus cleared of their chlorophyll content were then observed for their trichome density. The cleared leaf sections were mounted on a slide in a drop of lactic acid and observed under a stereomicroscope at a magnification of 100x (10x10). The trichomes on both abaxial and adaxial surfaces of the leaf sections were counted in three microscopic fields selected at random and expressed as the number of trichomes per microscopic field. Seedling vigor was recorded at 9 DAE on a 1-5 rating scale (1 = highly vigorous, plots showing a large number of fully expanded leaf blades and robust seedlings; 5 = poor seedling vigor, plots showing poor growth and weak seedlings).

1.8.1.2 *Rabi* Field Evaluation (2006)

The *Rabi* season field evaluation of shoot fly resistance QTL introgression near-isogenic lines was sown at ICRISAT, Patancheru, Andhra Pradesh, India, in the first week of November 2006. The *Rabi* experimental material consisted of two

sets of materials. Tne first set of materials was that used in *Kharif* screening with some 20 additional entries. The additional entries included selfed progenies of plants J2886 (-J1+J2?(AAAB)), J2946 (-J1+J2?(AAAB)), J2982 (+J1-J2?(BBBA)), J2801 (-QTL A), J2822 (-QTL A), J2808 (AB recombinant for QTL A), J2869 (+J1+J2), J2890 (+J1+J2), J2898 (+J1+J2), J2936 (+J1+J2), J2965 (+J1+J2), J2895 (-J1-J2), J2947 (-J1-J2), J2967 (-QTL J (*Xisp258*-H)), J2990 (-J1-J2), J2867 (-J1-J2), J2998 (??), Swarna, IS 1054, and IS 2312. These BTx623-background test materials were sown in a 9x9 alpha lattice design in 6 replications. Each genotype was sown in single-row plots of 2-m length with inter-row spacing of 75 cm and intra-plot spacing of one meter within the row. All other conditions were maintained at the same level as that of the *Kharif* season trial. Data were recorded on all plots in these BTx623-background materials for glossiness (9 DAE), seedling vigor I (9 DAE), seedling vigor II (16 DAE), number of eggs and numbers of plants with eggs at 14 and 21 DAE, and plants with deadhearts at 14, 21 and 28 DAE. The second set of materials evaluated were comprised of 110 entries of 296B-background near-isogenic shoot fly resistance QTL introgression lines, their parents, and controls laid out in an 11x10 alpha lattice design in 6 replications. Each genotype was sown in single-row plots of 2-m length with inter-row spacing of 75 cm and intra-plot spacing of one meter within the row. All other conditions were maintained at the same level as that of the BTx623-background trial. For these 296B-background materials, leaf glossiness was recorded at 9 DAE, seedling vigor score at 9 DAE and 16 DAE, egg count and plants with eggs at 21 DAE, and deadhearts count at 14 DAE, 21 DAE, and 28 DAE. The data on number of eggs was expressed as the number of eggs per 100 plants, and that for plants with eggs and deadhearts in terms of percentage of the total number of plants.

1.8.1.3 *Kharif* Field Evaluation (2007)

The 2007 *Kharif* season field evaluation of shoot fly resistance QTL introgression near-isogenic lines was sown at ICRISAT, Patancheru, Andhra Pradesh, India, in the first week of August 2007. The experimental material consisted of identical sets of materials used in 2006 *Kharif* and *Rabi* experiments with reduced numbers of replications. BTx623-background introgression lines were evaluated as 64 entries in 2-row plots of 4-m length and 4 replications in an 8x8 alpha lattice design. 296B-background introgression material was subdivided into two sets, one with 84 entries arranged in 2 replications sown in single-row plots of 2-m length in a 12x7 alpha lattice design, and the second with 20 entries arranged 3 replications in 2-row plots of 4-m length in a 5x4 alpha lattice design. All field

conditions were maintained at the same levels as that of the prior *Kharif* season trial. Data were recorded on glossiness (9 DAE), number of eggs (from single replication) and numbers of plants with eggs at 14 DAE and plants with deadhearts at 14 and 21 DAE were recorded on all plots in the BTx623-background material. For the 296B-background material (84-entry set), leaf glossiness (9 DAE), number of eggs and plants with eggs count at 14 DAE and deadhearts count at 14 and 21 DAE were recorded. For the 20-entry set of 296B-background materials, glossiness (9 DAE), number of eggs (14 DAE), number of plants with eggs (14 and 21 DAE) and deadhearts count (14 and 21 DAE) were recorded. The data on number of eggs was expressed as number of eggs per 100 plants, and those for plants with eggs and deadhearts were expressed in terms of percentage of the total number of plants.

1.8.2 Shoot fly Resistance Screening Techniques

To attain uniform shoot fly pressure under field conditions the interlard-fish meal technique (Nwanze, 1997) was followed for resistance screening. Four rows of a susceptible cultivar (Swarna) was sown 20 days before sowing the test material. This was done to allow multiplication of shoot fly for one generation. Ten days after seedling emergence of the test material, polythene bags containing moistened fish meal were kept in the test material at uniform intervals covering the entire area to attract the emerging shoot flies from infester rows. Plant protection measures were avoided until the shoot fly infestation period was complete. However, chemical spray was carried out when the level of shoot fly infestation in the susceptible check entries was more than 70%.

1.8.2.1 Observation

Observations on leaf glossiness (1-5 scale), seedling vigor (1-5 scale), trichome density (number per microscopic field), oviposition (%), and deadhearts (%), were recorded in both *Kharif* and *Rabi* screening environments.

1.8.2.1.1 Glossiness

Intensity of glossiness was recorded at 7 DAE (*Rabi*), 9 DAE (*Kharif*) on a 1 to 5 scale where 1 = high intensity of glossiness and 5 = non-glossy (Plates, 6a and 6b). Leaf glossiness was scored in the morning hours when there was maximum reflection of light.

1.8.2.1.2 Seedling vigor

Seedling vigor (height, leaf growth and robustness) was scored at 9 DAE (*Kharif*), 7 DAE to 16 DAE (*Rabi*), on a 1-5 scale where 1 = high seedling vigor (plants showing maximum height, leaf expansion and robustness) and 5 = low seedling vigor (plants showing minimum growth, leaf expansion and poor adaptation) (Plates, 7a and 7b). The seedlings being recorded at 7 and 16 DAE were designated as seedling vigor I and seedling vigor II, respectively.

1.8.2.1.3 Trichome density

For recording leaf trichome density (Plates, 8a and 8b), the central portion of third leaf from the base was taken from three randomly selected seedlings in each entry at 12 DAE in both the *Kharif* and *Rabi* screening environments. Thus a total of 54 observations per entry mean (3 plants x 3 microscopic fields x 6 replications) on upper and lower leaf blade surfaces were recorded in the *Kharif* 2006 and *Rabi* 2006 assessments of BTx623-background materials, while the total number of observations per entry mean for this character was reduced in the *Kharif* 2007 assessments.

1.8.2.1.4 Oviposition

Total number of plants with eggs in each entry was recorded twice with an interval of 7 days in the *Kharif* 2006 screen, at 14 and 21 DAE. Egg counts were taken at 15 and 23 DAE, delayed by 1-3 days, in the *Rabi* environment for BTx623-background material. In 296B-background material, a single egg count was taken at 23 DAE in the *Rabi* 2006 screen. The observations on oviposition recorded at these two stages are referred to here onwards as oviposition I and oviposition II. Oviposition counts were expressed in terms of percentage. A typical plant with eggs laid on the lower leaf blade surface is shown in Plate 9.

$$\text{Oviposition (\%)} = \frac{\text{Number of plants with Eggs}}{\text{Total number of plants}} \times 100$$

1.8.2.1.5. Deadhearts

Deadhearts count was recorded at least twice at 7-day intervals in all three screening environments. Deadhearts count was carried out at 14 and 21 DAE in the two *Kharif* screening environments. In the *Rabi* screening environment,

Plate 6(a): glossy leaves

Plate 6(b): non-glossy leaves

Plate 7a: seedling vigor (BTx623)

Plate 7b: seedling vigor (IS18551)

Plate 8a: trichomed

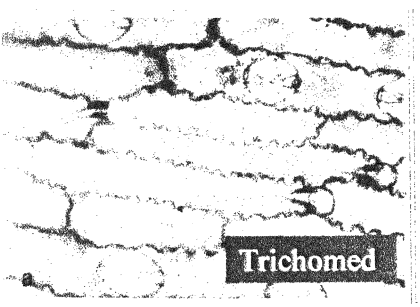


Plate 8b: non-trichomed

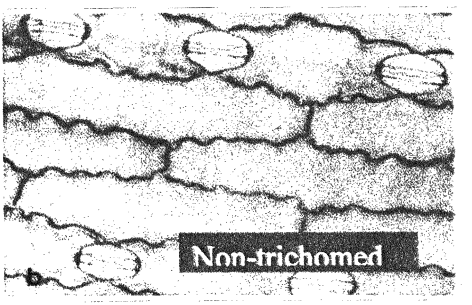


Plate 9: Shoot fly eggs on undersurface of the leaf blade

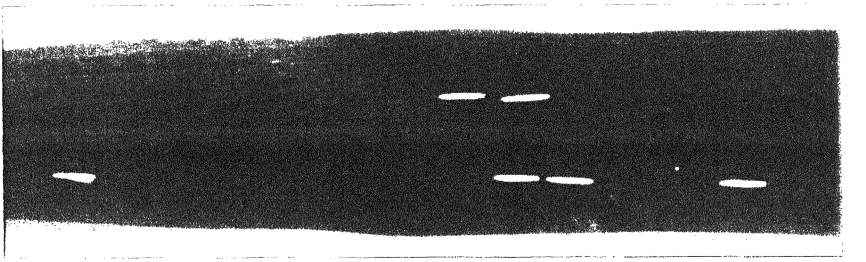
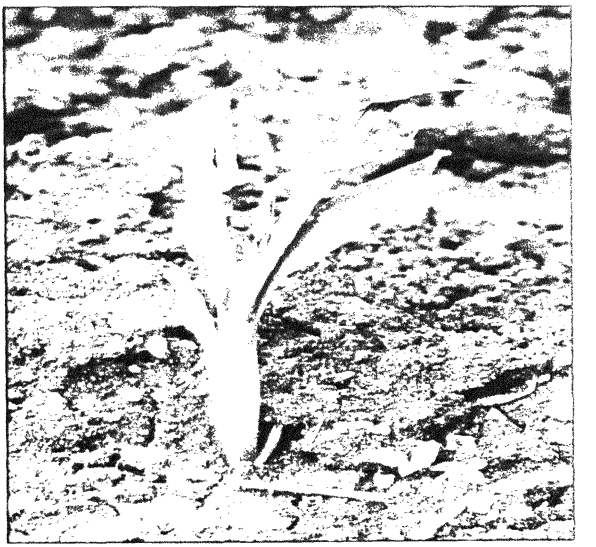


Plate 10: Dead heart formation on a shoot fly susceptible plant



deadhearts count was taken at three intervals, 14, 21 and 28 DAE. The observations on deadhearts (%) recorded at these three stages of seedling growth are referred here onwards as deadhearts I, deadhearts II and deadhearts III. A single plant with deadheart symptoms due to damage by shoot fly is shown in plate 10.

$$\text{Deadhearts (\%)} = \frac{\text{Number of plants with Deadhearts}}{\text{Total number of plants}} \times 100$$

1.9 Phenotypic Data Analysis

1.9.1 Analysis of Variance

The analysis of variance for observed components of resistance was performed using the residual maximum likelihood algorithm (ReML) introduced by Patterson and Thomson (1971), which provides best linear unbiased predictions (BLUPs) of the performance of the genotypes. ReML estimates the components of variance by maximizing the likelihood of all contrasts with zero expectation. Entry means were estimated by generalized least squares with weights depending on the estimated variance components according to Patterson (1997). The data was analyzed using the GENSTAT (9th edition) statistical software package.

Test of Significance of Means

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

where,

$$s_1^2 = \frac{\sum (x_{i1} - \bar{X}_1)^2}{n_1 - 1}$$ (Variance of sample 1 mean)

$$s_2^2 = \frac{\sum (x_{i2} - \bar{X}_2)^2}{n_2 - 1}$$ (Variance of sample 2 mean)

Degrees of freedom $(n_1 + n_2) - 2$

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULT

The experimental study was carried out to introgress shoot fly resistance QTLs, previously identified from a RIL mapping population of cross BTx623 x IS 18551, into elite shoot fly susceptible breeding lines BTx623 and 296B. The BC_1F_1 seed material of crosses BTx623 x IS 18551 and 296B x IS 18551 were obtained from Dr BVS Reddy. Simultaneously four recombinant inbred lines with maximum shoot fly resistance, *viz.*, RIL 153, RIL 166, RIL 189 and RIL 252 identified from Dr. Gowri Sajjanar's mapping population were utilized as donor parents.

2.1 Parental polymorphism testing

Parental polymorphism check was performed between the parents involved in the present introgression program, *viz.*, BTx623, 296B and IS 18551. Fifty-two SSRs, distributed across the 10 sorghum linkage groups — SBI-01 (A), SBI-02 (B), SBI-03 (C), SBI-04 (D), SBI-07 (E), SBI-09 (F), SBI-10 (G), SBI-08 (H), SBI-06 (I), and SBI-05 (J) — were tested for parental polymorphism between the parental lines BTx623 and IS 18551, to determine their rough allele sizes, which helped in later identification of the alleles on PAGE or capillary electrophoresis (Table 9). Among them *Xcup62*, *Xcup48*, *Xtxp18*, and *Xisp278* were monomorphic, and *Xisp257* showed multiple bands. About 30 SSRs tested for allelic scoring between the two parental lines 296B and IS 18551, 19 SSRs showed sufficient polymorphism, where as 11 SSRs were monomorphic and hence could not be used in this study (Table 10).

2.2 Backcross I

Forty-four (DNA samples J1-J44) BC_1F_1 seed of the cross with 296B were sown in July 2003 along with two parental controls *viz.*, 296B (J45 and J46) and IS 18551 (J47 and J48). These plants were screened with polymorphic simple sequence repeat (SSR) markers mapping to four QTL locations identified for shoot fly deadhearts incidence (Table 11). The markers included *Xtxp37* and *Xtxp75* for the QTL on SBI-01 (A), *Xtxp159* and *Xtxp40* for the QTL on SBI-07 (E), *Xtxp141* and *Xgap1* for the QTL on SBI-10 (G), and *Xtxp65* and *Xtxp15* for the QTLs on SBI-05 (J). Marker *Xtxp94* was monomorphic across parents 296B and IS 18551, and hence it was not used. Eight selected BC_1F_1 plants with shoot fly resistance QTLs in single and multiple combinations were advanced (one was in fact an F_2 plant resulting from self-pollination in the previous F_1 generation, as indicated by homozygosity for a donor parent marker allele). The selected individuals (Table

12) were backcrossed to 296B and also selfed; however, only the crossed seed was subsequently utilized in this study.

Simultaneously, 124 (DNA samples J184-J315) BC_1F_1 seed of the cross with BTx623 and 2 parental controls, *viz.*, BTx623 (J276 and J277 on plate 1, and J312 and J313 on plate 2) and IS 18551 (J278 and J279 on plate 1, and J314 and J315 on plate 2) were screened with *Xtxp37*, *Xtxp75*, *Xtxp159*, *Xtxp40*, *Xtxp141*, *Xgap1*, *Xtxp65*, *Xtxp94* and *Xtxp15* (Table 13). In this case, *Xtxp94* was polymorphic between BTx623 and IS 18551 and so was utilized in the screening. Donor parent marker allele homozygosity indicated that a substantial portion of these "BC_1F_1" individuals were in fact F_2 individuals produced by self pollinations of their female F_1 parent rather than backcrosses to their recurrent parent. The 16 selected individuals (three of them F_2s rather than BC_1F_1s) with various single- and multiple-QTL combinations were backcrossed to BTx623 and also selfed. Only the backcross seed was advanced to the next generation. The harvested panicles were dried for some time, threshed and packeted. The seed packets were labeled properly for easy identification. The amount of backcross seed obtained from each cross was counted, and selfed seed was weighed. The amount of harvested seed that was obtained and the number of seed advanced in the next generation is indicated (Table 14).

2.2.1 Screening of crossed recurrent parents

BTx623 and 296B parents (13 individual plants) involved in BC_1F_1 crosses were singled out and parental purity check was performed on them with foreground markers, comparing them against standard BTx623, 296B and IS 18551 DNA samples as controls. J90 and J91 were carrying all homozygous recurrent parent alleles as expected (296B). Similarly, J583, J594, J595, J616, J618 and J632 were observed to be carrying all recurrent parent (BTx623) alleles. However, J614 and J663 were outcrosses. Unfortunately, due to a data entry mistake in the BC_1F_1 harvesting list, individual plants J558, J562 and J566 (of RIL 252) were labeled as the recurrent parent. Results of the parental purity check disclosed this error (Table 15).

2.3 Backcross II

Seventy-six (J1233-J1308) "BC_2F_1" progeny (and parents) from 8 first generation backcross (296B) parents were genotyped (Table 16). Segregation for donor parent marker homozygosity indicated that many of the genotyped individuals were in fact products of selfing, not backcrossing, in the previous generation.

Eleven individuals with different QTL combinations were selected and crossed with the recurrent elite parent 296B and cross seed from these was collected. Six "BC_2F_1" individuals (two of them actually F_3 individuals resulting from two consecutive generations of failed backcrosses, and three of them BC_1F_2 individuals resulting from selfing in the previous generation, as indicated by donor parent marker allele homozygosity) were subsequently selected for advancement (Table 17) based on combined foreground and background marker data, and availability of crossed seed. Since marker *Xtxp94* is monomorphic between 296B and IS 18551, a polymorphic marker (*Xisp258*) located near to this was identified and used as a substitute for *Xtxp94*. Similarly, *Xtxp65* was substituted with *Xtxp23*.

Simultaneously, 294 (J901-J1195) "BC_2F_1" progeny, produced from attempted backcrosses of 16 first generation backcross (BTx623) parents, were analyzed for the presence of shoot fly resistance QTLs (Table 18). As for the 296B-background progenies, a substantial portion of these BTx623-background progenies appeared to have resulted from failed backcrosses (indicated by the presence of donor parent marker allele homozygosity at one or more loci). Fifty-one individuals found to have targeted shoot fly resistance QTLs in single and multiple combinations were backcrossed to recurrent parent BTx623 and the crossed seed from 37 of these (Tables 19a and 19b) was collected and preserved for advancement.

2.3.1 Background screening on BC_2F_1 adjacent flanking markers

Background screening was performed on the foreground-selected second generation backcross introgression progeny of parents 296B and BTx623. Eleven BC_2F_1 (296B-background) individuals (J1233, J1234, J1244, J1260, J1262, J1288, J1296, J1299, J1302, J1306 and J1308) were background screened at markers mapping adjacent to QTL flanking regions. From these, six individuals (J1234, J1244, J1262, J1288, J1296, and J1302) were selected for third backcross advancement (Table 17). For the QTL on SBI-01, background markers used were *Xtxp319* on top and *Xtxp32* on bottom; for SBI-07, *Xisp348* on top and *Xtxp312* on bottom were used; and for SBI-05, *Xisp258* on top and *Xtxp283* on bottom were used (Table 20).

Fifty-one (BC_2F_1 (BTx623-background) progeny were background screened using flanking markers linked adjacent to the target QTL regions (Tables 21 and 22). Markers *Xcup62* and *Xtxp88* were used as background selection flanking markers for the QTL on SBI-01 (A); *Xgap342* was used for the QTL on SBI-07 (E); *Xisp263*

and *Xcup07* were used for the QTL on SBI-10 (G); and *Xisp258* and *Xtxp225* were used for QTLs J1 and J2 on SBI-05 (J). From the available 46 background-screened genotypes, 24 advanced samples (Table 19a) were categorized to have single QTLs and background screening revealed the presence of mostly type-2 (homozygous for the recurrent parent allele at one of the flanking markers) and type-4 (heterozygous for the recurrent parent allele at both flanking markers) recombinants. However, J901, J909, and J920 (heterozygous for the donor allele of the SBI-01 target QTL) are type-1 recombinant individuals, which are homozygous for the recurrent parent allele at both flanking markers. A carrier chromosome of this type has by expectation the smallest proportion of donor genome and can be regarded as the final product of a gene introgression program. Other nine single-QTL samples in the background-screened plants list, most of them type-1 recombinants, *viz.*, J919 (SBI-07), J932 (SBI-07), J1127 (SBI-01), J1185 (J1 on SBI-05), J1187 (J1 on SBI-05), or type–2 recombinants, *viz.*, J993 (SBI-01), J1106 (SBI-07), J1186 (J1 on SBI-05), and J1188 (J1 on SBI-05), were good for advancement but not advanced as other background-screened genotypes were already planted (Table 21).

Similarly, a set of 13 genotypes in BTx623 background were selected for advancement having type-1 and/or type-2 recombinations in multiple QTL combinations/introgressions (Table 19b, Table 22), *viz.*, QTLs on SBI-01 and SBI-07 (J904, J924, and J1048), QTLs on SBI-07 and SBI-10 (J927 and J988), QTLs on SBI-07 and SBI-05 (J942 and J956), QTLs on SBI-07 and SBI-05 (J1) (J933, J950 and J1111), QTLs on SBI-01, SBI-07 and SBI-05 (J1) (J1024, J1046), and QTLs on SBI-10 and SBI-05 (J2) (J966).

2.4 Backcross III

2.4.1 Single QTL introgressions

One hundred and sixteen BC_3F_1 progeny (J1401-J1526 excluding J1467-J1473) from 24 background-screened single-QTL BC_2F_1 x BTx623 crosses were genotyped (Table 23). As in previous generations, there was ample evidence of failures in backcrossing (indicated by segregation of homozygous donor parent alleles at various loci). For the target QTL on SBI-10 (G), self seed was advanced from J1407 and J1409, and the resulting 40 BC_3F_2 progeny were genotyped at markers *Xgap1* and *Xtxp141* flanking this QTL to identify putative QTL introgression heterozygotes (Table 24). Heterozygous BC_3F_1 individuals for shoot fly resistance QTLs on SBI-01 (J1458, J1463, J1474, and J1481), on SBI-07 (J1413, J1422, J1424, J1434, J1440, and J1445), on SBI-05 (J1487 (J1), J1492

(J), J1495 (J), J1502 (J), J1505 (J), and J1506 (J)) and on SBI-05 (J2) (J1500, and J1508) were advanced by backcrossing with BTx623 (Table 25).

2.4.1.1 BC_3F_2 for target QTL G

In the BTx623 recurrent parent background, a data-tracking mistake occurred in the BC_3F_1 marker data sheet and was identified from the BC_4F_1 generation marker data. Following correction of the tracking error, plant numbers J1407 and J1409 were identified as having heterozygous alleles for markers flanking the target QTL on linkage group SBI-10 (G). As these plants were not considered to be putative QTL carrier genotypes at the time the BC_3F_1 marker data was originally generated, they were not backcrossed but their selfed seed was available. Ten BC_3F_2 seed each from J1407 and J1409 was sown, the seedlings genotyped (Table 24), and target QTL G homozygotes advanced by selfing to BC_3F_3 (but not backcrossed to produce BC_4F_1 seed due to shortage of time). Among the putative QTL homozygotes identified, selfed seed from J2614 was subsequently used in the field screening for as the +G QTL introgression line.

2.4.2 Multiple QTL introgressions

Eighty-eight "BC_3F_1" BTx623-background progeny (J1600-J1688) from 12 "BC_2F_1" x BTx623 crosses were genotyped at foreground marker loci (Table 26). Segregation of donor parent allele homozygotes indicated large-scale failure of backcrossing in the previous generation. Individuals heterozygous for markers flanking single target QTLs on SBI-01 (J1647, J1651, and J1654), SBI-07 (J1656), SBI-10 (J1666, J1667, and J1670), SBI-05 (J = J1+J2) (J1630, J1632, and J1637), SBI-05 (J1) (J1616, J1618, J1620, J1633, J1636, and J1638), and combinations of QTLs on SBI-01 and SBI-05 (J1) (J1646) were backcrossed to BTx623. Harvested seed was dried, threshed, packeted and labeled (Table 27).

DNA samples were prepared from 41 "BC_3F_1" 296B-background progeny (J1689-J1729) from 6 "BC_2F_1" x 296B crosses were genotyped at marker loci flanking shoot fly resistance QTLs on three linkage groups (Table 28). Selected individuals heterozygous for donor parent alleles at marker loci flanking target QTLs on SBI-01 (J1690, J1692, J1696, J1698, J1700, J1702, J1723, J1724, J1727, J1728, J1729), SBI-05 (J) (J1706 and J1712), on SBI-07 (J1695 and J1707), and combinations of QTLs on SBI-01 and SBI-05 (J1690, J1715) were backcrossed to 296B. Harvested seed was dried, threshed, packeted and labeled (Table 29).

2.4.3 Background screening on BC_3F_1 (BTx623-background) progenies

The BC_3F_1 female parents of target QTL introgression heterozygote backcrosses available for advance from both sets (Tables 25 and 27) were identified and brought into a single list. Nearly all of them ended up having single-QTL targets. There were large numbers of individuals in each shoot fly resistance single-QTL target category. Therefore background genotyping was performed initially on the QTL carrier linkage group and later on non-carrier linkage groups (Tables 31-35). This was done to reduce the number of BC_4F_1 families to be advanced, choosing for advance those progenies with the smallest amount of donor background genome. Backcrosses of seven "BC_3F_1" individuals heterozygous for markers immediately flanking the target QTL on SBI-01 (J1647 x J1930, J1651 x J1936, J1654 x J1952, J1458 x J1538, J1463 x BTx623, J1474 x J1550, and J1481 x J1540) were background screened with eight additional SBI-01 markers (*Xtxp316*, *Xtxp248*, *Xtxp319*, *Xtxp32*, *Xtxp357*, *Xcup73*, *Xtxp208* and *Xtxp302*). Two best option progenies were selected from among them. BC_4F_1 (actually BC_3F_1 due to failure of back cross in first generation of backcross resulted in formation of F_2 instead of BC_1F_1 formation) J1481 x J1540 family was the best option for advance as its female parent had homozygous recurrent parent alleles at seven of eight background loci on SBI-01. J1647 x J1930 was the second best option with its female parent having homozygous recurrent parent alleles at six of these eight loci (Table 31). The BC_4F_1 backcross seed produced on each of these two BC_3F_1 parents was advanced.

Female parents of seven backcrosses targeting the QTL on SBI-07 (J1413 x J1541, J1422 x J1558, J1424 x J1540, J1434 x J1541, J1440 x J1537, J1445 x J1536, J1656 x J1966) were background screened with three additional SBI-07 markers *Xtxp312*, *Xtxp295* and *Xisp344*, which were distributed below the target QTL in this linkage group. Five individuals having the best available carrier chromosome genotype were selected (recurrent parent allele homozygote at background loci *Xtxp295* and *Xisp344*, and heterozygous at *Xtxp312* as well as foreground loci *Xtxp159* and *Xtxp40*) (Table 31). These were further background screened with three SSRs on each of the nine non-carrier linkage groups (total 29) to further reduce the progeny number for advance to two (Table 32). Crosses J1422 x J1558 (female parent 85% homozygous for recurrent parent alleles and 15% heterozygous) and J1440 x J1537 (female parent 79% homozygous for recurrent parent alleles, 3% homozygous for donor parent alleles, and 18% heterozygous), were selected for advance.

Female parents of seven backcrosses targeting the J QTL (both J1 and J2 QTLs combined) on SBI-05 (J1495 x J1541, J1502 x J1537, J1505 x J1545, J1506 x J1525, J1630 x J1964, J1632 x J1965 and J1637 x J1969) were background screened with two additional SBI-05 markers *Xisp258* and *Xtxp262*. Six individuals having the best available carrier chromosome genotype (heterozygous at *Xisp258* and homozygous for the recurrent parent allele at *Xtxp262*) were observed (Table 33). These six individuals were further background genotyped with 31 SSRs to reduce to three the number of BC_4F_1 families to be advanced (Table 34). J1630 x J1964 (female parent 69% homozygous for recurrent parent alleles and 31% heterozygous), J1632 x J1965 and J1637 x J1969 (female parents 64% homozygous for recurrent parent alleles and 33% heterozygous) were chosen for advance. Donor allele homozygote segments were not detected in the female parents of all three of these BC_4F_1 families selected for advance (Table 34).

Female parents of seven backcrosses targeting the J1 QTL and three backcrosses targeting the J2 QTL on linkage group J (SBI-05) (J1487 x J1536, J1616 x J1947, J1618 x J1982, J1620 x J1925, J1633 x J1928, J1636 x J1933, J1638 x J1956; J1492 x J1541, J1500 x J1537, J1508 x J1549) were background screened with the two additional SSRs on linkage group SBI-05. All were selected as joint best options for their respective single-QTL targets (heterozygous at *Xisp258* and homozygous for the recurrent parent allele at *Xtxp262*) (Table 33). These were further background screened with 32 SSR loci distributed across the nine non-carrier linkage groups to reduce to three the plant number of BC_4F_1 families to be advanced (Table 35). J1638 x J1956 and J1636 x J1933 (female parents homozygous for 86% of recurrent parent alleles tested, 14% heterozygous), being the joint best options for the 'J1' QTL, and J1633 x J1928 (female parent homozygous for 83% of recurrent parent alleles tested, 17% heterozygous) as the second best 'J1' option, were selected for advance. From the three 'J2' target QTL genotypes that were background screened, J1492 x J1541 (actually heterozygous at both J1 and J2) was chosen for advance (female parent homozygous for 83% of recurrent parent alleles tested and 17% heterozygous).

2.4.4 Background screening on BC_3F_1 (296B)

"BC_3F_1" female parents of eleven 296B-background backcrosses targeting the QTL on linkage group A (SBI-01) were extensively background screened on this carrier linkage group with SSR markers *Xtxp316*, *Xtxp248*, *Xtxp319*, *Xtxp32*, *Xtxp88*, *Xtxp149*, *Xtxp302*, *Xgap206*, and *Xgap57* (Table 36). J1696, having the highest

percentage recurrent parent genome recovery (89% homozygous and 11% heterozygous) and four joint second best individuals (78% homozygous recurrent parent alleles and 22% heterozygous) were then background screened with 27 additional SSRs distributed across the nine non-carrier linkage groups (Table 37). Crosses J1692 x J2079 (female parent 63% homozygous for recurrent parent alleles, 34% heterozygous, and 3% missing data), J1690 x J2072 (female parent 63% homozygous for recurrent parent alleles, 32% heterozygous, and 5% missing data), and J1698 x J2019 (female parent 66% homozygous for recurrent parent alleles, 26% heterozygous, 5% homozygous for donor parent alleles, and 3% missing data) were selected for advance (Table 37).

"BC_3F_1" female parents of two 296B-background backcrosses targeting the QTL on linkage group E (SBI-07) were background screened with 4 SSRs on this carrier linkage group (*Xisp348*, *Xtxp312*, *Xtxp36* and *Xisp310*). J1707 x J2057 (female parent homozygous for recurrent parent alleles at two of these loci and heterozygous at the other two) was identified as the best option, and J1695 x J2062 (female parent homozygous for recurrent parent alleles at only one of these loci and heterozygous at the other three) was the second best available option (Table 36). Both were advanced.

Similarly, the BC_3F_1 female parent of a 296B-background backcross (J1712 x J2027) targeting the two putative shoot fly resistance QTLs (J1 and J2) on linkage group SBI-05 was background screened with 2 SSRs on the carrier linkage group (*Xisp215* and *Xtxp23*). It proved to be homozygous for the recurrent parent allele at distal marker Xtxp23 and heterozygous at *Xisp215* (Table 36). The backcross of this plant was advanced.

2.4.5 BC_3F_1 multiple QTL introgressions (early sown materials, sown along with RIL BC_2F_1)

Backcrosses made on a small number of foreground-selected "BC_3F_1" plants (J2101-2107 in BTx623 background and J2188-J2207 in 296B background) were sown before sowing of the materials described in the previous paragraphs, which were sown after completion of the background screening (Table 30). The female parents of some of these "BC_4F_1" plants were later background screened (after sowing of their "BC_4F_1" progenies) along with the above BC_3F_1 genotypes. The following plant numbers were sown early and later background screening was performed on their "BC_3F_1" female parents. BC_4F_1 plant numbers J2101-J2107 (BTx623 background), derived from cross J1646 x J1929, were observed to segregate for two QTLs on SBI-01 and SBI-05 (J1), as expected. Plant numbers

J2202-J2207 (296B-background BC_3F_1s) derived from cross J1712 x J2027, were expect to segregate for QTL J2 on SBI-05. Plant numbers J2208-J2220 (296B-background BC_4F_1s) derived from cross J1706 x J2059, segregated for one of two expected QTLs (that on SBI-07, but not for J2 on SBI-05). BC_2F_1 plants J2188-J2194 and BC_3F_1 plants J2195-J2201 (296B-background progeny of crosses J1707 x J2057 and J1695 x J2062, respectively) segregated as expected for the SBI-07 shoot fly resistance QTL. Female parents J1646 and J1706 were not background screened, whereas and J1695, J1707, and J1712 were background screened (Table 36).

2.5 Backcross IV

"BC_3F_1" x BTx623 crosses selected for advancement following background screening (Tables 25 and 27) targeted shoot fly resistance QTLs on three linkage groups as follows:

- QTL A on SBI-01 — J1481 x J1540 (BC_3F_1: J2251-J2270) and J1647 x J1930 (BC_4F_1: J2271-J2274);
- QTL E on SBI-07 — J1422 x J1558 and J1440 x J1537 (BC_4F_1: J2275-J2304);
- QTL J = J1+J2 on SBI-05 — J1630 x J1694, J1632 x J1965, and J1637 x J1969 (BC_4F_1: J2335-J2428), and J1492 x J1541 (BC_4F_1: J2459-J2488);
- QTL J1 on SBI-05 — J1638 x J1956, J1636 x J1933, and J1633 x J1928 (BC_4F_1: J2429-J2458).

From the foreground marker data (Table 38), individuals heterozygous for donor parent alleles at marker loci immediately flanking shoot fly resistance QTL alleles for the following targets were identified for background screening:

- QTL A on SBI-01 — J2252, J2256, J2257, J2260, J2264, J2266, J2267, J2269, J2270, J2271, J2273, and J2274;
- QTL E on SBI-07 — J2278, J2283, J2288, J2291, and J2300;
- QTLs J1 and J2 on SBI-05 — J2337, 2349, J2352, J2353, J2362, J2364, J2366, J2372, J2373, J2374, J2384, J2388 J2398, J2401, J2407, J2408, J2417, J2420, J2421, J2423, J2424, J2425, and J2427;
- QTL J1 on SBI-05 — J2430, J2432, J2438, J2439, J2442, J2444, 2447, J2448, J2470, and J2479; and,
- QTL J2 on SBI-05 — J2460, J2461, J2465, J2467, J2477, J2482, J2483, and J2486 (actually QTL J plants).

Similarly, "BC_3F_1" x 296B crosses selected for advancement following background screening (Table 29) targeted shoot fly resistance QTLs on two linkage groups as follows:

- QTL A on SBI-01 — J1692 x J2079 (BC_2F_1), J1690 x J2072 (BC_2F_1), J1698(F_4) x J2019 (BC_1F_1) (2489-2544); and,
- QTL A on SBI-01 combined with QTLs J1 and J2 on SBI-05 — J1690 x J2072 (BC_2F_1: 2545-2603).

In all cases, the "BC4F1" materials advanced in 296B background were actually products of earlier generation backcrosses due to failure of one or more backcrosses during the course of their breeding.

From the foreground marker data (Table 39), individuals heterozygous for donor parent alleles at marker loci immediately flanking shoot fly resistance QTL alleles for the following targets were identified for background screening:

- QTL A on SBI-01 — J2491, J2494, J2495, J2498, J2502, J2503, J2511, J2513, J2516, J2518, J2521, J2525, J2527, J2528, J2529, J2532, J2534, J2536, J2541, and J2543;
- QTLs A on SBI-01 and J1 + J2 on SBI-05 — J2601 and J2599;
- QTL J1 on SBI-05 — J2547, J2556, J2564, J2565, J2581,and J2593; and,
- QTLs J1 + J2 on SBI-05 — J2555, J2559, J2560, J2567, J2583, J2592, J2595, and J2600.

The above-listed foreground QTL introgression heterozygote selections in BTx623 background and 296B background were self-pollinated and their "BC_4F_2" seed samples harvested for possible advance pending the outcome of background screening.

2.5.1 BC_4F_1 Background Screening

Background screening was performed on foreground-selected "BC_4F_1" generation individuals to reduce the level of donor parent heterozygosity present on non-carrier linkage groups in the final generation of this study's QTL introgression program. This was done to help in choosing a reduced number of "BC_4F_2" populations to be advanced for the production of homozygous near-isogenic pairs (+QTL and -QTL) of the target shoot fly resistance QTLs in BTx623 and 296B backgrounds and to determine how much heterozygosity reduction has been achieved.

- BTx623-background BC_3F_1 progenies heterozygous for target QTL A on linkage group SBI-01 (*viz.*, J2252, J2256, J2257, J2260, J2264, J2266, J2267, J2269, and J2270) were screened with *Xtxp32* and comparable BC_4F_1 progenies (*viz.*, J2271, J2273, and J2274) were screened with markers *Xtxp32* and *Xtxp357* (Table 40).

- BTx623-background BC_4F_1 progenies heterozygous for target QTL E on linkage group SBI-07 (*viz.*, J2278, J2283, J2288 and J2291) were screened with *Xtxp317* and *Xtxp274* and J2300 was screened with *Xtxp317*, *Xtxp274* and *Xtxp211* (Table 40).
- BTx623-background BC_4F_1 progenies heterozygous for both target QTLs J1 and J2 on linkage group SBI-05 (*viz.*, J2337, J2349, J2352, J2353, J2362, J2364, J2366, J2372, J2373, J2374, and J2384) were screened with *Xtxp1*, *Xtxp207*, *Xisp10323*, and *Xtxp10*; while J2388, J2398, J2401, J2407, and J2408 were screened with *Xtxp1*, *Xtxp207*, *Xisp10323*, *Xtxp258*, *Xtxp20*, and *Xisp10263*; and J2417, J2420, J2421, J2423, J2424, J2425, and J2427 were screened with *Xtxp207*, *Xisp10323*, *Xtxp343*, *Xtxp10*, *Xtxp258*, and *Xisp10263* (Table 40).
- BTx623-background BC_4F_1 progenies heterozygous for target QTL J1 on linkage group SBI-05 (*viz.*, J2430, J2432, J2438, J2439, J2442, J2444, J2447, and J2448) were screened with *Xtxp1*, *Xtxp207*, *Xisp10323*, and *Xtxp258* (Table 40).
- Finally, BTx623-background BC_4F_1 progenies heterozygous for target QTLs J1 or J2 on linkage group SBI-05 (viz., J2460, J2461, J2465, J2467, J2470, J2477, J2479, J2482, J2483, and J2486) were screened with *Xisp10323*, *Xtxp258*, and *Xcup07* (Table 40).

In case of 296B-background materials, BC_2F_1 progenies heterozygous for target QTL A on linkage group SBI-01 were background genotyped with various groups of markers:

- J2491, J2494, J2495, J2498, J2502 and J2503 were background-genotyped with markers *Xtxp32*, *Xgap57*, *Xisp335*, *Xisp348*, *Xisp310*, *Xtxp312*, *Xtxp20*, *Xisp359*, *Xcup67*, *Xtxp354*, *Xisp264*, *Xtxp317*, *Xisp215*, and *Xtxp283b*;
- J2513, J2516, J2518, J2521, J2525, J2527, J2528, and J2529 were background-screened with markers *Xtxp32*, *Xgap57*, *Xtxp69*, *Xisp335*, *Xtxp312*, *Xtxp20*, *Xisp359*, *Xtxp354*, *Xisp215*, *Xtxp23*, and *Xtxp283b*; and,
- J2532, J2534, J2536, J2541, and J2543 were background-screened with *Xtxp32*, *Xgap57*, *Xtxp69*, *Xisp348*, *Xisp310*, *Xtxp312*, *Xisp264*, *Xtxp317*, *Xisp215*, and *Xtxp23* (Table 41).
- Similarly, 296B-background BC_2F_1 progenies heterozygous for target QTLs A on linkage group SBI-01 and target QTLs J1 and J2 on SBI-05 (*viz.*, J2601, J2599, J2601, J2547, J2556, J2564, J2565, J2581, J2593, J2555, J2559, J2560, J2567, J2583, J2592, J2595, and J2600) are background-screened

with *Xtxp32*, *Xgap57*, *Xtxp69*, *Xisp335*, *Xtxp312*, *Xtxp20*, *Xisp359*, *Xtxp354*, *Xisp215*, *Xtxp23*, and *Xtxp283b* (Table 41).

2.5.2 BC_4F_1 background screening: 296B-background target QTL E

Fifteen 296B-background BC_2F_1 and BC_3F_1 plants heterozygous for donor parent marker alleles flanking target QTL E on SBI-07 (derived from crosses J1695 x J2062, J1707 x J2057 and J1706 x J2059, Table 30), *viz.*, BC_2F_1 plants J2189, J2193, and J2194; and BC_3F_1 plants J2195, J2196, J2197, J2198, J2199, J2201, J2208, J2209, J2210, J2211, J2212, and J2213, were subjected to background screening with 29 SSR markers distributed across all ten sorghum linkage groups (Table 42). On linkage groups SBI-06 and SBI-05 only 2 markers were included. Two individuals were selected for advance by selfing to the BC_4F_2 generation: J2196 was the best option (87% homozygous for recurrent parent background marker alleles) and J2198 (BC_3F_2) (84% homozygous for recurrent parent background marker alleles).

2.6 "BC_4F_2" generation set I in BTx623 background

After foreground selection, self-pollination, background selection and harvest, the selfed progeny of selected "BC_4F_1" generation plants were sown in several sets and raised as the "BC_4F_2" generation in order to identify near-isogenic pairs homozygous for donor parent marker alleles or homozygous for their recurrent parent allele at loci flanking the individual target QTLs. The results from foreground screening of the first-sown set of these materials in BTx623-background, which were sown before completion of background screening of the foreground-selected BC_4F_1 individuals, are presented in Table 43.

The BTx623-background progenies advanced included:

- two families targeting shoot fly resistance QTL A on SBI-01: selfed progenies of J2252 (BC_3F_2: J2650-J2673) and J2271 (BC_4F_2: J2674-J2697);
- two families targeting shoot fly resistance QTL E on SBI-07: selfed progenies of J2278 (BC_4F_2: J2698-J2721) and J2300 (BC_4F_2: J2722-J2745);
- three families targeting shoot fly resistance QTLs J1 and J2 on SBI-05:- selfed progenies of J2337 (BC_4F_2: J2794-J2817), J2408 (BC_4F_2: J2818-J2841), and J2421 (BC_4F_2: J2842-J2865); and,

- two families targeting shoot fly resistance QTL J1 on SBI-05: selfed progenies of J2430 (BC_4F_2: J2746-J2769) and J2438 (BC_4F_2: J2770-J2793).

Following foreground genotyping (Table 43) of this first set of BC_3F_2 and BC_4F_2 plants in the genetic background of recurrent parent BTx623, the following sets of near-isogenic pairs of donor and recurrent parent allele homozygotes for each of the target QTL regions were identified for advance by selfing:

- Shoot fly resistance QTL A on SBI-01:
  - BTx623 allele homozygotes at flanking loci *Xtxp37* and *Xtxp75* (-A isolines): J2650, J2651, J2662, J2674, J2682, and J2683; and
  - IS 18551 allele homozygotes at flanking loci *Xtxp37* and *Xtxp75* (+A isolines): J2668, J2672, J2678, J2680, J2681, J2686, and J2688.
- Shoot fly resistance QTL E on SBI-07:
  - BTx623 allele homozygotes at flanking loci *Xtxp40* and *Xtxp159* (-E isolines): J2702, J2707, J2711, J2712, J2716, J2724, J2726, and J2741; and
  - IS 18551 allele homozygotes at flanking loci *Xtxp40* and *Xtxp159* (+E isolines): J2699, J2708, J2710, J2714, and J2743.
- Shoot fly resistance QTLs J1 and J2 on SBI-05:
  - BTx623 allele homozygotes at flanking loci *Xisp258*, *Xtxp65*, *Xtxp94* and *Xtxp15* (-J1-J2 isolines): J2799, J2814, and J2826;
  - IS 18551 allele homozygotes at flanking loci *Xisp258*, *Xtxp65*, *Xtxp94* and *Xtxp15* (+J1+J2 isolines): J2816, J2833, and J2834.
- Shoot fly resistance QTL J1 (but not J2) on SBI-05:
  - BTx623 allele homozygotes at flanking loci *Xisp258*, *Xtxp65*, *and Xtxp94* (-J1-J2 isolines): J2749, J2770, J2771, J2777, J2780, and J2785;
  - IS 18551 allele homozygotes at flanking loci *Xisp258*, *Xtxp65*, *and Xtxp94* (+J1-J2 isolines): J2752, J2758, J2760, J2767, and J2779.
- Shoot fly resistance QTL J2 (but not J1) on SBI-05:
  - IS 18551 allele homozygotes at flanking loci *Xtxp94* and *Xtxp15* (-J1+J2 isolines): J2827 only.

Self-pollinated seed (BC_3F_3 or BC_4F_3) harvested from these selected individuals was then used to sow replicated field screens at ICRISAT-Patancheru in *Kharif* (2006), *Rabi* (2006) and *Kharif* (2007) environments.

2.7 "BC_4F_2" generation set II (multiple) BTx623 and 296B backgrounds

The results from foreground screening of the second-sown set of selfed progeny of selected "BC_4F_1" generation plants in BTx623-background, which were sown after completion of background screening of the foreground-selected BC_4F_1 individuals, are presented in Table 44.

The BTx623-background progenies advanced for foreground marker genotyping and self-pollination included:

- the self-pollinated family of a single BC_4F_1 plant apparently segregating for shoot fly resistance QTL A on SBI-01: J2273 (BC_4F_2: J2800-J2823);
- the self-pollinated family of a single BC_4F_1 plant apparently segregating for shoot fly resistance QTL E on SBI-07: J2283 (BC_4F_2: J2824-J2847);
- the self-pollinated families of four BC_4F_1 plants apparently segregating for shoot fly resistance QTLs J1 and J2 on SBI-05: J2352 (BC_4F_2: J2848-J2895), J2407 (BC_4F_2: J2896-J2919), J2465 (BC_4F_2: J2920-J2944), and J2467 (BC_4F_2: J2945-J2968); and
- the self-pollinated families of a single BC_4F_1 plant apparently segregating for shoot fly resistance QTL J1 on SBI-05: J2439 (BC_4F_2: J2969-J2991).

Following foreground genotyping (Table 44) of this second set of BC_4F_2 plants in the genetic background of recurrent parent BTx623, the following sets of donor and recurrent parent allele homozygotes for each of the target QTL regions were identified for advance by selfing to produce BC_4F_3 near-isogenic line sets in BTx623 background:

- Shoot fly resistance QTL A on SBI-01:
  - BTx623 allele homozygotes at flanking loci *Xtxp37* and *Xtxp75* (-A isolines): J2801 and J2822; and
  - IS 18551 allele homozygote at flanking loci *Xtxp37* and *Xtxp75* (+A isolines): J2804 only.
- Shoot fly resistance QTL E on SBI-07: Due to an apparent sample tracking error (where the seed sample harvested from plant J2283 did not correspond to the marker data generated with that sample identification number), all plants in this progeny were BTx623 allele homozygotes at flanking loci *Xtxp40* and *Xtxp159*, so no plants from this progeny were identified for advance.
- Shoot fly resistance QTLs J1 and J2 on SBI-05:
  - BTx623 allele homozygotes at flanking loci *Xisp258*, *Xtxp65*, *Xtxp94* and *Xtxp15* (-J1-J2 isolines): J2867, J2895, J2901, J2967, J2947, and J2990; and

- IS 18551 allele homozygotes at flanking loci *Xisp258*, *Xtxp65*, *Xtxp94* and *Xtxp15* (+J1+J2 isolines): J2869, J2890, J2898, J2936, and J2965.

In addition, several homozygous recombinants involving the shoot fly resistance QTLs J1 and J2 on SBI-05 were identified for advancement by selfing for use in fine-mapping these two QTLs:

- three plants homozygous for recurrent parent BTx623 alleles at loci *Xisp258*, *Xtxp65*, and *Xtxp94*, and homozygous of donor parent IS 18551 alleles at locus *Xtxp15* (i.e., -J1+J2?): J2878, J2886 and J2946; and
- four plants homozygous for recurrent parent BTx623 alleles at locus *Xtxp15* and homozygous of donor parent IS 18551 alleles at loci *Xisp258*, *Xtxp65*, and *Xtxp94* (+J1-J2?): J2978, J2979, J2982 and J2983.

The results from foreground screening of selfed progeny of selected "BC_4F_1" generation plants in 296B-background, which were sown after completion of background screening of the foreground-selected "BC_4F_1" individuals are presented in Table 45. Many of these families of selfed progenies were not truly of the BC_4F_2 generation due to failure of backcrossing in one or more prior generations, and were instead comprised of BC_2F_2 or BC_3F_2 generation individuals. These 296B-background progenies sown for foreground marker genotyping and self-pollination included:

- the self-pollinated families of two BC_2F_1 plants apparently segregating for shoot fly resistance QTL A on SBI-01: J2513 (BC_2F_2: J3016-J3039) and J2529 (BC_2F_2: J3040-J3062);
- the self-pollinated families of two BC_3F_1 plants apparently segregating for shoot fly resistance QTL E on SBI-07: J2196 (BC_3F_2: J3280-J3304) and J2198 (BC_3F_2: J3305-J3329);
- the self-pollinated families of four BC_2F_1 plants apparently segregating for shoot fly resistance QTLs J1 and J2 on SBI-05: J2567 (BC_2F_2: J3207-J3244) and J2595* (BC_2F_2: J3245-J3279);
- the self-pollinated families of four BC_2F_1 plants apparently segregating for shoot fly resistance QTL J1 on SBI-05: J2564 (BC_2F_2: J3159-J3182) and J2593* (BC_2F_2: J3183-J3206); and
- the self-pollinated family of a single self BC_2F_1 plant apparently segregating for the combination of shoot fly resistance QTL A on SBI-01 and QTLs J1 and J2 on SBI-05: J2601* (BC_2F_2: J3063-J3158).

Following foreground genotyping (Table 45) of these BC_2F_2 and BC_3F_2 plants in the genetic background of recurrent parent 296B, the following sets of donor and

recurrent parent allele homozygotes for each of the target QTL regions were identified for advance by selfing to produce BC_nF_3 near-isogenic line sets in 296B background:

- Shoot fly resistance QTL A on SBI-01:
  - 296B allele homozygotes at flanking loci *Xtxp37* and *Xtxp75* (-A isolines): J3018, J3044, J3059, and J3062; and
  - IS 18551 allele homozygote at flanking loci *Xtxp37* and *Xtxp75* (+A isolines): J3022, J3042, J3048 and J3054;
- Shoot fly resistance QTL E on SBI-07:
  - 296B allele homozygotes at flanking loci *Xtxp40* and *Xtxp159* (-E isolines): J3282, J3283, J3284, J3290, J3295, J3297, J3300, J3301, J3315, J3317 ; and
  - IS 18551 allele homozygote at flanking loci *Xtxp40* and *Xtxp159* (+E isolines): J3289, J3296, J3307, J3308, J3319, J3323, and J3324.
- Shoot fly resistance QTL J1 on SBI-05:
  - 296B allele homozygotes at flanking loci *Xisp10258*, *Xtxp65*, and *Xtxp15* (-J1-J2 isolines): J3168, J3171, J3172, J3197; and
  - IS 18551 allele homozygotes at flanking loci *Xisp10258* and *Xtxp65* (+J1-J2 isolines): J3175 and J3202
- Shoot fly resistance QTLs J1 and J2 on SBI-05:
  - 296B allele homozygotes at flanking loci *Xisp10258*, *Xtxp65*, and *Xtxp15* (-J1-J2 isolines): J3215, J3222, J3231, J3244 ; and
  - IS 18551 allele homozygotes at flanking loci *Xisp10258*, *Xtxp65,* and *Xtxp15* (+J1+J2 isolines): J3213, J3233, J3235, and J3243.
- Shoot fly resistance QTLs on linkage groups SBI-01(A) and SBI-05(J):

There was erroneous marker data segregation for Ig A markers and Xtxp 15 marker and selection could be done for only QTL J1 plants.

  - 296B allele homozygotes at flanking loci *Xisp10258*, *Xtxp65* (-J1 isolines): J3065, J3066, J3068, J3071, J3074, J3081, J3082, J3085, J3088, J3093, J3103, J3106, J3124, J3134, J3154; and
  - IS 18551 allele homozygotes at flanking loci *Xisp10258* and *Xtxp65* (+J1 isolines): J3063, J3070, J3072, J3075, J3089, J3091, J3097, J3101, J3119, J3121, J3131, J3137, J3145, J3147, 3158.

In addition, several possible homozygous recombinants involving the shoot fly resistance QTLs J1 and J2 on SBI-05 were identified for advancement by selfing for use in fine-mapping these two QTLs:

- four plants homozygous for recurrent parent BTx623 alleles at loci *Xisp10258, and Xtxp65*, and homozygous of donor parent IS 18551 alleles at locus *Xtxp15* (i.e., -J1+J2?): J3180, J3186, J3200, and J3242; and
- one plant homozygous for recurrent parent BTx623 alleles at locus *Xtxp15* and *Xtxp65*, and homozygous of donor parent IS 18551 alleles at loci *Xisp10258*, (+J1?-J2): J3239.

Self-pollinated seed (BC_2F_3, BC_3F_3 or BC_4F_3) harvested from these selected BTx623- or 296B-background individuals was then used to sow replicated field screens of the near-isogenic line sets at ICRISAT-Patancheru in *Kharif* (2006), *Rabi* (2006) and *Kharif* (2007) environments to phenotypically assess the utility of the individual target QTLs of this experimental marker-assisted backcrossing program.

2.8 Recombinant inbred line donors

Ten seeds were sown of each of four Recombinant Inbred Lines (RILs) identified from the RIL mapping population of cross BTx623 x IS 18551 as being homozygous for multiple shoot fly resistance QTLs from IS 18551 (Table 47). Marker genotyping of the resulting seedlings revealed that the seed stock of RIL 166 (homozygous for target QTLs A, G, J) was outcrossed, wherease those of RIL 189 (A, E, J), RIL 252 (A, G, J), and RIL 153 (A, G, E, J) were true to their expected genotypes (Table 47). In the RIL BC_3F_1 backcross generation, these four RIL parents were again sown in duplicate. In this case flanking markers for QTL E (SBI-07) were not screened. Marker data in this second screen of the RILs coincided with the initial screen makers (Table 48). In both screens RIL 166 was found to be outcrossed and therefore did not have the expected marker genotype.

2.8.1 Recombinant inbred line backcross

2.8.1.1 RIL crossing

RIL 153 (homozygous for IS 18551 marker alleles flanking QTLs A, E, G, J), RIL 166 (homozygous for QTLs A, G, J), RIL 189 (homozygous for QTLs A, E, J), and RIL 252 (homozygous for A, G, J) were selected from the RIL mapping population of cross BTx623 x IS 18551 (Table 46). Ten seeds of each Recombinant Inbred Line listed above were sown and the resulting plants, *viz.*, RIL 153 (J511-J528), RIL 166 (J529-J547), RIL 189 (J548-J557), and RIL 252 (J558-J577), crossed to BTx623.

2.8.1.2 RIL F_1

F_1 hybrid progenies from plant x plant crosses of individual RIL donor parents and recurrent parent BTx623, *viz.*, RIL 153-J511 x J644 (RIL F_1: J1358-J1363), RIL 166-J532 x J586 (RIL F_1: J1364-J1371), RIL 189-J545 x J647 (RIL F_1: J1372-J1377) (progenitor sown for this was actually from RIL 166), and RIL 252-J629 x J562 (RIL F_1: J1351-J1357) were sown, genotyped at marker loci flanking target QTLs (Table 49), and selected F_1 individuals backcrossed to recurrent parent BTx623. BC_1F_1 seed lots produced from such backcrosses of selected plants from RIL F_1 families are listed in Table 50 and included:

- RIL 153 - J1359 x J1380 (A+G), and J1361 x J1391 (A+G).
- RIL 166 - J1364 x J1388 (G+J), J1366 x J1393 (G+J), J1367 x J1379 (G+J), and J1371 x J1390 (G+J);
- RIL 189 - J1373 x J1389 (A+J), J1374 x J1390 (A+J), J1375 x J1386 (A+J), and J1376 x J1378 (A+J); and
- RIL 252 - J1351 x J1382 (A+G+J), J1352 x J1377 (A+G+J), J1356 x J1381 (A+G+J), and J1357 x J1391 (A+G+J).

2.8.1.3 RIL BC_1F_1 generation

A total of 120 individuals of RIL BC_1F_1 progenies (Table 50), segregating for multiple QTL targets [*viz.*, A+G+J (RIL BC_1F_1: J1801-J1848); A+G (RIL BC_1F_1: J1849-J1872), G+J (RIL BC_1F_1: J1873-J1896), and A+J (RILBC$_1$F$_1$: J1897-J1920)] were sampled and their DNA genotyped at marker loci flanking these target QTLs (Table 51). The following plants from the RIL BC_1F_1 populations:

- RIL 252 donor - J1802 (A+G), J1804 (J2), J1806 (A+J), J1808 (G), J1817 (A+J1), and J1831 (A+G);
- RIL 153 donor - J1849 (A+G), J1852 (A+G), J1855 (G), J1857 (A), and J1861 (G);
- RIL 166 donor - J1878 (G), J1880 (G+J), J1890 (G), J1893 (G), and J1895 (G+J); and,
- RIL 189 donor - J1897 (A), J1917 (A+J1)

were advanced by backcrossing to BTx623, after which background screening was accomplished with 33 SSR markers (Table 52), and backcrosses (BC_2F_1 seed) of selected genotypes were advanced [Note: J1802, J1806, J1808, J1897 and J1917 are F2's (Table 52)].

2.8.1.4 RIL BC_2F_1 generation

Backcrosses of the best and second best selected RIL BC_1F_1 plants for various target QTL combinations were sown and the resulting seedlings genotyped at marker loci flanking target QTLs (Table 53):

- target QTL A - J1857 x J2013 with 79% BTx623 background (RIL BC_2F_1: 2108-2114);
- target QTL G - J1878 x J2015 (76% BTx623 background) (RIL BC_2F_1: 2115-2120), and J1893 x J2002 (76% BTx623 background) (RIL BC_2F_1: 2121-2127);
- target QTLs A+G - J1849 x J2000 (88% BTx623 background) (RIL BC_2F_1: 2128-2141), and J1831 x J2006 (73% BTx623 background) (RIL BC_2F_1: 2142-2155);
- target QTLs A+J1 - J1817 x J2012 (73% BTx623 background) (RIL BC_2F_1: 2156-2159), and J1917 (39% BTx623 background as actually a self (F_2) and not a backcross) (RIL BC_1F_1: 2160-2173);
- target QTLs G+J - J1895 x J1995 (76% BTx623 background) (RIL BC_2F_1: 2174-2177), and J1880 x J1985 (70% BTx623 background) (RIL BC_2F_1: 2178-2187).

The following foreground-selected RIL BC_2F_1 plants (Table 53) were chosen for advance by backcrossing to recurrent parent BTx623 (crosses indicated were made):

- target QTL A - J2111 x BTx623-3, J2114 x BTx623-25, J2145 x BTx623-16, J2138, J2171, J2128, and J2153 x BTx623-7;
- target QTL G - J2121 x BTx623-12, J2126 x BTx623-4, J2135 x BTx623-13, J2137 x BTx623-6, J2142 x BTx623-9, J2147 x BTx623-5, J2148 x BTx623-9, J2176 x BTx623-26 and J2178;
- target QTL J1 - J2157 x BTx623-15 and J2186 x BTx623-8;
- target QTL J2 - J2177 x BTx623-4;
- target QTLs A+G - J2149 x BTx623-17;
- target QTLs A+J1 - J2156 x BTx623-20 and J2173 x BTx623-2; and,
- target QTLs G+J1 - J2184 x BTx623-23 and J2185 x BTx623-11(G+J1).

A selected subset of the resulting backcrosses *viz.*, target A- J2111, J2114; target G- J2135, J2137; target A+J1- J2156; target J1-J2157, target J2- J2177; target G+J1- J2184, J2185 were chosen for sowing to advance to the RIL BC_3F_1 generation (Table 54).

2.8.1.5 RIL BC_3F_1 generation

Backcrosses of the best and second best selected RIL BC_3F_1 plants for various target QTL combinations were sown and the resulting seedlings genotyped at marker loci flanking target QTLs (Table 55):

RIL BC_2F_1 progenies,

- target QTL A - J2111 x BTx623-3 (RILBC$_3$F$_1$: 2651-2675), and J2114 x BTx623-25 (RILBC$_3$F$_1$: 2676-2700),
- target QTL G - J2135 x BTx623-13 (RILBC$_3$F$_1$: 2701-2719), and J2137 x BTx623-6 (RILBC$_3$F$_1$: 2720-2725),
- target QTLs A+J1 - J2156 x BTx623-20 (RILBC$_3$F$_1$: 2726-2747),
- target QTL J1 - J2157 x BTx623-15 (RILBC$_3$F$_1$: 2848-2776),
- target QTL J2 - J 2177 x BTx623-4 (RILBC$_3$F$_1$: 2777-2784),
- target QTLs G+J1 - J2184 x BTx623-23 (RILBC$_3$F$_1$: 2785-2789), and J2185 x BTx623-11 (RILBC$_3$F$_1$: 2790).

The following foreground-selected RIL BC_3F_1 plants (Tables 55, 56) were chosen for advance by selfing or backcrossing to recurrent parent BTx623 (crosses indicated were made):

- target QTL A - J2658 (self, actually BC2F2), J2698 (self, actually BC2F2), J2669 x BTx623, J2673 x BTx623, J2676 x BTx623, J2684 x BTx623, J2687 x BTx623, J2689 x BTx623, and J2699 x BTx623;
- target QTL G - J2722 x BTx623, J2723 x BTx623, and J2725 x BTx623;
- target QTLs A+J1 - J2728 x BTx623, and J2746 x BTx623;
- target QTL J1 - J2749 x BTx623, J2752 x BTx623, J2753 x BTx623, J2754 x BTx623, J2756 x BTx623, J2757 x BTx623, J2759 x BTx623, J2763 x BTx623, J2765 x BTx623, J2774 x BTx623, and J2790 x BTx623;
- target QTLs G+J1 – plant 2785 was homozygous for donor markers flanking both of these QTLs, but the plant died and hence could not be advanced by selfing.

Heterozygote plants were backcrossed to BTx623 and selfed (Table 56). Two BC_2F_3 QTL introgression homozygote entries (J2658 (+QTL-A) and J2698 (+QTL-A)) from this were utilized in field screening for target QTL-A.

2.9 Field evaluation of shoot fly resistance QTL introgression near-Isogenic lines

2.9.1 *Kharif* 2006 (K)

The field data was subjected to general t-paired tests, the mean values of each test entry, controls and checks were examined for significance of differences in

mean value performance. The parents, BTx623 and IS 18551, showed highly significant differences for nearly the entire range of observed shoot fly resistance traits *viz.*, glossiness, seedling vigor, oviposition I, eggs per 100 plants I, oviposition II, deadhearts I, and deadhearts II. The sole exception was for eggs per 100 plants II, which showed a non-significant difference, although the mean for susceptible parent BTx623 was numerically greater than that for resistance donor IS 18551 (Table 57).

The comparison of moderately resistant control IS 1054 with susceptible control Swarna was highly significant for glossiness, oviposition I, eggs per 100 plants I, oviposition II, deadhearts I, and deadhearts II. Comparison of highly resistant control IS 2312 with Swarna gave similar results. When means for resistant controls IS 1054 and IS 2312 were compared, differences in glossiness, oviposition I, eggs per 100 plants I, deadhearts I were highly significant whereas those for oviposition II and deadhearts II were significant. However, seedling vigor differences were non-significant in all three of these pair-wise comparisons.

Compared to susceptible parent BTx623, means of Recombinant Inbred Lines RIL 153 (A, E, G, J), RIL 189 (A, E, J) and RIL 252 (A, G, J) were highly significant for the glossiness trait. Means of RIL 153 were also highly significant for oviposition I, eggs per 100 plants I, oviposition II, deadhearts I, deadhearts II and significant for seedling vigor and eggs per 100 plants II. Means of RIL 189 were highly significant for seedling vigor, oviposition I, eggs per 100 plants I, oviposition II, deadhearts I, and deadhearts II but non-significant positive for eggs per 100 plants II. Similarly, compared to susceptible parent BTx623, means of RIL 252 were highly significant for seedling vigor, oviposition I, eggs per 100 plants I, deadhearts I and deadhearts II.

Compared to its susceptible recurrent parent BTx623, the 'plus-G' introgression isoline was highly significantly superior for glossiness, oviposition II and deadhearts II. This line was also significantly superior for seedling vigor, oviposition I, eggs per 100 plants I, eggs per 100 plants II, and deadhearts I.

Compared to their recurrent parent allele 'minus-J1' and 'minus-J' counterparts, the 'plus-J1' and 'plus-J' introgression lines showed highly significant differences for glossiness. The 'plus-J1' isolines also showed highly significant differences for oviposition I, eggs per 100 plants I, deadhearts I and deadhearts II and significant differences for oviposition II. However, the difference between the 'minus-J1' and 'plus-J1' isolines for eggs per 100 plants II was non-significant but positive. Similarly, compared to their 'minus-J' near-isogenic counterparts, the 'plus-J' genotypes showed highly significant differences for oviposition I, eggs per

100 plants I, and deadhearts II, and significant differences for oviposition II and dead hearts I. As for the isoline 'plus-J1', the 'plus-J' genotypes also gave a non-significant but positive difference for eggs per 100 plants II compared to their 'minus-J' near-isogenic counterparts.

Compared to their 'minus-A' counterparts, the 'plus-A' genotypes were significantly more glossy and had highly significantly better seedling vigor. However, the 'minus-A' isolines exhibited significantly lower oviposition I, eggs per 100 plants I, and eggs per 100 plants II in this screening environment, which were unexpected results.

Compared to their 'minus-E' counterparts, the 'plus-E' genotypes had highly significantly better seedling vigor. However, the 'minus-E' genotypes were highly significantly better than their 'plus-E' counterparts for oviposition I, eggs per 100 plants I, deadhearts I, and deadhearts II, and significantly better for oviposition II, and all of these results were unexpected. Differences between these two groups of genotypes for number of eggs per 100 plants II were non-significant but positive for 'minus-E'.

2.9.2 *Rabi* 2006/07 (R)

2.9.2.1 The BTx623-background 81-entry trial analysis

The parents, BTx623 and IS 18551, showed highly significant differences for the observed shoot fly resistance traits *viz.*, glossiness, seedling vigor I, seedling vigor II, oviposition I, eggs per 100 plants I, eggs per 100 plants II, deadhearts I, deadhearts II and deadhearts III (Table 58). Compared to susceptible check Swarna, moderately resistant check IS 1054 was highly significantly superior for nearly all the observed shoot fly resistance traits *viz.*, glossiness, seedling vigor II, oviposition I, eggs per 100 plants I, oviposition II, eggs per 100 plants II, deadhearts I, deadhearts II and deadhearts III. Similarly, highly resistant check, IS 2312 is highly significantly superior for all observed the shoot fly resistance traits *viz.*, glossiness, seedling vigor I, seedling vigor II, oviposition I, eggs per 100 plants I, eggs per 100 plants II, deadhearts I, deadhearts II and deadhearts III. The difference between IS 1054 and IS 2312 was highly significant for glossiness and seedling vigor I and oviposition II, and significant for oviposition I, and eggs per 100 plants I.

Compared to susceptible parent BTx623, means of RIL 153, showed highly significant differences for most observed traits, exhibiting superiority for glossiness, seedling vigor II, oviposition I, eggs per 100 plants I, eggs per 100

plants II, deadhearts I, deadhearts II and deadhearts III. Similarly, RIL 189 showed highly significant differences compared to BTx623 for glossiness, seedling vigor II, oviposition I, eggs per 100 plants I, eggs per 100 plants II, deadhearts I, deadhearts II and deadhearts III. Finally, RIL 252 showed highly significant differences compared to BTx623 for glossiness, seedling vigor I, oviposition I, eggs per 100 plants I, eggs per 100 plants II, deadhearts I, deadhearts II and deadhearts III.

Compared to its susceptible recurrent parent BTx623, the 'plus-G' QTL introgression isoline was highly significantly superior for glossiness, seedling vigor II, eggs per 100 plants I, eggs per 100 plants II, and was significantly superior for oviposition I, deadhearts II, and deadhearts III.

Differences between isolines 'plus-J1' and 'minus-J1' were highly significant for glossiness, deadhearts II, and deadhearts III and significant for oviposition I, dead hearts I, and eggs per 100 plants II. For each of the above traits the 'plus-J1' lines gave superior performance compared to their 'minus-J1' counterparts. However, differences between these near-isogenic genotypes were non-significant for eggs per 100 plants I and oviposition II. Further, the 'minus-J1' genotypes were significantly superior for seedling vigor I.

Compared to their near-isogenic counterpart ['-J1+J2? (AAAB)'], the '+J1-J2? (BBBA)' entries were highly significantly glossier, but had highly significantly poorer seedling vigor I and seedling vigor II scores, and lower counts of eggs per 100 plants I. The '+J1-J2?' entries also had significantly lower means for oviposition I and deadhearts I. Differences for eggs per 100 plants II, deadhearts II and deadhearts III were non-significant, but favored the '+J1-J2?' entries. However, the '-J1+J2?' entries unexpectedly exhibited highly significantly lower oviposition II values (Table 58).

Compared to their 'minus-J' (AAAA) counterparts, the 'plus-J' [+J1+J2 (BBBB)] isolines were highly significantly more glossy, and had highly significantly better values for seedling vigor II, oviposition I, eggs per 100 plants I, eggs per 100 plants II, deadhearts I, deadhearts II and deadhearts III. Only in case of seedling vigor I did the 'minus-J' isolines perform highly significantly better than their 'plus-J' counterparts.

Differences between the 'minus-A' and 'plus-A' isolines were non-significant for all observed traits although shoot fly infestation was numerically greater on the 'plus-A' lines. Differences were nearly significant for seedling vigor II.

Differences between the 'minus-E' isoline and its 'plus-E' counterpart were highly significant for seedling vigor I and seedling vigor II, significant for deadhearts III, and non-significant positive for deadhearts II, with the 'plus-E' isolines exhibiting the better levels of resistance. However, the 'minus-E' isolines exhibited highly significantly lower means for deadhearts I and significantly lower means for eggs per 100 plants I. These mixed results for the QTL-E isolines were unexpected.

2.9.2.2 The 296B-background 110-entry trial analysis

Differences between parents 296B and IS 18551 of the 296B-background trial were observed to be highly significant for glossiness, seedling vigor II, oviposition II, eggs per 100 plants II, deadhearts II, and deadhearts III. Compared to susceptible check Swarna, moderately resistant check IS 1054 was highly significantly superior for glossiness, seedling vigor I, seedling vigor II, oviposition II, eggs per 100 plants II, deadhearts I, deadhearts II and deadhearts III. IS 2312 is also highly significantly superior to Swarna for all of these traits (*viz.*, glossiness, seedling vigor I, seedling vigor II, oviposition II, eggs per 100 plants II, deadhearts I, deadhearts II and deadhearts III). Between moderately resistant check IS 1054 and highly resistant check IS 2312, there were highly significant differences for glossiness, oviposition II, eggs per 100 plants II, and deadhearts I (Table 59).

Compared to their 'minus-J1' counterparts, the 'plus-J1' isolines were highly significantly more glossy and had highly significantly better values for, oviposition II, deadhearts II, and dead hearts III; and significantly better values for eggs per 100 plants II and deadhearts I. However, the 'minus-J1' isolines had significantly better seedling vigor I scores (Table 58). Similarly, compared to their 'minus-J' counterparts, the 'plus-J' isolines were highly significantly more glossy and had highly significantly lower, deadhearts II values as well as significantly lower deadhearts I and deadhearts III values. Further, the differences between the '+J1?-J2' (BAA) and '-J1?+J2?' (AAB) isolines was highly significant for glossiness and deadhearts I; and significant for deadhearts II and deadhearts III, with all differences favoring the '+J1?-J2' isolines. Finally, the differences between the '+J?' (BBA) and '–J' (AAA) genotypes in 296B-background were highly significant for glossiness, and significant for eggs per 100 plants II, deadhearts II, and deadhearts III with differences for each of these traits favoring the '+J?' genotypes that are homozygous for at least the glossy alleles from donor parent IS 18551 at the top of linkage group SBI-05 (J).

Differences between the 'minus-A' and 'plus-A' isolines in 296B-background in this *rabi* 2006/07 screen were non-significant for all observed traits. Similarly, differences between the 'minus-E' and 'plus-E' isolines were significant only for seedling vigor I with 'plus-E being more vigorous, and were non-significant for all other observed traits in this trial (Table 59).

2.9.3 Kharif 2007 (K*)

2.9.3.1 BTx623-background 64-entry trial analysis

Differences between the parental lines BTx623 and IS 18551 were highly significant for all of the traits observed this season *viz.*, glossiness score, oviposition I, deadhearts I and deadhearts II (Table 60). Differences between these entries for eggs per 100 plants I from a single replication were also highly significant. IS 2312 and IS 1054 are highly significantly better than susceptible check entry Swarna for glossiness score, oviposition I, deadhearts I and deadhearts II. However, the differences between highly resistant check entry IS 2312 and moderately resistant check IS 1054 were highly significant only for glossiness score. RIL parents (RIL 153, RIL 189, and RIL 252) are highly significantly superior to recurrent parent BTx623 for glossiness score. RIL 153 and RIL 252 were also highly significantly superior to BTx623 for oviposition I, deadhearts I and deadhearts II, whlie RIL 252 was also significantly superior to BTx623 for eggs per 100 plants I. Compared to BTx623, RIL 189 was significantly better for oviposition I and deadhearts II, and highly significantly better for deadhearts I.

The BTx623-background isoline 'plus-G' was highly significantly better than its BTx623 recurrent parent for glossiness score, oviposition I, and deadhearts I, and significantly better for deadhearts II.

Compared to its 'minus-J1' counterpart, the 'plus-J1' isoline was highly significantly better for glossiness score and deadhearts I, and significantly better for oviposition I and deadhearts II. Similarly, the isoline 'plus-J' was highly significantly better than its 'minus-J' counterpart for glossiness and deadhearts II, and significantly better for deadhearts I. As in the *kharif* 2006 and *rabi* 2006/07 field screening results, the *kharif* 2007 results showed no significant superiority of the 'plus-A' and 'plus-E' isolines over their 'minus-A' and 'minus-E' counterparts.

2.9.3.2 The 296B-background 20-entry trial analysis

The control entries BTx623 and IS 18551 were highly significantly different for glossiness score and significant for oviposition II in the 20-entry *kharif* 2007 trial of 296B-background materials (Table 61). Similarly, recurrent parent 296B and donor parent IS 18551 were highly significantly different for glossiness score and oviposition II. These pair-wise combinations were not found to be significantly different for other observed traits although values for shoot fly resistance donor IS 18551 were numerically superior to those for both of the two susceptible controls.

Compared to its 'minus-J' counterpart, the 'plus-J' isoline was highly significantly better only for glossiness score. Similarly, the 'plus-J1' isoline was highly significantly better than its 'minus-J' counterpart for glossiness score and oviposition II, but differences between these entries were non-significant for oviposition I, eggs per 100 plants I, deadhearts I, and deadhearts II.

Compared to its 'minus-E' counterpart, the 296B-background 'plus-E; isoline was significantly (but marginally) better for deadhearts II. Differrences for the remaining observed traits were non-significant positive for these two genotypes. Finally, differences between the 'minus-A' and 'plus-A' isolines in 296B-background in this *kharif* 2007 screen were non-significant for all observed shoot fly resistance component traits (Table 61).

2.9.3.3 The 296B-background 84-entry trial analysis

The control entries BTx623 and IS 18551 were highly significantly different for glossiness score, oviposition I, eggs per 100 plants I, and deadhearts I, and significantly different for deadhearts II, with resistance donor IS 18551 performing better than shoot fly susceptible check BTx623 for all of these traits in this 2007 *kharif* season trial (Table 62). Similarly, parental lines 296B and IS 18551 were highly significantly different for glossiness score, oviposition I, eggs per 100 plants I, and deadhearts I, but non-significant for deadhearts II.

Compared to their 'minus-J' counterparts, the 'plus-J' isolines in 296B-background were highly significantly better for glossiness score, but differences between these two groups of materials were non-significant for all other observed components of shoot fly resistance under the high level of insect pressure in this trial. Similarly, the 'plus-J1' isolines were significantly better than their 'minus-J1'

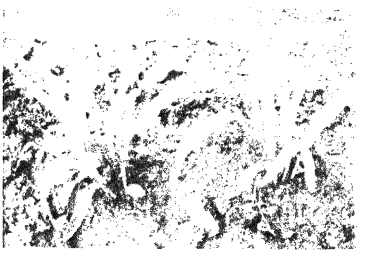
counterparts only for glossiness score. No significant superiority of the 'plus-A' and 'plus-E' isolines over their 'minus-A' and 'minus-E' counterparts in 296B-background were observed in this 84-entry field trial conducted at ICRISAT-Patancheru during the 2007 *kharif* season.

Plate 11: Observed glossiness characteristic for recurrent (BTx623) and donor (IS18551) parents, RILs and their near isogenic glossy lines.

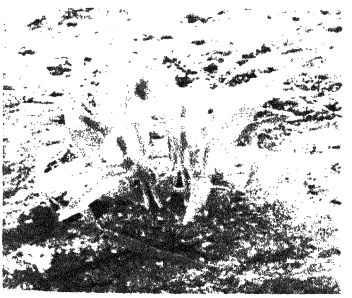
11(a): Shoot fly susceptible parent BTx623

11(b): Shoot fly resistant parent IS18551

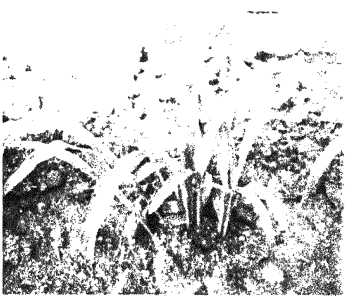
11(c): Recombinant inbred line 153 (A, E, G, J)



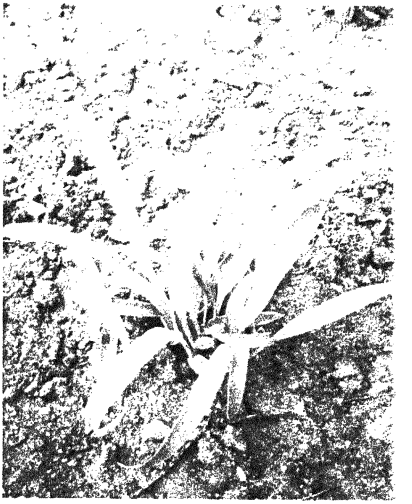
11(d): Recombinant inbred line 252(A, G, J)



11(e): Recombinant inbred line 189 (A, E, J)



(f) Shoot fly resistant BTx623 introgression Line +J1(*Xisp258-Xtxp94*)



11(g) Shoot fly susceptible BTx623 introgression Line -J1 counterpart (*Xisp258-Xtxp15*)

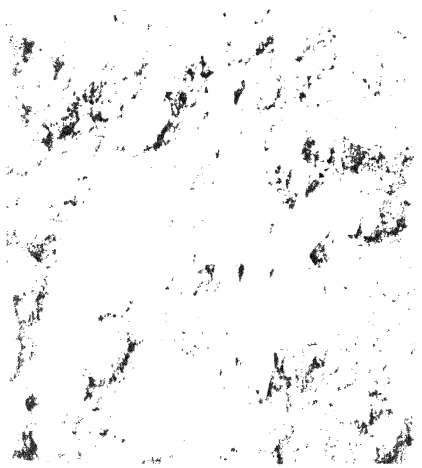
(h) Shoot fly resistant BTx623 introgression Line +J (*Xisp258-Xtxp15*)

11(i) Shoot fly susceptible BTx623 introgression Line -J counterpart (*Xisp258-Xtxp15*)

11(j). Shoot fly resistant BTx623 introgression Line +G

11(k). Shoot fly susceptible parent BTx623

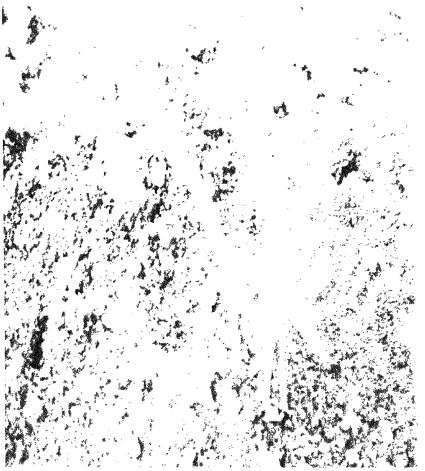
Plate 12. Observed glossiness characteristic for recurrent (296B) and donor (IS18551) parents, and their J1, J2 lines



12(a). Shoot fly susceptible parent 296B

12(b). Shoot fly resistant parent IS18551

12(c) Shoot fly resistant 296B introgression line +J1 (*Xisp258-Xfxp65*)

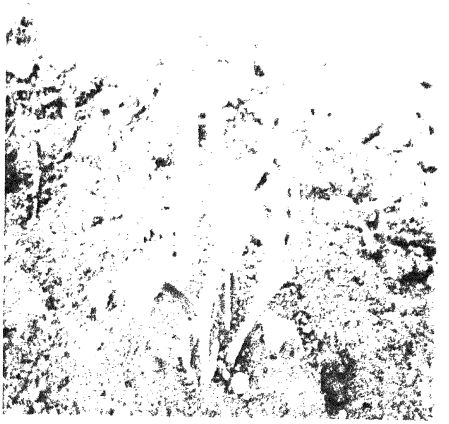


12(d) Shoot fly susceptible 296B –J counterpart (*Xisp258-Xfxp15*)

12(e) Shoot fly resistant 296B introgression line +J1?-J2 (BAA)

12(f) Shoot fly resistant 296B introgression line -J1+J2? (AAB)

12(g) Shoot fly resistant 296B introgression line +J (*Xisp258-Xtxp15*)



12(h) Shoot fly susceptible 296B–J Counterpart (*Xisp258-Xtxp15*)

TABLES

Table 9. Parental SSR marker polymorphism between shoot fly backcross introgression lines, BTx623 and IS 18551

S.No	SSR locus	Linkage group	BTx623 (on PAGE)	IS 18551 (on PAGE)	Polymorphism
1	*Xtxp316*	SBI-01 = A	350	460	polymorphic
2	*Xtxp248*	SBI-01 = A	240	220	polymorphic
3	*Xtxp319*	SBI-01 = A	160	140	polymorphic
4	*Xtxp32*	SBI-01 = A	135	140	polymorphic
5	*Xtxp302*	SBI-01 = A	190	230	polymorphic
6	*Xcup73*	SBI-01 = A	<205	210	ABI
7	*Xcup62*	SBI-01 = A	185	185	monomorphic
8	*Xtxp208*	SBI-01 = A	245	250	ABI
9	*Xtxp96*	SBI-02 = B	195	190	polymorphic
10	*Xtxp211*	SBI-02 = B	205	215	polymorphic
11	*Xtxp4*	SBI-02 = B	160	130	polymorphic
12	*Xtxp1*	SBI-02 = B	200	190	polymorphic
13	*Xtxp286*	SBI-02 = B	190	210	polymorphic
14	*Xtxp19*	SBI-02 = B	260	<305	polymorphic
15	*Xtxp207*	SBI-02 = B	185	190	ABI
16	*Xtxp4*	SBI-02 = B	175	145	polymorphic
17	*Xtxp9*	SBI-03 = C	140	105	polymorphic
18	*Xtxp285*	SBI-03 = C	265	250	sufficiently polymorphic
19	*Xisp323*	SBI-03 = C	170	180	sufficiently polymorphic
20	*Xcup11*	SBI-03 = C	170	175	polymorphic
21	*Xisp323*	SBI-03 = C	160	170	polymorphic
22	*Xtxp34*	SBI-03 = C	480	350	polymorphic
23	*Xtxp285*	SBI-03 = C	265	250	polymorphic
24	*Xtxp34*	SBI-03 = C	480	350	polymorphic
25	*Xtxp343*	SBI-04 = D	150	120	polymorphic
26	*Xtxp41*	SBI-04 = D	270	295	polymorphic
27	*Xtxp27*	SBI-04 = D	320	300	Polymorphic
28	*Xcup05*	SBI-04 = D	200	<205	ABI
29	*Xtxp21*	SBI-04 = D	180	170	polymorphic
30	*Xcup48*	SBI-04 = D	285	285	monomorphic
31	*Xisp344*	SBI-07 = E	260	280	polymorphic
32	*Xtxp295*	SBI-07 = E	155	<160	polymorphic
33	*Xtxp312*	SBI-07 = E	195	180	polymorphic
34	*Xtxp258*	SBI-07 = F	215	190	polymorphic
35	*Xtxp289*	SBI-07 = F	280	265	polymorphic
36	*Xcup02*	SBI-07 = F	190	195	ABI
37	*Xtxp20*	SBI-10 = G	210	195	polymorphic
38	*Xisp263*	SBI-10 = G	320	300	polymorphic
39	*Xtxp18*	SBI-08 = H	190	190	monomorphic
40	*Xtxp105*	SBI-08 = H	<305	300	scorable
41	*Xisp198*	SBI-08 = H	260	270	polymorphic
42	*Xisp278*	SBI-08 = H	290	290 (mult. bands)	monomorphic
43	*Xtxp6*	SBI-06 = I	118	81	polymorphic
44	*Xtxp317*	SBI-06 = I	170	160	polymorphic
45	*Xtxp265*	SBI-06 = I	210	200	polymorphic
46	*Xtxp274*	SBI-06 = I	350	320	polymorphic
47	*Xisp328*	SBI-06 = I	160	150	polymorphic
48	*Xisp264*	SBI-06 = I	180	167	polymorphic
49	*Xtxp57*	SBI-06 = I	251	241	polymorphic
50	*Xisp347*	SBI-06 = I	195	200	polymorphic
51	*Xisp258*	SBI-05 = J	195	180	polymorphic
52	*Xisp257*	SBI-05 = J	150(2b)+290(2b)	230+300	Not sure

Table 10. Parental SSR marker polymorphism between shoot fly backcross introgression lines, 296B and IS 18551

S.No	SSR locus	Linkage group	296B	IS 18551	Polymorphism
1	*Xtxp316*	SBI-01 = A	365	450	polymorphic
2	*Xtxp319*	SBI-01 = A	175	160	polymorphic
3	*Xtxp32*	SBI-01 = A	150	135	polymorphic
4	*Xtxp302*	SBI-01 = A	185	235	polymorphic
5	*Xtxp248*	SBI-01 = A	205	>210	ABI?
6	*Xcup73*	SBI-01 = A	225	>230	ABI?
7	*Xcup62*	SBI-01 = A	185	185	monomorphic
8	*Xtxp211*	SBI-02 = B	205	180	polymorphic
9	*Xtxp1*	SBI-02 = B	180	190	polymorphic
10	*Xtxp4*	SBI-02 = B	160	145	polymorphic
11	*Xtxp96*	SBI-02 = B	185	185	monomorphic
12	*Xtxp286*	SBI-02 = B	215	215	monomorphic
13	*Xtxp205*	SBI-03 = C	195	200	polymorphic
14	*Xcup11*	SBI-03 = C	175	185	polymorphic
15	*Xtxp34*	SBI-03 = C	335	350	polymorphic
16	*Xtxp9*	SBI-03 = C	110	110	monomorphic
17	*Xcup48*	SBI-04 = D	285	285	monomorphic
18	*Xtxp343*	SBI-04 = D	150	135	polymorphic
19	*Xisp344*	SBI-07 = E	280	305	polymorphic
20	*Xtxp312*	SBI-07 = E	150	190	polymorphic
21	*Xtxp312*	SBI-07 = E	155	180	polymorphic
22	*Xtxp295*	SBI-07 = E	200	>205	ABI?
23	*Xtxp258*	SBI-09 = F	230	200	polymorphic
24	*Xtxp289*	SBI-09 = F	270	270	monomorphic
25	*Xtxp141*	SBI-10 = G	165	165	monomorphic
26	*Xisp263*	SBI-10 = G	310	310	monomorphic
27	*Xisp198*	SBI-08 = H	285	285	monomorphic
28	*Xtxp18*	SBI-08 = H	190	190	monomorphic
29	*Xtxp105*	SBI-08 = H	295	295	monomorphic
30	*Xisp258*	SBI-05 = J	175	>170	ABI?

Table 11. SSR- marker genotyping of "BC_1F_1 progeny" from shoot fly susceptible and resistant parent lines 296B and IS18551

Linkage group	Marker/Sample	J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J11	J12*	J13	J14	J15	J16	J17	J18	J19	J20	J21	J22	J23	J24
SBI-01 (A)	*Xtxp37*	H	H	A	A	A	H	A	B	A	A	H	H	H	B	A	A	A	H	A	H	A	A	A	A
	Xtxp75	A	H	H	A	A	H	A	B	A	A	H	H	H	H	A	A	A	H	-	A	A	A	A	A
SBI-10(G)	*Xtxp141*	A	A	A	A	A	-	A	A	A	A	A	B	-	A	A	A	B	H	B	A	A	B	B	A
	Xgap1	H	A	A	H	H	-	A	B	H	H	A	H	H	B	H	A	H	A	H	H	H	A	H	H
SBI-07 (E)	*Xtxp159*	H	A	H	H	H	A	H	H	H	A	H	H	A	H	H	H	A	H	A	A	H	H	H	H
	Xtxp40	H	H	H	H	H	A	H	B	H	A	A	H	A	A	H	H	A	H	A	A	H	H	H	H
SBI-05 (J)	*Xtxp65*	H	H	H	H	H	A	H	B	H	A	H	H	H*	B	A	H	A	H	A	H	H*	A	A	A
	Xtxp15	H	H	H	H	A	H	H	B	-	H	H	H	A*	H	A	H	H	H	A	H	A*	A	A	A

Linkage group	Marker/Sample	J25	J26	J27	J28	J29	J30	J31	J32	J33	J34	J35	J36	J37	J38	J39	J40	J41	J42	J43	J44	J45	J46	J47	J48
SBI-01 (A)	*Xtxp37*	B	A	H	H	H	A	H	H	A	H	A	B	H	A	H	A	A	H	A	H	A	A	B	B
	Xtxp75	H	H	H	H	H	A	H	B	A	H	H	B	H	A	H	A	A	H	A	H	A	A	B	B
SBI-10(G)	*Xtxp141*	A	A	A	A	A	A	-	A	A	A	A	A	-	-	A	A	A	A	A	A	A	A	B	B
	Xgap1	A	A	H	A	A	-	A	A	A	A	B	B	A	H	H	H	H	B	A	A	A	A	B	B
SBI-07 (E)	*Xtxp159*	H	H	A	A	A	A	A	H	A	A	H	H	A	A	A	H	A	H	A	A	A	A	B	B
	Xtxp40	H	H	A	A	A	A	H	H	A	A	H	H	A	A	A	H	A	H	A	A	A	A	B	B
SBI-05 (J)	*Xtxp65*	H	A	H	A	H*	H	H	H	H	A	H	B	H*	A	H	A	A	H	H	-	A	A	B	B
	Xtxp15	H	A	H	A	A*	A	H	A	A	A	H	H	A*	H	A	A	A	A	A	A	A	A	B	B

J1-J44 = BC1F1 Progenies, J45 and J46 = recurrent parent 296B, and J47 and J48 = donor parent IS18551; J12 is self

Table 12. " BC_1F_1 progenies " of shoot fly susceptible and resistant parents, 296B and IS 18551 advanced to second backcross generation

S. No	"BC_1F_1" cross	Target QTL	No. of "BC_2F_1" seed harvested	No. of "BC_2F_1" seed sown	"BC_2F_1" progenies
1	J2 x J90	A+J	144	12	J1256-J1266
2	J3 x J90	E+J	6	6	J1286-J1290
3	J4 x J673	E+J	30	12	J1276-J1285
4	J6 x J91	A	93	16	J1296-J1308
5	J7 x J671-1	E+J	6	6	J1291-J1295
7	J12*(F_2) x J676	A+G+E+J	30	30	J1233-J1255 (BC_1F_1)
8	J27 x J705	A+J	49	6	J1267-J1269
9	J27 x J91	A+J	32	6	J1270-J1275

J12* = self product (F_2) due to failure of prior backcross resulting in selfing (and production of F_2 seed rather than BC_1F_1)

Table 14: "BC1F1 progenies" of shoot fly susceptible and resistant parents, BTx623 and IS 18551 advanced to second backcross generation

S. No	" BC_1F_1" Cross	Target QTL	No. of "BC_2F_1" seed harvested	No. of "BC_2F_1" seed Sown	"BC_2F_1" progenies
1	J231 x J620	A+E	100	12	J901-J912
2	J282 x J598	A+E	59	12	J913-J924
3	J284 x J601	G+E	13	13	J925-J929
4	J208 x J595	E+J	57	9	J930-J934
5	J271 x J666	E+J	150	9	J935-J943
6	J298 x J631	E+J	152	9	J944-J952
7	J310 x J647	E+J	124	9	J953-J959
8	J228 x J627	G+J	21	21	J960-J979
9	J234 x J637	A+G+E	14	14	J980-J990
10	J240*(F_2) x J618	A+E+J	76 (BC_1F_1)	48	J991-J1033 (BC_1F_1)
11	J286 x J659	A+E+J	50	24	J1034-J1051
12	J247*(F_2) x J651	G+E+J	76 (BC_1F_1)	70	J1052-J1122 (BC_1F_1)
13	J251*(F_2) x J596	A	109 (BC_1F_1)	23	J1123-J1145 (BC_1F_1)
14	J273 x J626	E	74	23	J1146-J1167
15	J198 x J614	G	42	24	J1168-J1185
16	J294 x J595	J	32	24	J1186-J1204

J240*, J247*, J251* = self products due to failure of backcross resulting in selfing and production of F_2 seed rather than BC_1F_1

Table 13. SSR- marker genotyping of "BC_1F_1 progeny" from shoot fly susceptible and resistant parent lines BTx623 and IS18551

Marker/ Sample	J184	J185	J186	J187	J188	J189	J190	J191	J192	J193	J194	J195	J196	J197	**J198**	J199	J200	J201	J202	J203	J204	J205	J206	J207	**J208**	J209	J210	J211	J212	J213	J214	J215	J216
Xtxp 37	H	H	H	H	A	B	H	H	A	A	A	H	H	A	A	H	H	A	A	H	A	H	H	A	A	A	H	H	A	H	A	H	H
Xtxp75	A	A	H	A	A	A	H	A	H	H	A	H	A	A	A	H	H	H	A	H	A	H	A	A	A	A	H	H	A	H	A	H	A
Xtxp141	H	H	H	H	H	B	A	B	H	B	H	B	H	H	**H**	H	A	B	B	H	B	A	B	H	H	A	A	H	B	A	H	B	A
Xgap1	-	H	H	H	H	B	A	A	H	-	A	H	H	H	**H**	-	A	H	-	H	H	A	A	A	A	A	-	B	A	H	H	A	A
Xtxp 159	H	H	H	H	H	A	H	H	H	A	A	A	B	H	A	B	A	H	H	B	H	A	H	H	**H**	A	A	A	H	H	A	A	H
Xtxp40	H	H	H	H	H	H	H	H	A	A	-	-	H	-	-	B	A	H	-	H	H	H	A	H	**H**	A	A	H	H	H	A	A	A
Xtxp65	H	A	A	H	A	A	H	H	A	H	-	-	A	-	-	A	H	H	-	H	H	A	H	H	**H**	H	H	B	A	A	-	A	A
Xtxp94	H	A	A	H	H	H	H	H	A	A	-	-	H	-	-	A	H	H	-	H	H	A	B	H	**H**	H	H	B	H	H	A	A	A
Xtxp15	A	A	A	A	H	A	H	A	A	H	-	-	A	-	-	A	H	H	-	H	H	A	H	H	**H**	H	H	B	A	A	-	A	A

Marker/ Sample	J217	J218	J219	J220	J221	J222	J223	J224	J225	J226	J227	**J228**	J229	J230	**J231**	J232	J233	**J234**	J235	J236	J237	J238	J239	J240	J241	J242	J243	J244	J245	J246	J247	J248	J249
Xtxp 37	H	A	H	A	H	H	A	A	H	H	B	-	B	H	**H**	B	H	**H**	H	OFF	A	H	H	B	B	A	H	H	A	H	A	H	H
Xtxp75	H	H	H	A	H	H	A	A	H	H	H	H	H	H	**H**	B	H	**H**	H	H	H	H	H	B	H	A	A	A	A	H	A	H	H
Xtxp141	-	A	B	H	A	A	B	A	A	A	B	**H**	A	B	A	B	B	**H**	B	-	B	B	A	H	B	B	B	-	B	A	B	B	A
Xgap1	H	H	H	-	A	-	A	B	A	A	A	**H**	-	A	A	A	H	**H**	H	H	H	H	H	B	H	A	H	A	H	A	B	H	A
Xtxp 159	A	H	H	A	A	-	A	H	A	H	H	A	A	H	**H**	B	A	**H**	B	H	H	H	H	H	H	A	A	A	A	H	B	H	A
Xtxp40	A	H	H	A	H	H	H	A	H	H	H	H	A	H	**H**	H	A	**H**	B	H	A	H	B	H	H	-	A	-	A	-	B	H	A
Xtxp65	H	H	H	-	A	H	-	A	A	A	H	**H**	A	A	A	H	H	A	A	A	A	A	A	H	A	-	H	-	A	-	H	H	A
Xtxp94	-	-	-	-	-	-	-	-	A	A	H	**H**	H	A	A	H	A	A	A	A	H	A	A	H	A	-	H	-	-	-	H	H	A
Xtxp15	H	H	H	-	A	H	-	A	A	A	H	**H**	A	A	A	H	H	A	A	A	A	A	A	H	A	-	H	-	A	-	H	H	A

Marker/ Sample	J250	J251	J252	J253	J254	J255	J256	J257	J258	J259	J260	J261	J262	J263	J264	J265	J266	J267	J268	J269	J270	**J271**	J272	**J273**	J274	J275	J276	J277	J278	J279	J280	J281	**J282**
Xtxp 37	H	H	A	H	A	-	H	H	H	H	A	-	H	-	H	A	H	H	H	-	H	A	H	A	H	A	A	A	B	B	A	H	**H**
Xtxp75	A	H	A	H	A	B	A	H	A	H	H	A	H	H	H	A	H	A	B	A	B	A	H	A	H	A	A	A	B	B	A	H	**H**
Xtxp141	B	B	B	B	A	B	A	H	B	B	B	B	B	A	B	A	B	B	A	A	A	A	B	A	H	H	A	A	B	B	A	B	A
Xgap1	A	-	A	H	A	A	A	H	H	A	A	H	A	H	H	H	H	H	A	A	A	A	H	A	H	A	A	A	B	B	A	H	A
Xtxp 159	B	A	H	H	H	A	A	H	A	-	H	H	A	H	A	H	H	H	B	H	H	**H**	A	**H**	H	H	A	A	B	B	H	H	**H**
Xtxp40	-	A	-	H	-	A	H	H	A	H	B	H	A	A	A	H	A	H	B	H	A	**H**	A	**H**	H	A	A	A	B	B	H	H	**H**
Xtxp65	-	A	-	A	H	A	H	H	H	H	H	A	H	B	A	A	A	B	H	A	A	**H**	A	A	H	A	A	A	B	B	H	A	A
Xtxp94	-	A	-	H	H	A	B	H	H	H	H	A	H	B	A	A	A	B	H	A	A	**H**	A	A	H	A	A	A	B	B	H	A	A
Xtxp15	-	A	-	A	H	A	H	H	H	H	H	A	H	B	A	A	A	B	H	A	A	**H**	A	A	H	A	A	A	B	B	H	A	A

Contd.......

Marker/ Sample	J283	**J284**	J285	**J286**	J287	J288	J289	J290	J291	J292	J293	**J294**	J295	J296	J297	**J298**	J299	J300	J301	J302	J303	J304	J305	J306	J307	J308	J309	**J310**	J311	J312	J313	J314	J315
Xtxp 37	H	-	A	**H**	H	A	A	A	A	A	H	H	H	H	A	H	H	B	-	H	A	A	H	A	H	A	A	H	A	A	A	B	B
Xtxp75	A	H	H	**H**	H	H	H	-	-	-	-	-	-	A?	H	A	H	H	H	H	A	H	H	A	H	A	A	A	A	A	A	B	B
Xtxp141	B	**H**	B	-	-	B	A	A	-	-	H	A	B	A	A	A	A	A	A	H	H	A	H	A	H	A	A	A	A	A	A	B	B
Xgap1	A	**H**	H	A	H	A	A	H	H	A	H	-	A	H	H	-	-	A	A	-	H	H	A	A	H	A	A	A	A	A	A	B	B
Xtxp 159	H	**H**	A	**H**	H	A	A	A	H	H	A	A	H	H	A	**H**	A	H	A	A	H	H	H	A	H	A	H	**H**	A	A	A	B	B
Xtxp40	H	**H**	A	**H**	H	A	H	A	H	A	A	A	B	H	A	**H**	H	H	A	A	H	A	A	A	A	A	H	**H**	A	A	A	B	B
Xtxp65	H	A	A	**H**	A	A	A	H	-	A	A	**H**	H	A	A	**H**	H	A	A	H	A	H	A	H	B	H	A	**H**	A	A	A	B	B
Xtxp94	H	H	H	**H**	A	A	A	H	A	A	A	**H**	H	A	A	**H**	H	A	A	H	A	H	A	H	B	H	H	**H**	A	A	A	B	B
Xtxp15	H	A	A	**H**	A	A	A	A	A	H	A	**H**	A	A	H	**H**	H	A	A	H	H	A	A	A	H	A	A	**H**	A	A	A	B	B

J184-J275 and J280-J311 = BC1F1 progenies; J276, J277, J312 and J313 = recurrent parent BTx623; and J278, J279, J314 and J315 = donor parent IS 18551

*J240, J247, J251 are selfs(F2)

Table 15. Screening of recurrent parents(296B and BTx623) backcrossed to "BC_1F_1 progenies"

LG or QTL Target		SBI-01 = A	SBI-01 = A	SBI-07 = E	SBI-07 = E	SBI-10 = G	SBI-10 = G	SBI-05 = J	SBI-05 = J	SBI-05 = J	
S.No	Parent	*Xtxp37*	*Xtxp75*	*Xtxp159*	*Xtxp40*	*Xtxp141*	*Xgap1*	*Xtxp65*	*Xtxp94*	*Xtxp15*	Remarks
1	J90	296B	296B	296B	296B	296B	296B	296B	296B	296B	296B
2	J91	296B	296B	296B	296B	296B	296B	296B	296B	296B	296B
3	J583	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623
4	J594	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623
5	J595	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623
6	J614	BTx623	BTx623	296B	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	Out cross
7	J616	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623
8	J618	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623
9	J632	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623
10	J663	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	Off	BTx623	-	Out cross
11	J558	IS18551	IS18551	BTx623	BTx623	IS18551	IS18551	IS18551	IS18551	IS18551	RIL 252: A -- G J
12	J562	IS18551	IS18551	BTx623	BTx623	IS18551	IS18551	IS18551	IS18551	-	RIL 252: A -- G J
13	J566	IS18551	IS18551	BTx623	BTx623	IS18551	IS18551	IS18551	IS18551	IS18551	RIL 252: A -- G J
Controls											
14	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623	BTx623
15	296B	296B	296B	296B	296B	296B	296B	296B	296B	296B	296B
16	IS 18551	IS18551	IS18551	IS18551	IS18551	IS18551	IS18551	IS18551	IS18551	IS18551	IS 18551

Table 16. Foreground marker data of "BC_2F_1 progeny" from shootfly susceptible and resistant parents 296B and IS 18551

Target A+E+J Marker/Sample	J1233	J1234	J1235	J1236	J1237	J1238	J1239	J1240	J1241	J1242	J1243	J1244	J1245	J1246	J1247	J1248	J1249	J1250	J1251	J1252	J1253	J1254	J1255
Xtxp37	A	H	A	H	B	B	B	H	H	H	off	H	A	off	A	A	H	H	H	H	A	H	H
Xtxp75	A	H	A	H	B	H	H	H	A	H	B	H	A	B	A	A	H	H	H	H	A	H	A
Xtxp159	H	H	H	A	A	H	H	H	H	A	B	H	A	B	A	H	A	A	A	H	H	A	A
Xtxp40	H	H	H	A	A	A	H	-	A	A	B	H	H	B	A	H	H	H	A	A	B	H	A
Xisp258	H	B	A	A	H	H	H	A	H	H	A	H	A	off	A	H	A	H	A	A	A	A	A
Xtxp65	H	B	A	A	H	H	H	A	H	H	H	A	A	H	H	A	A	H	A	H	A	A	A
Xtxp15	H	H	A	A	H	H	off	H	A	H	H	H	H	-	A	H	H	H	A	A	A	H	A
Xtxp23	H	H	A	H	H	H	B	H	A	H	off	H	H	off	A	H	H	H	A	H	H	A	A

Target A+J Marker/Sample	J1256	J1257	J1258	J1259	J1260	J1261	**J1262**	J1263	J1264	J1265	J1266	J1244	J1268	J1269	J1270	J1271	J1272	J1273	J1274	J1275
Xtxp37	A	A	A	A	H	A	**H**	A	A	H	H	H	A	-	H	B	H	A	A	A
Xtxp75	A	A	A	A	H	B	**H**	A	H	A	H	H	A	A	A	B	A	B	A	A
Xisp258	A	A	H	H	H	A	**H**	H	H	B	A	A	A	H	H	H	A	A	A	H
Xtxp65	A	A	A	A	H	H	**H**	A	H	H	H	B	A	A	H	H	H	H	A	A
Xtxp15	A	H	H	H	-	A	A	H	A	B	off	A	A	H	H	-	A	H	A	A
Xtxp23	A	A	A	A	A	off	A	A	-	A	A	A	A	A	A	A	A	off	A	A

Target E+J Marker/Sample	J1276	J1277	J1278	J1279	J1280	J1281	J1282	J1283	J1284	J1285	J1286	J1287	J1288	J1289	J1290	J1291	J1292	J1293	J1294	J1295
Xtxp159	B	A	A	H	H	B	B	B	B	B	A	H	H	B	H	A	A	B	B	-
Xtxp40	B	A	A	H	A	B	B	B	B	H	A	B	H	B	A	A	H	B	B	H
Xisp258	A	A	A	A	A	A	A	A	A	A	H	A	H	B	A	A	A	off	off	off
Xtxp65	A	H	A	H	B	H	A	A	A	A	A	A	H	A	H	B	A	A	A	A
Xtxp15	off	H	A	A	H	B	A	-	-	H	H	B	H	H	H	A	H	-	-	-
Xtxp23	off	A	H	H	H	off	off	off	off	H	A	A	A	A	A	A	H	off	off	off

Target A Marker/Sample	**J1296**	J1297	J1298	J1299	J1300	J1301	**J1302**	J1303	J1304	J1305	J1306	J1307	J1308
Xtxp37	**H**	H	B	H	A	B	**H**	A	H	A	H	A	H
Xtxp75	**H**	H	H	H	A	B	**H**	A	-	B	H	H	H

J1234*= self-product due to second successive failure of backcross resulting in selfing (and production of F_3 rather than BC_1F_1 seed)

J1244*= self-product due to second successive failure of backcross resulting in selfing (and production of F_3 rather than BC_1F_1 seed) since background marker data in Table 20 includes "B" values.

J1288*= self-product due to failure of second backcross resulting in selfing (and production of BC_1F_2 rather than BC_2F_1 seed) since background marker data in Table 20 includes "B" values.

Table 17. "BC_2F_1 progenies" of shoot fly susceptible and resistant parents, 296B and IS 18551 advanced after background screening

S.No	"BC_2F_1" Cross	Target QTLs	Seed sown	"BC_3F_1" progenies
1	J1234*(F_3) x J1336	A+E+J2	17(F_3)	J1689-J1697 (BC_1F_1)
2	J1244*(F_3) x J888	A+E	12	J1698-J1702 (BC_1F_1)
3	J1262 x J880	A+J1	1	J1703 (BC_3F_1)
4	J1288*(BC_1F_2) x J1337	E+J	20	J1704-J1717 (BC_2F_1)
5	J1296* (BC_1F_2) x J880	A	5	J1718-J1723 (BC_2F_1)
6	J1302* (BC_1F_2) x J891	A	5	J1724-J1729 (BC_2F_1)

J1234*= self-product due to second successive failure of backcross resulting in selfing (and production of F_3 rather than BC_1F_1 seed) since foreground marker data in Table 16 includes "B" values.

J1244*= self-product due to second successive failure of backcross resulting in selfing (and production of F_3 rather than BC_1F_1 seed) since background marker data in Table 20 includes "B" values.

J1288*= self-product due to failure of second backcross resulting in selfing (and production of BC_1F_2 rather than BC_2F_1 seed) since background marker data in Table 20 includes "B" values.

J1296*= self-product due to failure of second backcross resulting in selfing (and production of BC_1F_2 rather than BC_2F_1 seed) since background marker data in Table 20 includes "B" values.

J1302*= self-product due to failure of second backcross resulting in selfing (and production of BC_1F_2 rather than BC_2F_1 seed) since background marker data in Table 20 includes "B" values.

Table 18. Foreground marker genotyping of "BC_2F_1 progeny" from shoot fly susceptible and resistant parents BTx623 and IS 18551

Target A+E	J901	J902	J903	J904	J905	J906	J907	J908	J909	J910	J911	J912	J913	J914	J915	J916	J917	J918	J919	J920	J921	J922	J923	J924
Marker/Sample																								
Xtxp159	H	A	A	H	H	A	H	H	H	A	A	A	H	A	A	H	H	A	H	H	H	H	A	H
Xtxp40	H	A	H	H	H	-	H	H	H	H	A	A	A	A	A	A	H	A	-	H	B	H	A	H
Xtxp141	A	H	H	H	H	A	H	A	A	H	A	A	H	A	A	A	A	A	H	A	B	H	A	H
Xgap1	-	H	H	H	A	H	H	H	H	A	A	A	A	A	A	H	H	-	H	H	B	H	H	H

Target E+G	J925	J926	J927	J928	J929
Marker/Sample					
Xtxp159	A	A	H	H	A
Xtxp40	A	A	H	H	A
Xtxp141	A	H	H	A	B
Xgap1	A	H	H	A	B

Target E+J	J930	J931	J932	J933	J934	J935	J936	J937	J938	J939	J940	J941	J942	J943	J944	J945	J946	J947	J948	J949	J950	J951	J952	J953	J954	J955	J956	J957	J958	J959
Marker/Sample																														
Xtxp159	H	A	H	H	A	B	B	A	H	H	A	H	H	A	A	A	H	H	H	A	H	A	A	A	-	H	H	A	A	-
Xtxp40	H	H	H	H	A	H	H	A	A	H	H	B	H	A	A	A	H	H	A	H	H	A	A	A	H	H	H	A	A	A
Xtxp65	H	A	H	H	H	H	A	H	A	B	A	A	H	-	-	A	A	A	A	H	H	A	A	A	B	H	H	A	H	A
Xtxp94	A	A	A	H	A	H	A	H	H	B	-	A	H	A	H	A	A	A	A	H	H	A	A	A	-	H	H	A	H	A
Xtxp15	A	A	A	A	A	A	A	H	H	H	A	A	H	H	H	A	A	A	A	A	A	A	A	H	-	H	H	A	H	H

Target G+J	J960	J961	J962	J963	J964	J965	J966	J967	J968	J969	J970	J971	J972	J973	J974	J975	J976	J977	J978	J979
Marker/Sample																				
Xtxp141	B	A?	off	-	A	A	H	A	A	A	H	H	H	A	B	off	H	-	-	A
Xgap1	B	A	A	A	A	A	H	A	H	A	-	A	H	H	B	H	H	A	A	H
Xtxp65	H	H	A	A	H	H	A	H	A	H	A	H	B	B	H	A	H	H	H	A
Xtxp94	H	H	A	H	H	H	H	H	A	H	A	H	H	B	A	H	H	A	H	A
Xtxp15	A	H	A	H	H	B	H	H	H	A	H	H	A	-	-	-	-	-	-	-

Target A+E+G	J980	J981	J982	J983	J984	J985	J986	J987	J988	J989	J990
Marker/Sample											
Xtxp37	H	H	H	A	B	H	H	B	H	H	H
Xtxp75	A	A	A	A	A	A	A	A	A	-	-
Xtxp159	A	B	A	A	A	B	A	H	H	H	A
Xtxp40	B	H	H	B	B	A	B	A	H	A	B
Xtxp141	-	H	A	H	A	A	A	-	H	B	B
Xgap1	H	H	H	H	A	A	A	H	H	H	H

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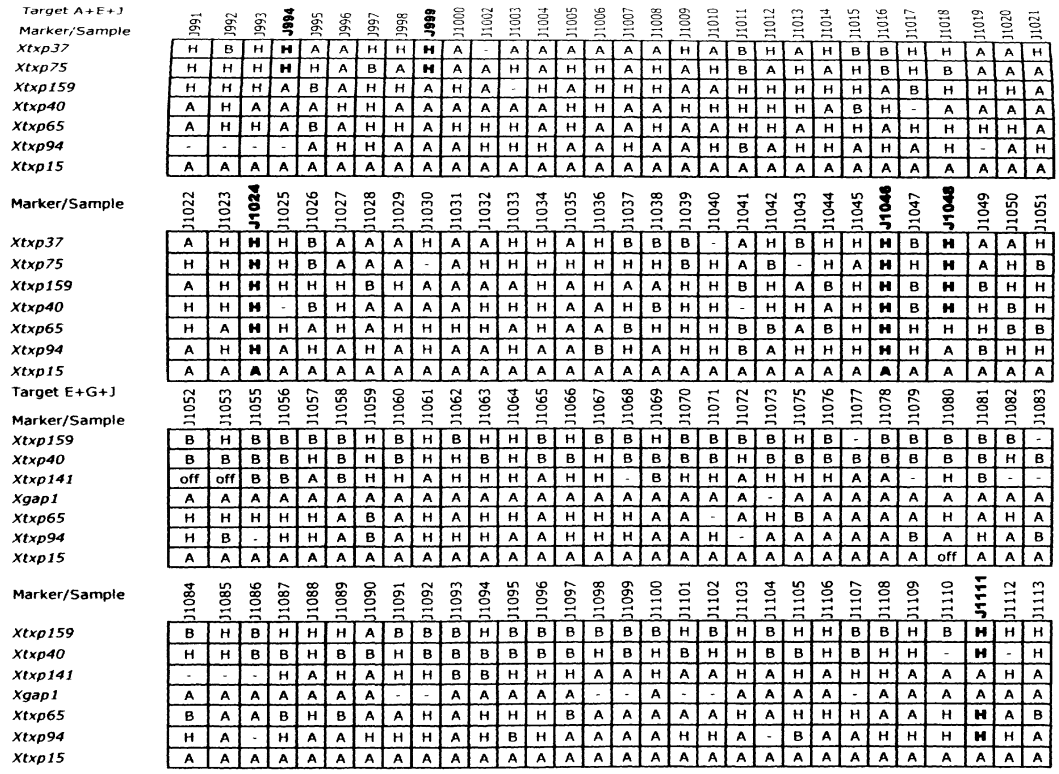
Target A+E+J
Marker/Sample
J991 J992 J993 J994 J995 J996 J997 J998 J999 J1000 J1002 J1003 J1004 J1005 J1006 J1007 J1008 J1009 J1010 J1011 J1012 J1013 J1014 J1015 J1016 J1017 J1018 J1019 J1020 J1021
Xtxp37 H B H H A A H H H A - A A A A A A H A B H A H B B H H A A H
Xtxp75 H H H H H A B A H A A H A H H A H A H B A H A H B H B A A A
Xtxp159 H H H A B A H H A H A - H A H H H A A H H H H H A B H H H A
Xtxp40 A H A A A H H A A A A A A H H A A H H H H H A B H - A A A A
Xtxp65 A H H A B A H H A H H H A H A A H A A H H A H H A H H H H A
Xtxp94 - - - - A H H A A A H H H A A H A A H B A H H A H A H - A H
Xtxp15 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 A
Marker/Sample
J1022 J1023 J1024 J1025 J1026 J1027 J1028 J1029 J1030 J1031 J1032 J1033 J1034 J1035 J1036 J1037 J1038 J1039 J1040 J1041 J1042 J1043 J1044 J1045 J1046 J1047 J1048 J1049 J1050 J1051
Xtxp37 A H H H B A A A H A A H H A H B B B - A H B H H H B H A A H
Xtxp75 H H H H B A A A - A H H H H H H H B H A B - H A H H H A H B
Xtxp159 A H H H H H B H A A A H A H A A H H B H A B H H B H B H H H
Xtxp40 H H H - B H A A A H H H A A H B H H - H H A H H B H H B H H
Xtxp65 H A H H A H A H H H H A H A A B H H H B B A B H H H H H B B
Xtxp94 A H H A H A H A H A A H A A B H A H H B A H H H H H A B H H
Xtxp15 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 A
Target E+G+J
Marker/Sample
J1052 J1053 J1055 J1056 J1057 J1058 J1059 J1060 J1061 J1062 J1063 J1064 J1065 J1066 J1067 J1068 J1069 J1070 J1071 J1072 J1073 J1075 J1076 J1077 J1078 J1079 J1080 J1081 J1082 J1083
Xtxp159 B H B B B B H B H B H H B H B B H B B B B H B - B B B B B -
Xtxp40 B B B B H B H B H H B H B B H B B B B H B B B B B B B H B
Xtxp141 off off B B A B H H A H H H A H H - B H H A H H H A A - H B - -
Xgap1 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
Xtxp65 H H H H H A B A H A H H A H H H A A - A H B A A A A H A H A
Xtxp94 H B - H H A B A H H H A A H H A A H - A A A A A B A H A B
Xtxp15 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 off A A A
Marker/Sample
J1084 J1085 J1086 J1087 J1088 J1089 J1090 J1091 J1092 J1093 J1094 J1095 J1096 J1097 J1098 J1099 J1100 J1101 J1102 J1103 J1104 J1105 J1106 J1107 J1108 J1109 J1110 J1111 J1112 J1113
Xtxp159 B H B H H H A B B B H B B B B B B H B H B H H B B H B H H H
Xtxp40 H H B B H B B H B B B B B B H B H B H H B B H B H H - H - H
Xtxp141 - - - H A H A H H B B H H H A A H A A H A H H A A A H A
Xgap1 A A A A A A A - - A A A A A - - A - - A A A A - A A A A A A
Xtxp65 B A A B H B A A H A H H H B A A A A A H A H H H A A H H A B
Xtxp94 H A - H A A H H H A H B H A A A A H H A - B A A H H H H A
Xtxp15 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 A

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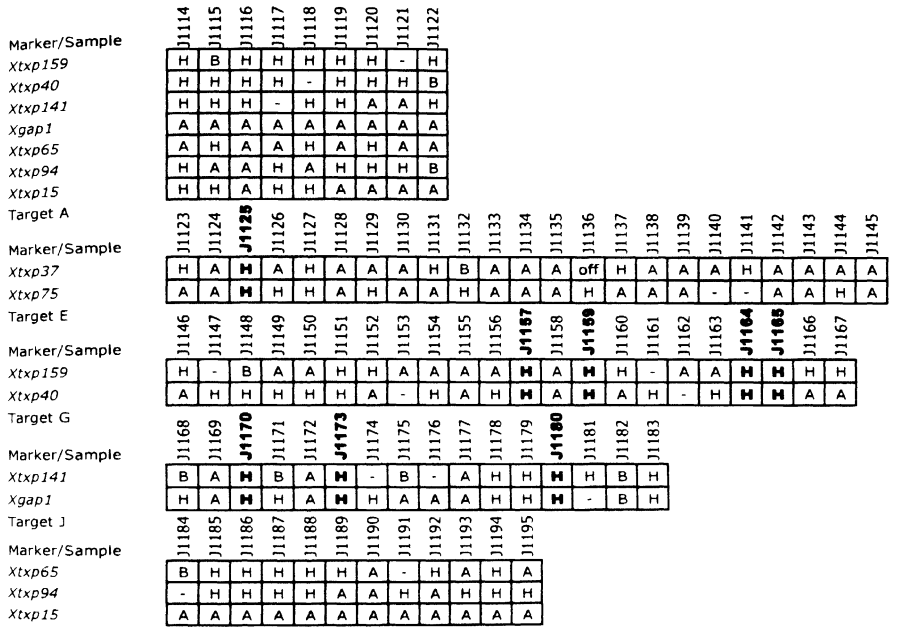


J1054 and J1074 = plants not available

J1024* = Failure of backcross and production of F_3 seed which is evidenced from the foreground marker data in Table 26.

J1111* = Failure of backcross in this generation resulting in selfing and formation of BC_1F_2 than BC_2F_1 (and failure in earlier generation) as the background marker data in Table 22 includes "B" value.

J988* = Failure of backcross in this generation resulting in selfing and formation of BC_2F_2 instead of BC_3F_1 since the background marker data in Table 22 includes "B" value.

Table 19a. Single-QTL "BC_2F_1 progenies" of shoot fly susceptible and resistant parents, BTx623 and IS 18551 advanced after background screening

S. No.	"BC_2F_1" Cross	Target QTL	Number of "BC_3F_1" seed sown	"BC_3F_1" progenies
1	J926 x J808	G	2	J1515
2	J1170 x J854	G	17	J1516-J1526
3	J1173 x J820	G	4	J1401-J1404
4	J1180 x J804	G	6	J1405-J1410
5	J902 x J801	E	7	J1411-J1417
6	J946 x J816	E	1	J1418
7	J1157 x J814	E	8	J1419-J1424
8	J1159 x J831	E	9	J1425-J1432
9	J1164 x J810	E	7	J1433-J1439
10	J1165 x J833	E	7	J1440-J1445
11	J901 x J857	A	7	J1446-J1449
12	J909 x J822	A	4	J1450-J1453
13	J917 x J865	A	7	J1454-J1460
14	J920 x J806	A	7	J1461-J1466
15	J947 x J817	A (mistake)	7	J1467-J1473
16	J994*(BC_1F_1) x J813	A	3	J1474 (BC_2F_1)
17	J999*(BC_1F_1) x J810	A	6	J1475-J1480 (BC_2F_1)
18	J1125*(BC_1F_1) x J835	A	1	J1481 (BC_2F_1)
19	J937 x J804	J	7	J1482-J1488
20	J961 x J819	J	1	J1489
21	J967 x J823	J	2	J1490-J1491
22	J971 x J813	J	7	J1492-J1498
23	J949 x J852	J1	7	J1499-J1505
24	J969 x J830	J1	6	J1506-J1510

J994*, J999*, J1125*= Failure of backcross in earlier generation (first generation) resulted in production of BC_2F_1 seed rather than BC_3F_1 in this generation

Target QTL E+J mistaked to "A" in J947 which is clear from tables 18 and 23

Table 19b. Multiple-QTL "BC_2F_1 progenies" of shoot fly susceptible and resistant parents, BTx623 and IS 18551 advanced after background screening

S.No.	"BC_2F_1" cross	Target QTLs	Number of "BC_3F_1" seeds sown	"BC_3F_1" progenies
1	J904 x J857	A+E	7	J1600-J1606
2	J924 x J810	A+E	7	J1607-J1614
3	J933 x J838	E+ J1	5	J1615-J1618
4	J942 x J819	E+J	8	J1619-J1626
5	J956 x J813	E+J	8	J1627-J1638
6	J1024*(F_2) x J810	A+E+J1	1	J1639 (F_3)
7	J1046 x J832	A+E+J1	8	J1640-J1647
8	J1048 x J832	A+E	7	J1648-J1656
9	J927 x J814	E+G	12	J1657-J1667
10	J988*(BC_2F_1) x J804	E+G	5	J1668-J1672(BC_2F_2)
11	J1111*(BC_1F_1) x J1326	E+J1	5	J1673-J1678(BC_1F_2)
12	J950 x J939	E+J1	5	J1679-J1688
13	J966 x J817	G+J2	2	J1511-J1512

J1024* = Failure of backcross in earlier and present generation resulting in production of F_3 seed rather than BC_1F_1 in this generation as foreground marker data in Table 26 includes "B" value.

J1111* = Failure of backcross in this generation resulting in selfing and formation of BC_1F_2 than BC_2F_1 (and failure in earlier generation) as the background marker data in Table 22 includes "B" value.

J988* = Failure of backcross in this generation resulting in selfing and formation of BC_2F_2 instead of BC_3F_1 since the background marker data in Table 22 includes "B" value.

Table 20. Background screening of flanking markers on foreground-selected "BC_2F_1" progenies of parents 296B and IS 18551

	Plant	*Xtxp319* on top	*Xtxp32* on bottom	
Linkage group: A				
1	J1234	A	H	
2	J1244*	A	B	self (F_3)
3	J1262	H	H	
4	J1288*	B	A	self (BC_1F_2)
5	J1296*	A	B	self (BC_1F_2)
6	J1302*	A	B	self (BC_1F_2)
7	J1306*	A	B	self (BC_1F_2)
	296B	A	A	
	IS 18551	B	B	

	Plant	*Xisp348* on top	*Xtxp312* on bottom	
Linkage group: E				
8	J1234	H	H	
9	J1244*	H	H	self (F_3)
	296B	A	A	
	IS 18551	B	B	

	Plant	*Xisp258* on top	*Xtxp283* on bottom	
Linkage group: J				
10	J1244*	B	A	self (F_3)
	296B	A	A	
	IS 18551	B	B	

Table 21. Background screening of flanking markers on foreground-selected "BC_2F_1 progenies" of BTx623 and IS 18551 (Single-QTL plants)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
Crossed BC_2F_1	**J926**	**J1170**	**J1173**	**J1180**	**J902**	**J946**	**J1157**	**J1159**	**J1164**	**J1165**	J1106	**J947**	J919	J932	J1151	**J901**	**J909**	**J917**	**J920**	J993	**J994**	**J999**	**J1125**	J1033	J1127	J908	**J937**	**J961**	**J967**	**J971**	J958	J964	**J949**	**J969**	J1185	J1186	J1187	J1188
Target QTL	**G**	**G**	**G**	**G**	**E**	**E**	**E**	**E**	**E**	**E**	E	**E**	E	E	E	**A**	**A**	**A**	**A**	A	**A**	**A**	**A**	A	A	A	**J**	**J**	**J**	**J**	J	J	**J1**	**J1**	J1	J1	J1	J1
Marker																																						
Xcup62																**A**	**A**	**H**	**A**	H	-	**H**	**H**	A	A	A												
Xtxp37																**H**	**H**	**H**	**H**	H	**H**	**H**	**H**	H	H	H												
Xtxp75																**H**	**H**	**H**	**H**	H	**H**	**H**	**H**	H	H	H												
Xtxp88																**A**	**A**	**A**	**A**	A	**B**	**A**	**A**	A	A	A												
Xgap342					**A**	**A**	**H**	**A**	**H**	**H**	H	**A**	A	A	A																							
Xtxp159					**H**	**H**	**H**	**H**	**H**	**H**	H	**H**	H	H	H																							
Xtxp40					**H**	**H**	**H**	**H**	**H**	**H**	H	**H**	H	H	H																							
Xisp263	**H**	**H**	**H**	**H**																																		
Xtxp141	**H**	**H**	**H**	**H**																																		
Xgap1	**H**	**H**	**H**	**H**																																		
Xcup07	**A**	**H**	**A**	**A**																																		
Xisp258																											**H**	**H**	**H**	**H**	H	H	**H**	**H**	A	A	A	A
Xtxp65																											**H**	**H**	**H**	**H**	H	H	**H**	**H**	H	H	H	H
Xtxp94																											**H**	**H**	**H**	**H**	H	H	**H**	**H**	H	H	H	H
Xtxp15																											**H**	**H**	**H**	**H**	H	H	**A**	**A**	A	A	A	A
Xtxp225																											**H**	**H**	**H?**	**H**	H	H	**H?**	**H?**	A	H?	A	H

Table 22. Results from background screening of flanking markers on foreground-selected "BC_2F_1 progenies" of BTx623 and IS 18551 (Multiple-QTL

Crossed BC_2F_1	J904	J924	J1048	J927	J988*	J927	J933	J1111*	J950	J942	J956	J966	J1024	J1046
Target QTLs	A+E	A+E	A+E	E+G	E+G	E+G	E+J1	E+J1	E+J1	E+J	E+J	G+J2	A+E+J1	A+E+J1
Xcup62	H	H	A										H	A
Xtxp37	H	H	H										H	H
Xtxp75	H	H	H										H	H
Xtxp88	A	A	A										A	A
Xgap342	A	A	A	A	A	A	H	B	H	A	A		H	A
Xtxp159	H	H	H	H	H	H	H	H	H	H	H		H	H
Xtxp40	H	H	H	H	H	H	H	H	H	H	H		H	H
Xisp263				H	B	H						H		
Xtxp141				H	H	H						H		
Xgap1				H	H	H						H		
Xcup07				A	A	A						H		
Xisp258							H	H	H	H	H	A	H	H
Xtxp65							H	H	H	H	H	A	H	H
Xtxp94							H	H	H	H	H	H	H	H
Xtxp15							A	A	A	H	H	H	A	A
Xtxp225							A	A	A	A	H	H	A	A

Table 23. Foreground marker data from BC_2F_1 progeny of shoot fly susceptible and resistant parents, BTx623 and IS 18551 (Single-QTL targets)

G /Sample	J1401	J1402	J1403	J1404	J1405	J1406	**J1407**	J1408	**J1409**	J1410	J1515	J1516	J1517	J1518	J1519	J1520	J1521	J1522	J1523	J1524	J1525	J1526	J1511	J1512
1	A	A	A	A	H	B	**H**	A	**H**	B	-	A	A	A	A	A	A	A	A	A	A	A	B	H
	A	A	A	A	H	B	**H**	B	**H**	B	H	A	A	A	A	A	A	A	A	A	A	A	B	H
E /Sample	J1411	J1412	**J1413**	J1414	J1415	J1416	J1417	J1418	J1419	J1420	J1421	**J1422**	J1423	**J1424**	J1425	J1426	J1427	J1428	J1429	J1430	J1431	J1432	J1433	**J1434**
9	B	A	**H**	B	A	A	H	B	B	A	A	**H**	A	**H**	A	A	A	A	-	A	A	A	H	**H**
	A	H	**H**	H	A	A	A	B	B	A	H	**H**	A	**H**	H	H	A	A	H	A	H	H	A	**H**
/Sample	J1435	J1436	J1437	J1438	J1439	**J1440**	J1441	J1442	J1443	J1444	**J1445**													
9	A	A	H	A	A	**H**	A	A	A	H	**H**													
	H	H	A	H	A	**H**	A	H	H	H	**H**													
A /Sample	J1446	J1447	J1448	J1449	J1450	J1452	J1453	J1454	J1455	J1456	J1457	**J1458**	J1459	J1460	J1461	J1462	**J1463**	J1464	J1465	J1466	J1467	J1468	J1469	J1470
	A	A	B	A	B	A	A	H	A	A	-	**H**	B	B	B	B	**H**	A	B	B	B	B	B	B
	A	A	A	A	H	A	A	A	A	A	H	**H**	B	B	A	B	**H**	A	A	H	A	A	A	A
/Sample	J1471	J1472	J1473	**J1474**	J1475	J1476	J1477	J1478	J1479	J1480	**J1481**													
	H	B	A	**H**	A	A	A	A	A	A	**H**													
	A	A	A	**H**	A	A	A	A	A	A	**H**													
I Sample	J1482	J1483	J1484	J1485	J1486	**J1487**	J1488	J1489	J1490	J1491	**J1492**	J1493	J1494	**J1495**	J1496	J1497	J1498	J1499	J1500	J1501	**J1502**	J1503	J1504	**J1505**
	H	H	H	A	B	**H**	A	B	H	-	**H**	A	A	**H**	B	A	H	H	A	A	**H**	B	H	**H**
	B	A	A	A	B	**H**	A	B	A	H	**H**	A	A	**H**	H	A	A	H	H	A	**H**	B	B	**H**
	A	H	A	A	A	A	A	H	A	A	**H**	H	A	**H**	H	A	A	H	H	A	**H**	H	H	**H**
Sample	**J1506**	J1507	**J1508**	J1509	J1510																			
	H	H	A	B	B																			
	H	B	**H**	B	H																			
	H	H	**H**	H	B																			

/genotyping mistake in target E from J1467-1473 as clear in (table 19a),
1481 are BC_2F_1 plants

Table 24. Foreground marker data generated from "BC_3F_2 progeny" of shoot fly susceptible and resistant parents, BTx623 and IS 18551 segregating for target QTL on SBI-10

Marker/ Sample	J2611	J2612	J2613	J2614	J2615	J2616	J2617	J2618	J2619	J2620	J2621	J2622	J2623
Xtxp141	H	B	H	[redacted]	B	H	H	B	A	B	B	H	H
Xgap1	H	B	H	[redacted]	B	B	H	B	A	B	H	A	H

Marker/ Sample	J2624	J2625	J2626	J2627	J2628	J2629	J2630	J2631	J2632	J2633	J2634	J2635	J2636
Xtxp141	B	B	H	A	H	H	H	H	H	B	H	A	H
Xgap1	H	B	H	H	H	B	A	H	A	H	B	A	H

Marker/ Sample	J2637	J2638	J2639	J2640	J2641	J2642	J2643	J2644	J2645	J2646	J2647	J2648	J2649
Xtxp141	H	B	A	H	B	B	B	B	B	H	B	H	H
Xgap1	H	B	H	B	A	H	B	B	B	B	B	B	B

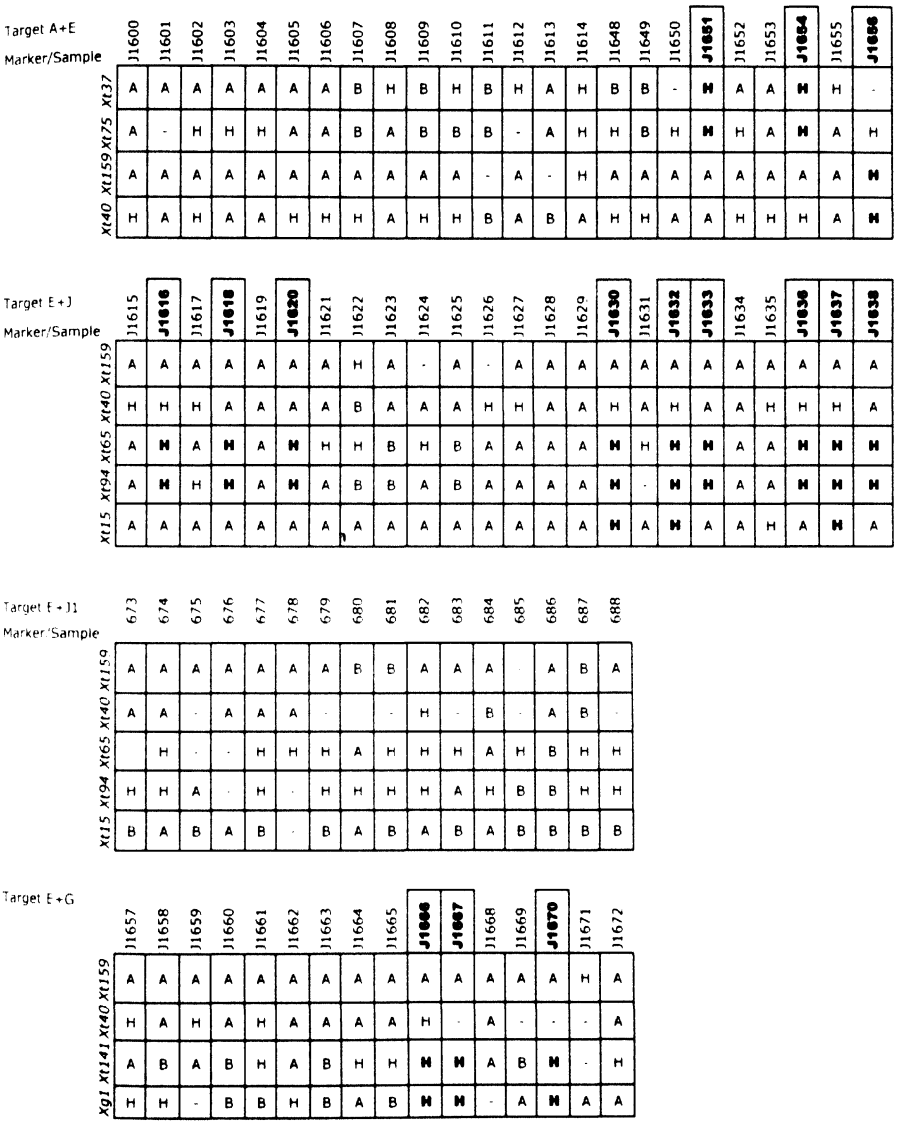
Marker/ Sample	J2650	BTx623	IS 18551
Xtxp141	H	A	B
Xgap1	B	A	B

Table 25. "BC_3F_1 Progenies" of shoot fly susceptible and resistant parents, BTx623 and IS 18551 (single-QTL targets) advanced after background screening

S.No.	"BC_3F_1" Cross	Target QTL	Number of "BC_4F_1" Seed Produced	"BC_3F_2" seed (g) harvested	"BC_4F_1" progenies ("BC_4F_2") advanced	"BC_3F_2" progenies
1	J1413 x J1541	E	25	16		
2	**J1422 x J1558**	**E**	**185**	**35**	**J2275-J2294**	**Nil**
3	J1424 x J1540	E	77	8		
4	J1434 x J1541	E	151	14		
5	**J1440 x J1537**	**E**	**93**	**29**	**J2295-J2304**	**Nil**
6	J1445 x J1536	E	53	26		
7	J1458 x J1538	A	73	33		
8	J1463 x BTx623	A	80	30		
9	J1474*(BC_2F_1) x J1550	A	54 (BC_3F_1)	51 (BC_2F_2)		
10	**J1481*(BC_2F_1) x J1540**	**A**	**164 (BC_3F_1)**	**51 (BC_2F_2)**	**J2251-2270**	**Nil**
12	**J1492 x J1541**	**J**	**95**	**25**	**J2459-J2488**	**Nil**
13	J1495 x J1541	J	117	10		
15	J1502 x J1537	J	7	33		
16	J1505 x J1545	J	90	56		
17	J1506 x J1525	J	23	30		
11	J1487 x J1536	J1	154	61		
14	J1500 x J1537	J2	105	62		
18	J1508 x J1549	J2	127	44		
21	**J1407**	**G**	**-**	**18**	**Nil**	**J2611-J2630**
22	**J1409**	**G**	**-**	**72**	**Nil**	**J2631-J2650**
23	J2614 x BTx623 (BC_3F_2)	G	54	24 (BC_3F_3)		

J1474*, J1481*= Failure of backcross in earlier generation (resulted in selfing) and production of BC3F1 and BC2F2 seed rather than BC4F1 and BC3F2 in this generation)

Table 26. Foreground marker genotyping of "BC_3F_1 Progeny" from shootfly susceptible and resistant parents, BTx623 and IS 18551 (multiple-QTL targets)



Target A+E+J1	**J1639**	J1640	J1641	J1642	J1643	J1644
Xt37	**H**	-	H	B	A	B
Xt75	**B**	H	H	B	A	H
Xt159	**A**	A	A	A	-	A
Xt40	**A**	H	H	H	H	H
Xt65	**B**	A	B	H	A	A
Xtxp94	**B**	A	B	H	H	H
Xtxp15	**A**	A	A	A	A	A

	J1645	**J1646**	**J1647**	Target G+J	J1511	J1512
Xt37	A	**H**	**H**	*Xt141*	B	-
Xt75	-	**H**	**H**	*Xg1*	B	H
Xt159	A	A	A	*Xt65*	-	A
Xt40	H	H	A	*Xt94*	B	H
Xt65	A	**H**	A	*Xt15*	A	A
Xtxp94	A	**H**	A			
Xtxp15	-	A	A			

J1639 = Frequent failures of backcross and formation of F3 instead of BC1F1 which is clear from this marker data
Plants J1673-J1678 were expected to be $BC2F_1$ individuals, but most were derived from selfs not back crosses

Table 27. "BC_3F_1 Progenies" of shoot fly susceptible and resistant parents, BTx623 and IS 18551 (Multiple-QTL targets) advanced after background screening

S.No.	"BC_3F_1" Cross	Target QTL	Number of "BC_4F_1 "seed harvested	"BC_3F_2"seed (g)	"BC_4F_1" progenies (BC_4F_2) advanced	"BC_3F_2 "progenies
1	**J1647 x J1930**	**A**	**10**	**34**	**J2271-J2274**	**Nil**
2	J1651 x J1936	A	67	2		
3	J1654 x J1952	A	175	33		
4	J1646 x J1929	A+J1	13	37		
5	J1656 x J1966	E	43	5		
6	J1666 x J1938	G	98	13		
7	J1667 x J1944	G	43	33		
8	J1670(BC_2F_2) x J1957	G	118(BC3F1)	75(BC3F2)		
9	**J1630 x J1964**	**J**	**205**	**20**	**J2335-J2387**	**Nil**
10	**J1632 x J1965**	**J**	**75**	**28**	**J2388-J2408**	**Nil**
11	**J1637 x J1969**	**J**	**194**	**44**	**J2409-J2428**	**Nil**
12	J1616 x J1947	J1	210	35		
13	J1618 x J1982	J1	54	12		
14	J1620 x J1925	J1	126	47		
15	**J1633 x J1928**	**J1**	**12**	**63**	**J2451-J2458**	**Nil**
16	**J1636 x J1933**	**J1**	**2**	**43**	**J2449-J2450**	**Nil**
17	**J1638 x J1956**	**J1**	**115**	**31**	**J2429-J2448**	**Nil**

J1670* = Failure of backcross in earlier generation and production of BC_3F_1 in this generation instead of BC_4F_1.

Table 28. Foreground marker data generated from "BC_3F_1" progeny of 296B and IS 18551 (multiple-QTL targets)

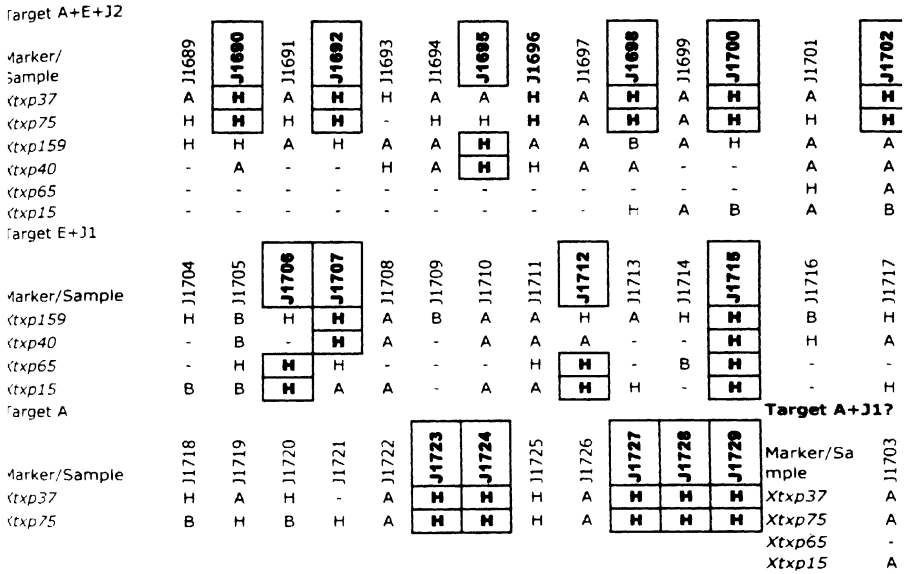


Table 29. "BC_3F_1 progenies" of shoot fly susceptible and resistant parents, 296B and IS 18551 (Multiple-QTL targets) advanced after background screening

S.No.	"BC_3F_1" crosses made	Target QTL	Number of "BC_4F_1" seed harvested	"BC_3F_2" seed (g)	"BC_4F_1" progenies advanced	"BC_3F_2" progenies advanced
1	**J1690 (BC_1F_1) x J2072**	**A+J**	**183 (BC_2F_1)**	**43 (BC_1F_2)**	**J2489-J2511,J2545-J2603**	**Nil**
2	**J1692 (BC_1F_1) x J2079**	**A**	**217 (BC_2F_1)**	**64 (BC_1F_2)**	**J2512-J2529**	**Nil**
3	J1695 (BC_1F_1) x J2062	E	7 (BC_2F_1)	60 (BC_1F_2)	J2188-J2194	
4	J1696 (BC_1F_1) x J2031	A	5 (BC_2F_1)	49 (BC_1F_2)		
5	**J1698 (F_4) x J2019**	**A**	**48 (BC_1F_1)**	**78 (F_5)**	**J2530-J2544**	**Nil**
6	J1700(F_4) x J2073	A	180 (BC_1F_1)	57 (F_5)		
7	J1702 (F_4) x J2031	A	59 (BC_1F_1)	83 (F_5)		
8	J1706(BC_2F_1) x J2059	J	25 (BC_3F_1)	62 (BC_2F_2)	J2208-J2220	
9	**J1707(BC_2F_1) x J2057**	**E**	**39 (BC_3F_1)**	**49 (BC_2F_2)**	**J2195-J2201**	**Nil**
10	J1712(BC_2F_1) x J2027	J	7 (BC_3F_1)	45 (BC_2F_2)	J2202-J2207	
11	J1715(BC_2F_1) x J2026	A+J	3 (BC_3F_1)	23 (BC_2F_2)		
12	J1723(BC_2F_1) x J2028	A	123 (BC_3F_1)	18 (BC_2F_2)		
13	J1724(BC_2F_1) x J2067	A	38 (BC_3F_1)	29 (BC_2F_2)		
14	J1727(BC_2F_1) x J2034	A	36 (BC_3F_1)	84 (BC_2F_2)		
15	J1728(BC_2F_1) x J2033	A	125 (BC_3F_1)	94 (BC_2F_2)		
16	J1729(BC_2F_1) x J2025	A	152 (BC_3F_1)	44 (BC_2F_2)		

J1698-J1702 are four generation self products due to failure of back cross and resulting in F5 formation, confirmed in Table 28 which include "B" values
Background data was not available for J1706 and J1715

Table 30. Foreground marker data generated from "BC_4F_1 progeny" of BTx623 and IS 18551, and 296B and IS 18551, sown along with RIL-derived backcross progeny

"BC_4F_1" progenies of BTx623 and IS 18551

QTL Target A+J1

		J2101	J2102	J2103	J2104	J2105	J2106	J2107
J1646 x J1929	Marker/sample							
(actually BC_4F_1)	*Xtxp37*	A	H	A	A	A	H	H
	Xtxp75	A	H	H	H	H	H	H
	Xtxp65	H	A	H	-	H	A	A
	Xtxp94	H	H	H	A	H	H	H
	Xtxp15	A	A	A	A	A	A	A

"BC_4F_1" progenies of BTx623 and IS 18551

QTL Target E

		J2188	J2189	J2190	J2191	J2192	J2193	J2194
J1695 x J2062	Marker/sample							
(actually BC_1F_1)	*Xtxp159*	A	H	H	A	A	H	H
Missing data	*Xtxp40*	A	H	H	H	A	H	H

		J2195	J2196	J2197	J2198	J2199	J2200	J2201
J1707 x J2057								
(actually BC_2F_1) ok	Marker/sample	H	H	H	H	H	A	H
	Xtxp40	H	H	H	H	H	H	H

QTL Target E+J

		J2208	J2209	J2210	J2211	J2212	J2213	J2214	J2215	J2216	J2217	J2218	J2219	J2220
J1706 x J2059	Marker/sample													
(actually BC_2F_1)	*Xtxp159*	H	H	H	H	H	H	A	H	A	A	A	H	H
Missing data	*Xtxp40*	H	H	H	H	H	H	A	A	A	-	H	H	H
	Xtxp65	A	H	H	A	H	H	H	B	H	O	A	A	A
	Xtxp15	A	A	A	A	A	A	A	A	A	O	A	A	A

QTL Target J

		J2202	J2203	J2204	J2205	J2206	J2207
J1712 x J2027	Marker/sample						
(actually BC_2F_1)	*Xtxp65*	A	A	A	A	A	A
	Xtxp15	H	H	A	H	A	H

*J1492 x J1541 is actually "J QTL"

Table 31. Results from background screening on carrier linkage groups A (SBI-01) and E (SBI-07) among foreground-selected BC_3F_1 progenies of BTx623 and IS 18551

S.No.	BC3F1 Cross	Linkage Group	*Xtxp316*	*Xtxp248*	*Xtxp319*	*Xtxp32*	*Xtxp357*	*Xcup73*	*Xtxp208*	*Xtxp302*	Total A's	Total H's	Total B's	Result
1	**J1647 x J1930**	**SBI-01**	**A**	**A**	**A**	**H**	**H**	**A**	**A**	**A**	**6**	**2**	**0**	**Second best option**
2	J1651 x J1936	SBI-01	B	B	B	A	H	A	A	A	4	1	3	Self
3	J1654 x J1952	SBI-01	B	B	B	A	A	A	A	A	5	0	3	Self
4	J1458 x J1538	SBI-01	A	B	H	A	H	H	A	A	4	3	1	Self
5	J1463 x BTx623	SBI-01	B	B	B	H	H	A	A	A	3	2	3	Self
6	J1474 x J1550	SBI-01	A	B	H	H	A	A	A	A	5	2	1	Self
7	**J1481 x J1540**	**SBI-01**	**A**	**A**	**A**	**H**	**A**	**A**	**A**	**A**	**7**	**1**	**0**	**Best option**
8	BTx623	A	A	A	A	A	A	A	A	A	8	0	0	Recurrent parent
9	IS 18551	B	B	B	B	B	B	B	B	B	0	0	8	Donor parent

S.No.	BC3F1 Cross	Linkage group	*Xtxp312*	*Xtxp295*	*Xisp344*	Total A's	Total H's	Total B's	Result
1	**J1413 x J1541**	**SBI-07**	**H**	**A**	**A**	**2**	**1**	**0**	**Joint best option**
2	**J1422 x J1558**	**SBI-07**	**H**	**A**	**A**	**2**	**1**	**0**	**Joint best option**
3	**J1424 x J1540**	**SBI-07**	**H**	**A**	**A**	**2**	**1**	**0**	**Joint best option**
4	J1434 x J1541	SBI-07	-	A	A	2	0	0	Missing data
5	**J1440 x J1537**	**SBI-07**	**H**	**A**	**A**	**2**	**1**	**0**	**Joint best option**
6	**J1445 x J1536**	**SBI-07**	**H**	**A**	**A**	**2**	**1**	**0**	**Joint best option**
7	J1656 x J1966	SBI-07	B	A	A	2	0	1	Self
8	BTx623	A	A	A	A	3	0	0	Recurrent parent

Table 32. Results from background screening of foreground-selected BC_3F_1 introgressions of target QTL E (Linkage Group SBI-07) derived from BTx623 and IS 18551

BC_3F_1 introgression heterozygote crosses available for possible advance

S.No	Marker	Linkage group	J1413 x J1541	**J1422 x J1558**	J1424 x J1540	J1440 x J1537	J1445 x J1536	BTx623	IS 18551
1	*Xtxp248*	SBI-01	H	**A**	A	A	A	A	B
2	*Xtxp32*	SBI-01	A	**A**	A	A	A	A	B
3	*Xtxp208*	SBI-01	A	**A**	A	B	B	A	B
4	*Xtxp211*	SBI-02	H	**A**	A	H	H	A	B
5	*Xtxp1*	SBI-02	A	**A**	A	A	A	A	B
6	*Xtxp207*	SBI-02	A	**A**	A	A	A	A	B
7	*Xtxp34*	SBI-03	A	**A**	A	A	A	A	B
8	*Xtxp285*	SBI-03	A	**A**	A	A	A	A	B
9	*Xtxp228*	SBI-03	A	**A**	A	A	A	A	B
10	*Xtxp343*	SBI-04	A	**A**	A	A	A	A	B
11	*Xcup05*	SBI-04	A	**A**	A	A	A	A	B
12	*Xtxp27*	SBI-04	A	**A**	A	A	A	A	B
13	Xtxp40	SBI-07	H	H	H	H	H	A	B
14	*Xtxp159*	SBI-07	H	H	H	H	H	A	B
15	*Xtxp312*	SBI-07	H	H	H	H	H	A	B
16	*Xtxp295*	SBI-07	A	**A**	A	A	A	A	B
17	*Xisp344*	SBI-07	A	**A**	A	A	A	A	B
18	*Xtxp10*	SBI-09	A	**A**	A	A	A	A	B
19	*Xtxp258*	SBI-09	A	**A**	A	A	A	A	B
20	*Xtxp230*	SBI-09	A	**A**	A	A	A	A	B
21	*Xtxp67*	SBI-09	A	**A**	A	A	A	A	B
22	*Xtxp289*	SBI-09	B	**A**	A	A	A	A	B
23	*Xtxp20*	SBI-10	A	**A**	A	A	A	A	B
24	*Xisp263*	SBI-10	A	**A**	A	A	A	A	B
25	*Xcup07*	SBI-10	A	**A**	A	A	A	A	B
26	*Xisp198*	SBI-08	A	**A**	A	A	A	A	B
27	*Xtxp18*	SBI-08	A	**A**	A	A	A	A	B
28	*Xtxp105*	SBI-08	A	**A**	A	A	A	A	B
29	*Xtxp317*	SBI-06	A	H	B	H	B	A	B
30	*Xtxp274*	SBI-06	A	H	B	H	B	A	B
31	*Xtxp57*	SBI-06	H	**A**	A	A	H	A	B
32	*Xisp258*	SBI-05	A	**A**	A	A	A	A	B
33	*Xtxp303*	SBI-05	B	**A**	A	A	A	A	B
34	*Xtxp70*	SBI-05	A	**A**	A	A	A	A	B
		A	26	**29**	29	27	26	34	0
		B	2	**0**	2	1	3	0	34
		H	6	**5**	3	6	5	0	0
		Other	0	**0**	0	0	0	0	0
		Sum	34	**34**	34	34	34	34	34
				Best	3rd Best	2nd Best			

Table 33. Results from background screening on carrier linkage group J (SBI-05) among foreground-selected "BC_3F_1 progenies" of BTx623 and IS 18551

S.No	BC_3F_1 Cross	QTL Target	*Xtxp65*	*Xtxp94*	*Xtxp15*	*Xisp258*	*Xtxp262*	Total A's	Total H's	Total B's	Result
1	**J1495 x J1541**	**J**	**H**	**H**	**H**	**H**	**A**	**1**	**4**	**0**	**Joint best option**
2	**J1502 x J1537**	**J**	**H**	**H**	**H**	**H**	**A**	**1**	**4**	**0**	**Joint best option**
3	J1505 x J1545	J	H	H	H	B	A	1	3	1	Self
4	**J1506 x J1525**	**J**	**H**	**H**	**H**	**H**	**A**	**1**	**4**	**0**	**Joint best option**
5	**J1630 x J1964**	**J**	**H**	**H**	**H**	**H**	**A**	**1**	**4**	**0**	**Joint best option**
6	**J1632 x J1965**	**J**	**H**	**H**	**H**	**H**	**A**	**1**	**4**	**0**	**Joint best option**
7	**J1637 x J1969**	**J**	**H**	**H**	**H**	**H**	**A**	**1**	**4**	**0**	**Joint best option**
8	BTx623	J	A	A	A	A	A	5	0	0	Recurrent parent
9	IS 18551	J	B	B	B	B	B	0	0	3	Donor parent

S.No	BC_3F_1 Cross	QTL Target	*Xtxp65*	*Xtxp94*	*Xtxp15*	*Xisp258*	*Xtxp262*	Total A's	Total H's	Total B's	Result
1	**J1487 x J1536**	**J1**	**H**	**H**	**A**	**H**	**A**	**2**	**3**	**0**	**Joint best option**
2	**J1616 x J1947**	**J1**	**H**	**H**	**A**	**H**	**A**	**2**	**3**	**0**	**Joint best option**
3	**J1618 x J1982**	**J1**	**H**	**H**	**A**	**H**	**A**	**2**	**3**	**0**	**Joint best option**
4	**J1620 x J1925**	**J1**	**H**	**H**	**A**	**H**	**A**	**2**	**3**	**0**	**Joint best option**
5	**J1633 x J1928**	**J1**	**H**	**H**	**A**	**H**	**A**	**2**	**3**	**0**	**Joint best option**
6	**J1636 x J1933**	**J1**	**H**	**H**	**A**	**H**	**A**	**2**	**3**	**0**	**Joint best option**
7	**J1638 x J1956**	**J1**	**H**	**H**	**A**	**H**	**A**	**2**	**3**	**0**	**Joint best option**
8	**J1492 x J1541**	**J2**	**A**	**H**	**H**	**H**	**A**	**2**	**3**	**0**	**Joint best option**
9	**J1500 x J1537**	**J2**	**A**	**H**	**H**	**H**	**A**	**2**	**3**	**0**	**Joint best option**
10	**J1508 x J1549**	**J2**	**A**	**H**	**H**	**H**	**A**	**2**	**3**	**0**	**Joint best option**
11	BTx623	J	A	A	A	A	A	5	0	0	Recurrent parent
12	IS 18551	J	B	B	B	B	B	0	0	3	Donor parent

J1492 is actually J QTL.

Table 34. Results from background screening of foreground-selected BC_3F_1 introgressions of target QTL J (Linkage group SBI-05) derived from BTx623 and IS 18551

			BC3F1 introgression heterozygote crosses available for possible advance							
S.No	Marker	Linkage group	J1495 x J1541	J1502 x J1537	J1506 x J1525	**J1630 x J1964**	J1632 x J1965	J1637 x J1969	BTx623	IS 18551
1	*Xtxp248*	SBI-01	A	A	A	**A**	A	A	A	B
2	*Xtxp32*	SBI-01	A	A	H	**A**	A	A	A	B
3	*Xtxp208*	SBI-01	A	A	A	**A**	A	A	A	B
4	*Xtxp211*	SBI-02	A	B	A	**A**	A	A	A	B
5	*Xtxp1*	SBI-02	B	H	B	H	H	A	A	B
6	*Xtxp207*	SBI-02	B	A	H	H	H	H	A	B
7	*Xtxp34*	SBI-03	A	B	A	**A**	A	A	A	B
8	*Xtxp33*	SBI-03	A	O	A	H	H	H	A	B
9	*Xtxp228*	SBI-03	A	H	A	**A**	A	A	A	B
10	*Xtxp343*	SBI-04	A	A	A	**A**	A	H	A	B
11	*Xcup05*	SBI-04	A	A	A	**A**	A	A	A	B
12	*Xtxp27*	SBI-04	H	A	B	**A**	A	A	A	B
13	*Xtxp312*	SBI-07	A	A	A	**A**	A	A	A	B
14	*Xtxp295*	SBI-07	A	A	H	**A**	A	A	A	B
15	*Xisp344*	SBI-07	A	A	H	**A**	A	A	A	B
16	*Xtxp10*	SBI-09	H	A	A	H	A	H	A	B
17	*Xtxp258*	SBI-09	A	A	A	**A**	O	O	A	B
18	*Xtxp289*	SBI-09	A	A	A	**A**	A	A	A	B
19	*Xtxp20*	SBI-10	A	B	A	**A**	H	A	A	B
20	*Xisp263*	SBI-10	A	A	A	**A**	H	H	A	B
21	*Xcup07*	SBI-10	A	A	A	**A**	A	A	A	B
22	*Xisp198*	SBI-08	A	A	A	**A**	A	A	A	B
23	*Xtxp18*	SBI-08	A	A	A	**A**	A	A	A	B
24	*Xtxp105*	SBI-08	A	A	A	**A**	A	A	A	B
25	*Xtxp6*	SBI-06	A	A	A	**A**	A	A	A	B
26	*Xtxp274*	SBI-06	H	A	B	**A**	A	A	A	B
27	*Xtxp57*	SBI-06	A	A	A	**A**	A	A	A	B
28	*Xisp258*	SBI-05	H	H	H	H	H	H	A	B
29	*Xtxp65*	SBI-05	H	H	H	H	H	H	A	B
30	*Xtxp94*	SBI-05	H	H	H	H	H	H	A	B
31	*Xtxp303*	SBI-05	H	H	H	H	H	H	A	B
32	*Xtxp15*	SBI-05	H	H	H	H	H	H	A	B
33	*Xtxp283b*	SBI-05	H	H	H	H	H	H	A	B
34	*Xtxp23*	SBI-05	off	H	H	H	H	H	A	B
35	*Xtxp70*	SBI-05	A	B	A	**A**	A	A	A	B
36	*Xtxp262*	SBI-05	A	A	A	**A**	A	A	A	B
		A	24	22	22	**25**	23	23	36	0
		B	2	4	3	**0**	0	0	0	36
		H	9	9	11	**11**	12	12	0	0
		Other	1	1	0	**0**	1	1	0	0
		Sum	36	36	36	**36**	36	36	36	36
						Best nd besrd best				

Table 35. Results from background screening of foreground-selected BC_3F_1 introgressions of target QTLs J1 and J2 (Linkage group SBI-05) derived from BTx623 and IS 18551

		BC3F1 introgression heterozygote crosses available for possible advance											
S. No	Marker	Linkage group	J1487 x J1536	J1616 x J1947	J1618 x J1982	J1620 x J1925	**J1633 x J928**	**J1636 x J1933**	**J1638 x J1956**	**J1492 x J1541(actuall y target J)**	J1500 x J1537	J1508 x J1549	BTx623	IS 18551
1	*Xtxp248*	SBI-01	A	A	A	A	**A**	**A**	**A**	**A**	A	A	A	B
2	*Xtxp32*	SBI-01	A	A	A	B	**A**	**A**	**A**	**A**	A	H	A	B
3	*Xtxp208*	SBI-01	A	A	A	B	**A**	**A**	**A**	**A**	A	A	A	B
4	*Xtxp211*	SBI-02	A	A	A	A	**A**	**A**	**A**	**A**	A	A	A	B
5	*Xtxp1*	SBI-02	A	A	A	A	**A**	**A**	H	**A**	H	B	A	B
6	*Xtxp207*	SBI-02	A	A	A	A	**A**	**A**	H	**A**	A	B	A	B
7	*Xtxp228*	SBI-03	B	A	A	B	**A**	**A**	**A**	**A**	A	A	A	B
8	*Xtxp34*	SBI-03	A	A	A	A	**A**	**A**	**A**	**A**	B	A	A	B
9	*Xtxp285*	SBI-03	A	A	A	A	**A**	**A**	**A**	**A**	H	A	A	B
10	*Xtxp343*	SBI-03	A	A	A	A	**A**	**A**	**A**	**A**	A	A	A	B
11	*Xcup05*	SBI-04	A	H	B	A	**A**	**A**	**A**	**A**	A	A	A	B
12	*Xtxp27*	SBI-04	A	H	H	A	**A**	**A**	**A**	**A**	A	A	A	B
13	*Xtxp312*	SBI-07	A	H	A	A	**A**	**A**	**A**	**A**	A	A	A	B
14	*Xtxp295*	SBI-07	A	A	A	A	**A**	**A**	**A**	**A**	A	B	A	B
15	*Xisp344*	SBI-07	A	A	A	A	**A**	**A**	**A**	**A**	A	B	A	B
16	*Xtxp10*	SBI-09	A	A	A	A	H	H	**A**	**A**	A	A	A	B
17	*Xtxp230*	SBI-09	H	A	A	A	**A**	**A**	**A**	**A**	A	A	A	B
18	*Xtxp289*	SBI-09	A	A	A	A	**A**	**A**	**A**	**A**	A	A	A	B
19	*Xtxp20*	SBI-10	A	A	A	A	H	H	**A**	**A**	H	A	A	B
20	*Xisp263*	SBI-10	A	A	A	A	H	**A**	**A**	**A**	A	A	A	B
21	*Xcup07*	SBI-10	A	H	A	A	**A**	**A**	**A**	H	A	H	A	B
22	*Xisp198*	SBI-08	A	A	A	H	**A**	**A**	**A**	**A**	A	A	A	B
23	*Xtxp18*	SBI-08	A	A	A	H	**A**	**A**	**A**	**A**	A	A	A	B
24	*Xtxp105*	SBI-08	A	A	A	A	**A**	**A**	**A**	**A**	A	A	A	B
25	*Xtxp6*	SBI-06	B	A	A	B	**A**	**A**	**A**	**A**	A	A	A	B
26	*Xtxp317*	SBI-06	H	A	A	H	**A**	**A**	**A**	**A**	A	A	A	B
27	*Xtxp274*	SBI-06	H	A	A	H	**A**	**A**	**A**	**A**	A	A	A	B
28	*Xtxp57*	SBI-06	H	A	A	A	**A**	**A**	**A**	**A**	A	A	A	B
29	*Xisp258*	SBI-05	H	H	H	H	H	H	H	H	H	H	A	B
30	*Xtxp65*	SBI-05	H	H	H	H	H	H	H	H	A	A	A	B
31	*Xtxp94*	SBI-05	H	H	H	H	H	H	H	H	H	H	A	B
32	*Xtxp303*	SBI-05	H	H	H	H	**A**	**A**	**A**	H	B	H	A	B
33	*Xtxp15*	SBI-05	A	A	A	A	**A**	**A**	**A**	H	H	H	A	B
34	*Xtxp283b*	SBI-05	A	A	A	A	**A**	**A**	**A**	H	A	H	A	B
35	*Xtxp70*	SBI-05	A	A	A	A	**A**	**A**	**A**	**A**	B	A	A	B
36	*Xtxp262*	SBI-05	A	A	A	A	**A**	**A**	**A**	**A**	A	A	A	B
		A	26	28	30	24	**30**	**31**	**31**	**29**	27	25	36	0
		B	2	0	1	4	**0**	**0**	**0**	**0**	3	4	0	36
		H	8	8	5	8	**6**	**5**	**5**	**7**	6	7	0	0
		Other	0	0	0	0	**0**	**0**	**0**	**0**	0	0	0	0
		Sum	36	36	36	36	**36**	**36**	**36**	**36**	36	36	36	36
							2nd-bestJ1	**BestJ1**	**BestJ1**	**BestJ2**				

Table 36. Results of background screening on carrier linkage groups A, E and J (SBI-01, SBI-07 and SBI-05) of foreground-selected "BC_3F_1 progenies" of 296B and IS 18551

S.No.	BC3F1 Cross	QTL Target	*Xtxp316*	*Xtxp248*	*Xtxp319*	*Xtxp32*	*Xtxp88*	*Xtxp149*	*Xtxp302*	*Xgap206*	*Xgap57*	Total A's	Total H's	Total B's	Result
1	**J1690 x J2072**	**A**	**A**	**A**	**A**	**H**	**A**	**A**	**A**	**A**	**H?**	**7**	**1**	**0**	**Joint second best option**
2	**J1692 x J2079**	**A**	**A**	**A**	**A**	**H**	**A**	**A**	**A**	**A**	**H**	**7**	**2**	**0**	**Joint second best option**
3	**J1696 x J2031**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**H**	**8**	**1**	**0**	**Best option for advance**
4	**J1698 x J2019**	**A**	**A**	**A**	**A**	**H**	**A**	**A**	**A**	**A**	**H**	**7**	**2**	**0**	**Joint second best option**
5	J1700 x J2073	A	A	A	A	H	A	A	A	A	B	7	1	1	Self
6	**J1702 x J2031**	**A**	**A**	**A**	**A**	**H**	**A**	**A**	**A**	**A**	**H**	**7**	**2**	**0**	**Joint second best option**
7	J1723 x J2028	A	A	H	A	H	A	H	H?	A	H	4	4	0	
8	J1724 x J2067	A	A	A	A	H	H	H	A	A	H	5	4	0	
9	J1727 x J2034	A	A	H	A	H	H	H	A	A	H	4	5	0	
10	J1728 x J2033	A	A	H	A	H	A	H	A	A	H	5	4	0	
11	J1729 x J2025	A	A	A	A	H	H	H	A	A	H	5	4	0	
12	296B	A	A	A	A	A	A	A	A	A	A	9	0	0	Recurrent parent
13	IS 18551	A	B	B	B	B	B	B	B	B	B	0	0	9	Donor parent

S.No.	BC3F1Cross	QTL Target	*Xisp348*	*Xtxp312*	*Xtxp36*	*Xisp310*						Total A's	Total H's	Total B's	Result
1	**J1695 x J2062**	**E**	**H**	**H**	**H**	**A**						**1**	**3**	**0**	**Second best option**
2	**J1707 x J2057**	**E**	**H**	**H**	**A**	**A**						**2**	**2**	**0**	**Best option**
3	296B	E	A	A	A	A						4	0	0	Recurrent parent
4	IS 18551	E	B	B	B	B						0	0	4	Donor parent

			Xtxp65	*Xtxp94*	*Xtxp15*	*Xisp215*	*Xtxp23*					Total A's	Total H's	Total B's	
1	**J1712 x J2027**	**J**	**H**	**H**	**H**	**H**	**A**					**1**	**4**	**0**	**Best option**
2	296B	J	A	A	A	A	A					5	0	0	Recurrent parent
3	IS 18551	J	B	B	B	B	B					0	0	3	Donor parent

Table 37. Results from background screening of foreground-selected "BC_3F1" introgressions of target QTL A (Linkage group SBI-01) derived from 296B and IS 18551

BC_3F_1 introgression heterozygote crosses available for possible advance									
S.No	Marker	Linkage Group	**J1690 x J2072**	**J1692 x J2079**	J1696 x J2031	J1698 x J2019	J1702 x J2031	296B	IS 18551
1	*Xtxp316*	SBI-01	**A**	**A**	A	A	A	A	B
2	*Xtxp248*	SBI-01	**A**	**A**	A	A	A	A	B
3	*Xtxp319*	SBI-01	**A**	**A**	A	A	A	A	B
4	*Xtxp75*	SBI-01	H	H	H	H	H	A	B
5	*Xgap57*	SBI-01	H?	H	H	H	H	A	B
6	*Xtxp37*	SBI-01	H	H	H	H	H	A	B
7	*Xtxp32*	SBI-01	H	H	A	H	H	A	B
8	*Xtxp88*	SBI-01	**A**	**A**	A	A	A	A	B
9	*Xtxp149*	SBI-01	**A**	**A**	A	A	A	A	B
10	*Xtxp302*	SBI-01	**A**	**A**	A	A	A	A	B
11	*Xgap206*	SBI-01	**A**	**A**	A	A	A	A	B
12	*Xtxp207*	SBI-02	**A**	**A**	A	A	A	A	B
13	*Xtxp25*	SBI-02	**A**	**A**	A	A	A	A	B
14	*Xtxp4*	SBI-02	**A**	**A**	A	A	A	A	B
15	*Xtxp69*	SBI-03	H	**A**	H	H	H	A	B
16	*Xtxp31*	SBI-03	**A**	**A**	A	A	B	A	B
17	*Xtxp228*	SBI-03	**A**	**A**	A	A	A	A	B
18	*Xcup48*	SBI-04	**A**	**A**	A	A	A	A	B
19	*Xisp335*	SBI-04	H	H	B	A	H	A	B
20	*Xtxp27*	SBI-04	**A**	**A**	A	A	A	A	B
21	*Xisp348*	SBI-07	**A**	H	H	H	A	A	B
22	*Xisp310*	SBI-07	**A**	A	B	H	A	A	B
23	*Xtxp312*	SBI-07	H	H	A	B	A	A	B
24	*Xtxp67*	SBI-09	**A**	**A**	A	A	A	A	B
25	*Xtxp10*	SBI-09	**A**	**A**	A	A	A	A	B
26	*Xtxp230*	SBI-09	**A**	**A**	A	A	A	A	B
27	*Xtxp20*	SBI-10	H	H	B	A	A	A	B
28	*Xisp359*	SBI-10	H	H	B	A	A	A	B
29	*Xcup67*	SBI-10	**A**	-	B	A	A	A	B
30	*Xtxp47*	SBI-08	**A**	**A**	A	A	A	A	B
31	*Xtxp354*	SBI-08	H	H	H	A	A	A	B
32	*Xtxp250*	SBI-08	**A**	**A**	A	A	A	A	B
33	*Xisp264*	SBI-06	**A**	**A**	B	H	H	A	B
34	*Xtxp317*	SBI-06	**A**	**A**	B	H	B	A	B
35	*Xisp347*	SBI-06	-	H	H	H	H	A	B
36	*Xisp215*	SBI-05	H	H	B	H?	H	A	B
37	*Xtxp23*	SBI-05	H	**A**	H	B	H	A	B
38	*Xtxp283b*	SBI-05	H	H	B	A	A	A	B
		A	**21**	**21**	18	22	23	35	0
		B	**0**	**0**	9	2	2	0	35
		H	**12**	**13**	8	10	10	0	0
		Other	5	4	3	4	3	3	3
		Sum	**38**	**38**	38	38	38	38	38
			2nd-best A	**Best A**		3rd-best A			

Table 38. Foreground marker data from "BC_4F_1 progeny" of shoot fly susceptible and resistant parents, BTx623 and IS 18551 (Single-QTL Targets)

Target A																										
Marker/Sample	J2251	**J2252**	J2253	J2254	J2255	**J2256**	**J2257**	J2258	J2259	**J2260**	J2261	J2262	J2263	**J2264**	J2265	**J2266**	**J2267**	J2268	**J2269**	**J2270**	**J2271**	J2272	**J2273**	**J2274**	BTx623	IS18551
Xtxp37	-	**H**	A	A	A	**H**	**H**	A	H	**H**	A	A	H	**H**	A	**H**	**H**	A	**H**	**H**	**H**	H	**H**	**H**	A	B
Xtxp75	H	**H**	A	A	H	**H**	**H**	A	H	**H**	A	A	A	**H**	A	**H**	**H**	A	**H**	**H**	**H**	A	**H**	**H**	A	B

Target E																										
Marker/Sample	J2275	J2276	J2277	**J2278**	J2279	J2280	J2281	J2282	**J2283**	J2284	J2285	J2286	J2287	**J2288**	J2289	J2290	**J2291**	J2292	J2293	J2294	J2295	J2296	J2297	J2298	J2299	**J2300**
Xtxp159	A	A	A	**H**	H	A	A	A	**H**	A	A	A	A	**H**	H	A	**H**	A	A	H	A	H	H	A	A	**H**
Xtxp40	A	A	A	**H**	A	A	A	A	**H**	A	A	A	A	**H**	H	A	**H**	H	A	-	A	A	A	A	A	**H**

Marker/Sample	J2301	J2302	J2303	J2304	BTx623	IS18551
Xtxp159	A	A	A	A	A	B
Xtxp40	H	A	A	A	A	B

Target G																										
Marker/Sample	J2305	J2306	J2307	J2308	J2309	J2310	J2311	J2312	J2313	J2314	J2315	J2316	J2317	J2318	J2319	J2320	J2321	J2322	J2323	J2324	J2325	J2326	J2327	J2328	J2329	J2330
Xtxp141	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
Xgap1	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

Marker/Sample	J2331	J2332	J2333	J2334	BTx623	IS 18551
Xtxp141	A	A	A	A	A	B
Xgap1	A	A	A	A	A	B

Target J																										
Marker/Sample	J2335	J2336	**J2337**	J2338	J2339	J2340	J2341	J2342	J2343	J2344	J2345	J2346	J2347	J2348	**J2349**	J2350	J2351	**J2352**	**J2353**	J2354	J2355	J2356	J2357	J2358	J2359	J2360
Xtxp65	H	A	**H**	A	B	H	H	H	H	A	H	H	A	A	**H**	A	A	**H**	**H**	A	H	H	H	A	H	H
Xtxp94	H	A	**H**	H	B	H	H	H	A	A	H	H	A	A	**H**	A	A	**H**	**H**	A	H	B	H	A	H	H
Xtxp15	B	H	**H**	B	B	A	A	A	A	A	A	A	A	H	**H**	A	B	**H**	**H**	B	A	H	A	H	B	A

Contd....

Marker/Sample	J2361	J2362	J2363	J2364	J2365	J2366	J2367	J2368	J2369	J2370	J2371	J2372	J2373	J2374	J2375	J2376	J2377	J2378	J2379	J2380	J2381	J2382	J2383	J2384	J2385	J2386
Xtxp65	H	H	H	H	A	H	A	H	B	H	H	H	H	H	-	A	A	A	A	B	B	B	H	H	H	H
Xtxp94	H	H	H	H	A	H	A	B	B	H	A	H	H	H	-	A	A	A	A	B	B	B	H	H	H	H
Xtxp15	A	H	A	H	A	H	H	B	B	A	A	H	H	H	B	H	A	H	H	H	B	H	B	H	B	B

Marker/Sample	J2387	J2388	J2389	J2390	J2391	J2392	J2393	J2394	J2395	J2396	J2397	J2398	J2399	J2400	J2401	J2402	J2403	J2404	J2405	J2406	J2407	J2408	J2409	J2410	J2411	J2412
Xtxp65	H	H	H	A	A	H	H	H	A	H	A	H	A	A	H	A	H	-	A	A	H	H	H	A	B	A
Xtxp94	B	H	H	A	A	A	H	H	A	H	A	H	A	A	H	A	A	-	A	A	H	H	A	A	B	A
Xtxp15	H	H	A	H	H	A	H	A	A	A	H	H	H	A	H	H	H	B	A	H	H	H	A	A	H	H

Marker/Sample	J2413	J2414	J2415	J2416	J2417	J2418	J2419	J2420	J2421	J2422	J2423	J2424	J2425	J2426	J2427	J2428	BTx623	IS18551
Xtxp65	H	A	H	A	H	A	A	H	H	H	H	H	H	A	H	A	A	B
Xtxp94	H	A	H	A	H	A	A	H	H	A	H	H	H	A	H	A	A	B
Xtxp15	B	A	B	A	H	H	A	H	H	A	H	H	H	A	H	A	A	B

Target J1

Marker/Sample	J2429	J2430	J2431	J2432	J2433	J2434	J2435	J2436	J2437	J2438	J2439	J2440	J2441	J2442	J2443	J2444	J2445	J2446	J2447	J2448	J2449	J2450	J2451	J2452	J2453	J2454
Xtxp65	A	H	H	H	A	A	H	A	A	H	H	A	A	H	H	H	A	H	H	H	B	A	A	H	A	A
Xtxp94	A	H	A	H	A	H	A	A	A	H	H	A	A	H	A	H	H	A	H	H	B	A	H	H	A	A
Xtxp15	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

Target J2(actually J
QTL plants)

Marker/Sample	J2459	J2460	J2461	J2462	J2463	J2464	J2465	J2466	J2467	J2468	J2469	J2470	J2471	J2472	J2473	J2474	J2475	J2476	J2477	J2478	J2479	J2480	J2481	J2482	J2483	J2484
Xtxp65	A	H	H	A	A	A	H	A	H	A	A	H	A	H	A	B	A	A	H	A	H	A	A	H	H	A
Xtxp94	A	H	H	A	A	H	H	A	H	H	A	H	A	H	A	B	A	A	H	A	H	A	A	H	H	A
Xtxp15	A	H	H	H	A	H	H	A	H	H	H	A	A	A	H	H	A	A	H	H	A	A	A	H	H	A

Target J2(actually J)

Target J1

Marker/Sample	J2455	J2456	J2457	J2458	BTx623	IS18551	J2485	J2486	J2487	J2488	BTx623	IS18551
Xtxp65	A	A	A	H	A	B	A	H	H	A	A	B
Xtxp94	A	A	H	A	A	B	A	H	A	H	A	B
Xtxp15	A	A	A	A	A	B	H	H	A	H	A	B

Table 39. Foreground marker genotyping of "BC_4F_1 progeny" from shoot fly susceptible and resistant parents 296B and IS 18551

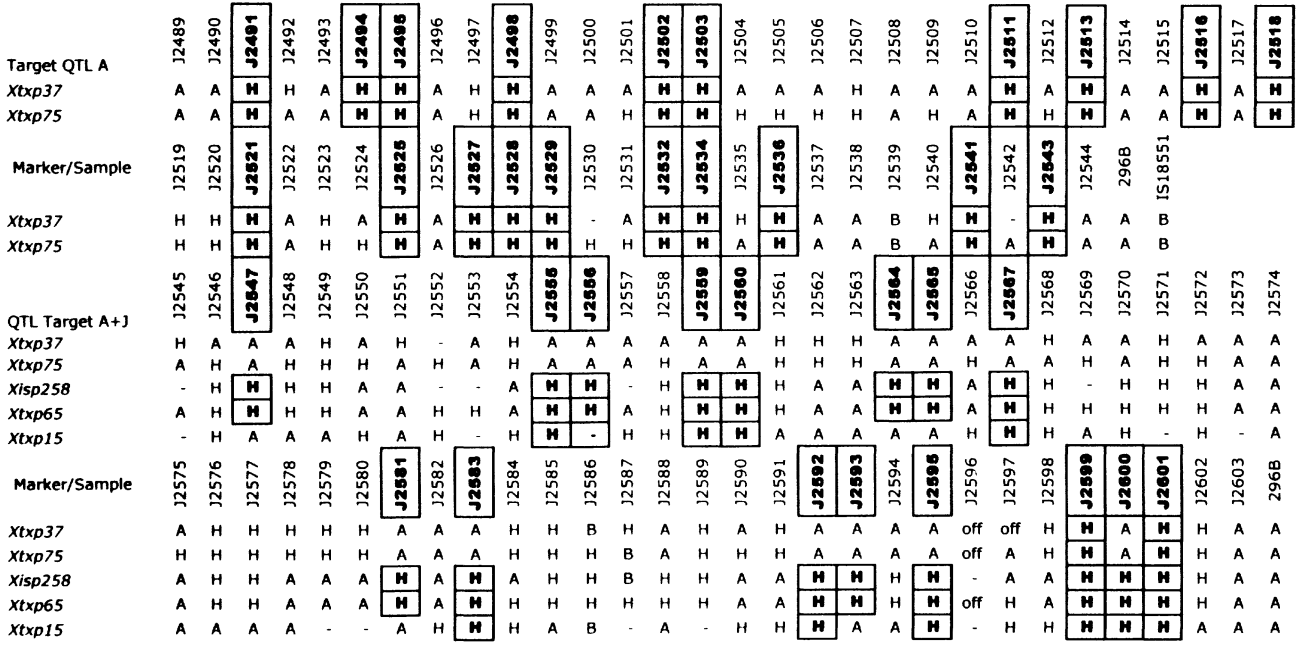


Table 40. Results from background screening of foreground-selected "BC_4F_1" introgressions derived from BTx623 and IS 18551

BC_4F_1 progenies of BTx623 x IS 18551 target QTL A heterozygotes

BC_3F_1 cross	Target QTL	"BC_3F_1" plant		*Xtxp32*	*Xtxp357*	Remarks	
J1481 x J1540 (actually BC_2F_1 cross)	A	**J2252***	**Set 1, good**	**A**		**OK**	**sown for advance, OK**
		J2256		A		OK	
		J2257		A		OK	
		J2260		A		OK	
		J2264		A		OK	
		J2266		H		rejected	
		J2267		H		rejected	
		J2269		A		OK	
		J2270		A		OK	
		"BC_4F_1" plant					
J1647 x J1930	A	J2271*	Set 1, Not a good choice for advance	H	H	rejected	sown for advance, but now should be rejected
		J2273**	**Better option sown in second set**	**A**	**A**	**selected for advance**	
		J2274		H	H	rejected	

BC_4F_1 progenies of BTx623 x IS 18551 target QTL E heterozygotes

BC_3F_1 cross	Target QTL	"BC_4F_1" plant		*Xtxp317*	*Xtxp274*	*Xtxp211*		
J1422 x J1558	E	**J2278***	**Set 1, good choice**	**A**	**A**		**OK**	
		J2283**	**sown in second set**	**A**	**A**		**OK**	
		J2288		A	A		OK	
		J2291		A	A		OK	
J1440 x J1537	E	J2300*	Set 1, Not a good choice for advance	H	H	O	rejected	sown for advance, but should now be rejected

Contd.....

BC_4F_1 progenies of BTx623 x IS 18551 target QTL J heterozygotes

BC_3F_1 cross	Target QTL	"BC_4F_1" plant		*Xtxp1*	*Xtxp207*	*Xisp1032.*	*Xtxp343*	*Xtxp10*	*Xtxp258*	*Xtxp20*	*Xisp1026.*	*Xcup07*	
J1630 x J1964	J	J2337*	Set 1, OK but not best	H	H	O		A					
		J2349		H	B	O		B					rejected self
		J2352**	**Better option sown in second set**	**A**	**A**	**H**		**A**					**Best, 1**
		J2353		H	H	O		A					
		J2362		A	H	O		B					rejected self
		J2364		A	B	O		B					rejected self
		J2366		H	B	O		A					rejected self
		J2372		B	H	O		A					rejected self
		J2373		A	A	O		A					
		J2374		H	H	H		A					OK, 3
		J2384		A	A	O		A					
J1632 x J1965	J	J2388		H	H	H			A	A	A		
		J2398		H	H	H			A	A	A		
		J2401		A	A	H			H	A	A		OK, 2
		J2407**	**Better option sown in second set**	**A**	**H**	**H**			**A**	**A**	**A**		**Best, 2**
		J2408*	**Set 1, poorest of 3 for LG J**	**H**	**H**	**A**			**H**	**H**	**H**		
J1637 x J1969	J	J2417	Better option		A	H	A	A	A		H		OK, 2
		J2420			A	B	B	B	H		H		rejected self
		J2421*	Set 1, OK but not best		H	O	A	A	H		A		
		J2423			H	O	H	A	A		H		
		J2424			H	O	H	A	A		A		
		J2425			H	H	A	A	A		H		OK, 3
		J2427			H	H	H	A	H		A		OK, 4
J1492 x J1541	J	J2460-J				A			O			B	rejected self
		J2461-J				O			A			A	
		J2465-J**	**Good option sown in second set**			**A**			**A**			**A**	**Best**
		J2467-J**	**Good option sown in second set**			**A**			**A**			**A**	**Best**
		J2470-J1				O			O			A	
		J2477-J				O			A			A	
		J2479-J1				A			O			A	
		J2482-J				A			O			A	
		J2483-J				O			A			A	
		J2486-J				A			O			A	

Contd.....

BC_4F_1 progenies of BTx623 x IS 18551 target QTL J1 heterozygotes

BC_3F_1 Cross	Target QTL	"BC_4F_1" plant			*Xtxp1*	*Xtxp207*	*Xisp10323*	*Xtxp343*	*Xtxp10*	*Xtxp258*	*Xtxp20*	*Xisp10263*	*Xcup07*		
J1638 x J19	J1	J2430*	Set 1, OK but not best		A	A	O			A					
		J2432			A	H	O			A					
		J2438*	Set 1, OK but not best		H	A	A			O					
		J2439**	**Better option**	**sown in second set**	**A**	**A**	**A**			**A**				**Selected for advance**	
		J2442			H	H	O			O					
		J2444			A	A	O			O					
		J2447			A	B	A			O				rejected self	
		J2448	Better option		H	H	A			A				OK, 2	2nd best

* = Genotypes advanced in set 1 ** = Genotypes advanced in set 2

Table 41. Results from background screening of foreground-selected target QTL A and J "BC_4F_1" introgressions derived From 296B and IS 18551

BC_3F_1 cross	Target QTL	"BC_2F_1" plant		*Xtxp32*	*Xgap57*	*Xtxp69*	*Xisp10335*	*Xisp10348*	*Xisp10310*	*Xtxp312*	*Xtxp20*	*Xisp10359*	*Xcup67*	*Xtxp354*	*Xisp10264*	*Xtxp317*	*Xisp215*	*Xtxp23*	*Xtxp283b*	Remarks	
BC_4F_1 progenies of 296B x IS 18551 target QTL A heterozygotes																					
J1692 x J2079	A	J2491		H	H		A	A	A	A	H	H	H	A			A		H	OK, 6H	
(actually BC_1F_1 cross)		J2494		A	H		A	A	A	A	H	H	H	H			A		H	OK, 6H	
		J2495		H	H		H	H	A	A	H	A	A	A			H		A	OK, 6H	
		J2498		H	H		A	A	A	A	H	H	H	B			A		H	rejected self	
		J2502		H	H		A	H	A	H	H	H	H	A			H		H	OK, 9H	
		J2503		H	H		A	A	O?	A	A	H	H	A			A		H		
		J2511		A	H		A	A	O?	A	H	H	A	A			A		H		
		"BC_2F_1" plant																			
J1690 x J2072	A	**J2513****	second set	**H**	**H**	**H**	**A**			**H**	**A**	**A**		**A**			**A**	**A**	**A**	OK, 4H	Best
(actually BC_1F_1 cross)		J2516		A	H	H	H			H	A	A		H			A	A	A	OK, 4H	
		J2518		A	H	H	H			A	H	H		A			A	H	A	OK, 6H	
		J2521		H	H	-	H			H	A	A		A			A	H	A	OK, 5H	
		J2525		H	H	-	A			A	A	A		H			H	A	A	OK, 4H	
		J2527		H	H	-	A			H	A	A		A			A	H	A	OK, 4H	
		J2528		H	H	A	A			H	A	A		H			A	A	A	OK, 4H	
		J2529**	second set	**H**	-	-	**A**			**H**	**A**	**A**		**A**			**A**	**A**	**A**	OK, 2H	Best
		"BC_1F_1" plant																			
J1698 x J2019	A	J2532		A	H	H		A	A	H					H	H	H	H		OK, 7H	
(actually F_4 cross)		J2534		H	H	A		H	A	H					A	A	A	H		OK, 5H	Best
		J2536		H	H	H		A	A	H					A	-	H	H		OK, 6H	
		J2541		A	H	A		H	H	H					A	A	H	H		OK, 6H	
		J2543		H	H	H		H	H	H					H	H	H	H		Poorest	

Contd.....

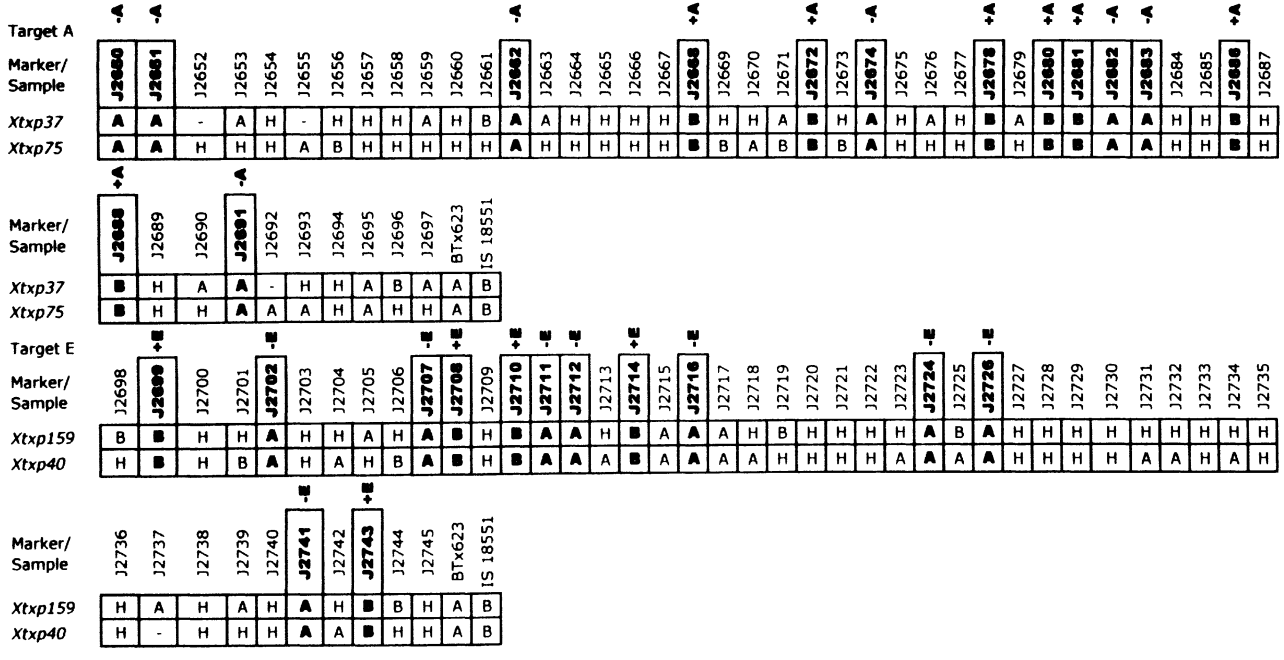
BC_4F_1 progenies of 296B x IS 18551 target QTLs A+J heterozygotes

BC_4F_1		BC_2F_1 plant		*Xtxp32*	*Xgap57*	*Xtxp69*	*Xisp10335*	*Xisp10348*	*Xisp10310*	*Xtxp312*	*Xtxp20*	*Xisp10359*	*Xcup67*	*Xtxp354*	*Xisp10264*	*Xtxp317*	*Xisp215*	*Xtxp23*	*Xtxp283b*	Remarks	
J1690 x J2072 (actually BC_1F_1 cross)	A+J	J2601**		A	H	H	H			A	A	A		H			H	H	A	OK, 6H	Best
		BC_2F_1 plant																			
J1690 x J2072	A+J1+J2	J2599		H	H	H	H			A	A	A		H			H	H	A	OK, 7H	2nd best
(actually BC_1F_1 cross)		**J2601****	second set	**A**	**H**	**H**	**H**			**A**	**A**	**A**		**H**			**H**	**H**	**A**	OK, 6H	**Best**
		BC_2F_1 plant																			
J1690 x J2072	J1	2547		A	A	H	H			A	H	H		H			H	A	H	OK, 7H	
(actually BC_1F_1 cross)		2556		A	A	H	A			A	-	H		H			H	A	H	OK, 5H	
		2564**	second set	**A**	**A**	**A**	**A**			**H**	**A**	**A**		**A**			**A**	**A**	**A**	OK, 1H	**Best**
		2565		A	A	H	A			A	H	H		H			A	A	-	OK, 4H	
		2581		A	A	H	H			A	A	A		A			H	H	A	OK, 4H	
		2593**	second set	**A**	**A**	**A**	**H**			**H**	-	**A**		**A**			**H**	**A**	**A**	OK, 3H	**2nd best**
		BC_2F_1 plant																			
J1690 x J2072	J1+J2	2555		A	A	H	H			H	H	H		A			H	H	H	OK, 8H	
(actually BC_1F_1 cross)		2559		A	A	A	H			H	A	A		H			H	H	A	OK, 5H	
		2560		H	A	A	A			A	H	H		H			H	H	H	OK, 6H	
		2567**	second set	**A**	**A**	**A**	**A**			**H**	**A**	**A**		**A**			**H**	**H**	**A**	OK, 3H	**2nd best**
		2583		A	A	A	A			H	H	H		A			H	H	H	OK, 6H	
		2592		H	A	H	A			A	H	H		H			H	H	H	OK, 7H	
		2595**	second set	**H**	**A**	**A**	**A**			**A**	**A**	**A**		**A**			**H**	**H**	**A**	OK, 2H	**Best**
		2600		A	A	H	H			A	H	H		H			H	H	H	OK, 8H	

"BC4F1" introgression heterozygote crosses available for possible advance

S. No	Marker	Linkage group	J 2189 x 296B-11 BC_2F_1 cross	J 2193 x 296B-10 BC_2F_1 cross	J 2194 x 296B-16 BC_2F_1 cross	J 2195 x 296B-6 BC_2F_1 cross	**J 2196 x 296B-7** BC_3F_1 cross	J 2197 x 296B-15 BC_3F_1 cross	**J 2198** BC_3F_1 self	J 2199 x 296B-26 BC_3F_1 cross	J 2201 x 296B-9 BC_3F_1 cross	J 2208 x 296B BC_3F_1 cross	J 2209 BC_3F_1 cross	J 2210 x 296B-3 BC_3F_1 cross	J2211 x 296B-2 BC_3F_1 cross	J2212 x 296B-1 BC_3F_1 cross	J2213 x 296B-25 BC_3F_1 cross	296B	IS18551
1	*Xtxp248*	SBI-01=A	A	A	A	A	**A**	A	**A**	A	H	H	B	H	H	H	B	A	B
2	*Xtxp32*	SBI-01=A	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
3	*Xtxp302*	SBI-01=A	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
4	*Xgap206*	SBI-01=A	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
5	*Xtxp207*	SBI-02=B	A	A	A	A	**A**	A	**A**	-	A	A	A	A	A	A	A	A	B
6	*Xtxp25*	SBI-02=B	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
7	*Xtxp4*	SBI-02=B	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
8	*Xtxp69*	SBI-03=C	H	H	A	A	**A**	A	**A**	A	-	A	A	A	A	A	A	A	B
9	*Xtxp31*	SBI-03=C	A	A	A	A	**A**	A	**A**	A	-	A	H	A	A	A	H	A	B
10	*Xtxp228*	SBI-03=C	A	A	A	H	H?	H	H	A	A	H	H	H	H	H	B	A	B
11	*Xcup48*	SBI-04=D	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
12	*Xisp335*	SBI-04=D	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
13	*Xtxp27*	SBI-04=D	A	H	A	H	**A**	H	**A**	H	A	H	B	H	H	H	B	A	B
14	*Xisp348*	SBI-07=E	H	H	-	H	H	H	H	H	H	H	A	H	H	H	H	A	B
15	*Xisp310*	SBI-07=E	O	A	O	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
16	*Xtxp312*	SBI-07=E	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
17	*Xtxp67*	SBI-09=F	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
18	*Xtxp10*	SBI-09=F	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	-	A	B
19	*Xtxp230*	SBI-09=F	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
20	*Xtxp20*	SBI-10=G	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
21	*Xisp359*	SBI-10=G	A	A	H	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
22	*Xcup67*	SBI-10=G	A	A	H	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
23	*Xtxp47*	SBI-08=H	A	A	H	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
24	*Xtxp354*	SBI-08=H	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
25	*Xtxp250*	SBI-08=H	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
26	*Xisp264*	SBI-06=I	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
27	*Xtxp317*	SBI-06=I	A	A	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	-
28	*Xisp215*	SBI-05=J	A	H	A	A	**A**	A	**A**	A	A	A	A	A	A	A	A	A	B
29	*Xtxp23*	SBI-05=J	H	A	H	A	**A**	A	-	-	A	A	A	A	A	A	-	-	B
		A	25	25	23	26	**27**	26	**26**	25	25	25	25	25	25	25	22	28	0
		B	0	0	0	0	**0**	0	**0**	0	0	0	2	0	0	0	3	0	28
		H	3	4	4	3	**1**	3	**2**	2	2	4	2	4	4	4	2	0	0
		Other	1	0	2	0	**1**	0	**1**	2	2	0	0	0	0	0	2	1	1
		Sum	29	29	29	29	**29**	29	**29**	29	29	29	29	29	29	29	29	29	29
							1st best		**second best**										

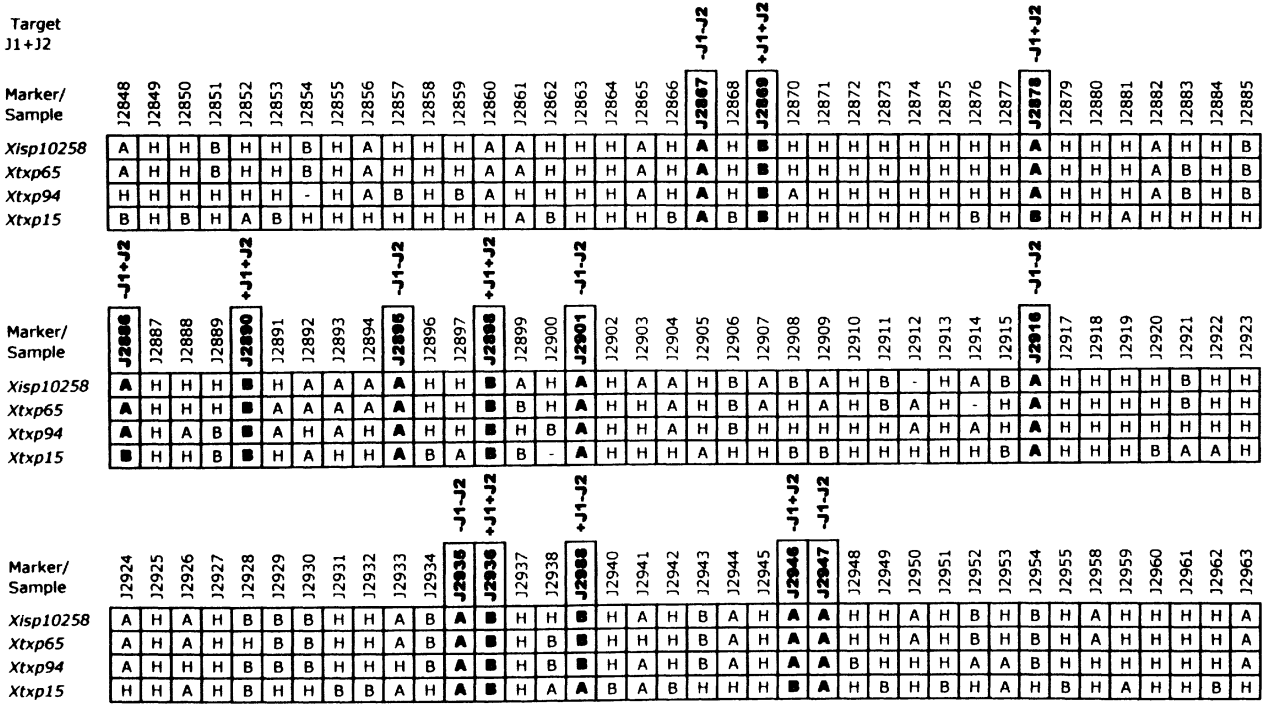
Table 43. Foreground marker genotyping data of "BC_4F_2" populations (first set) derived from BTx623 and IS 18551



Contd.....

Target J
Marker/ Sample
Xisp258
Xtxp65
Xtxp94
Xtxp15
J2794
J2795
J2796
J2797
J2798
J2799
J2800
J2801
J2802
J2803
J2804
J2805
J2806
J2807
J2808
J2809
J2810
J2811
J2812
J2813
J2814
J2815
J2816
J2817
J2818
J2819
J2820
J2821
J2822
J2823
J2824
J2825
J2826
J2827
J2828
J2829
J2830
J2831
J
-4
-4
+4
AAAB
AAAB
Marker/ Sample
Xisp258
Xtxp65
Xtxp94
Xtxp15
J2832
J2833
J2834
J2835
J2836
J2837
J2838
J2839
J2840
J2841
J2842
J2843
J2844
J2845
J2846
J2847
J2848
J2849
J2850
J2851
J2852
J2853
J2854
J2855
J2856
J2857
J2858
J2859
J2860
J2861
J2862
J2863
J2864
J2865
BTx623
IS 18551
+4
+4
+4
+4
Target J1
Marker/ Sample
Xisp258
Xtxp65
Xtxp94
Xtxp15
J2746
J2747
J2748
J2749
J2750
J2751
J2752
J2753
J2754
J2755
J2756
J2757
J2758
J2759
J2760
J2761
J2762
J2763
J2764
J2765
J2766
J2767
J2768
J2769
J2770
J2771
J2772
J2773
J2774
J2775
J2776
J2777
J2778
J2779
J2780
J2781
J2782
J2783
J1
J1
J1
+J1
+J1
+J1
+J1
+J1
+J1
+J1
J1
Marker/ Sample
Xisp258
Xtxp65
Xtxp94
Xtxp15
J2784
J2785
J2786
J2787
J2788
J2789
J2790
J2791
J2792
J2793
BTx623
IS 18551
J1
J2788
+J1
+J1
+J1

Table 44. Foreground marker genotyping data of "BC_4F_2" populations (second set) derived from BTx623 and IS 18551



Contd....

Marker/ Sample	J2964	J2968	J2967	J2966	+J1+J2
Xisp10258	H	H	H	B	B
Xtxp65	H	H	H	B	B
Xtxp94	H	H	A	H	B
Xtxp15	H	H	A	B	A

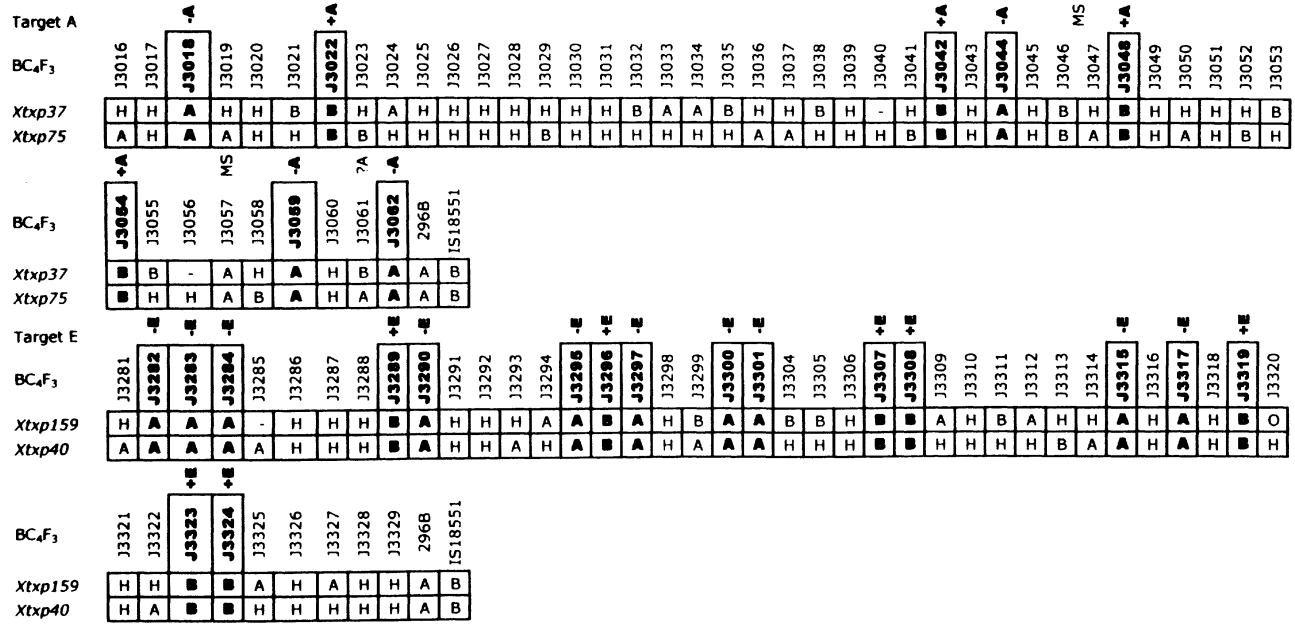
Marker/ Sample	J2969	J2970	J2971	J2972	+J1-J2	J2973	J2974	J2975	J2976	J2977	J2978	+J1-J2	J2979	+J1-J2	J2980	J2981	J2982	+J1-J2	J2983	+J1-J2	J2984	J2985	J2986	J2987	J2989	J2990	-J1-J2	J2991	BTx623	IS 18551
Target A																														
Xtxp15	A	A	A	A	A	A	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A	A	B	A	H	A	A	H	A	B
Xtxp94	A	A	H	A	A	H	B	H	H	H	A	A	A	A	A	B	H	H	H	H	H	H	H	H	H	A	A	H	A	B
Xtxp65	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H	A	B
Xisp10258	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	B	H	H	H	H	H	A	B

Marker/ Sample	J2800	J2801 -A	J2802	J2803	J2804 +A	J2805	J2806	J2807	J2808 A?	J2809	J2810	J2811	J2812	J2813	J2814	J2815	J2816	J2817	J2818	J2819	J2820	J2821	J2822 -A	J2823
Xtxp37	H	A	H	H	B	H	H	H	A	A	B	H	H	H	H	H	H	H	H	H	H	H	A	H
Xtxp75	A	A	H	A	B	H	H	H	B	H	H	A	H	B	A	H	A	H	H	H	H	H	A	H

Unexpected results: No desirable segregants

Marker/ Sample	J2824	J2825	J2826	J2827	J2828	J2829	J2830	J2831	J2832	J2833	J2834	J2835	J2836	J2837	J2838	J2839	J2840	J2841	J2842	J2843	J2844	J2845	J2846	J2847
Xtxp159	A	A	A	A	A	A	A	O	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Xtxp40	A	-	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

Table 45. Foreground marker genotyping data of "BC_4F_2" populations (second set) derived from 296B and IS 18551



Ms=Male sterile

Contd....

Unexpected genotype data for LGA markers and for Xtxp15, so could only select for LG11

Target A+J	BC$_4$F$_3$	J3083 +J1	J3102	J3103 -J1	J3068 -J1	J3068 -J1	J3067	J3068 -J1	J3069	J3070 +J1	J3071 -J1	J3072 +J1	J3073	J3074 -J1	J3078 +J1	J3076	J3077	J3078	J3079	J3080	J3081 -J1	J3082 -J1	J3083	J3084	J3068 -J1	J3086	J3087	J3088 -J1	J3088 +J1	J3090	J3091	J3092	J3083 -J1	J3094	J3095	J3096	J3097 +J1	J3098	J3099	J3100
		+J1		-J1	-J1			-J1		+J1	-J1	+J1		-J1	+J1						-J1				-J1			-J1	+J1				+J1			-J1			+J1		
Xtxp37	A	A		A	A			A		A	A	A		A	A						A				A			A	A				A			A			A	
Xtxp75	A	A		A	A			A		A	A	A		A	A						A				A			A	A				A			A			A	
Xisp10258	B	H		A	A			H		B	A	A		H	A						H				H			H	B				H			H			H	
*Xtxp65**	B	B		A	A			H		A	B	B		H	A						H				H			H	B				H			H			H	
*Xtxp15**	A	A		A	A			A		A	A	A		A	A						A				A			A	A				A			A			A	

Target A+J	BC$_4$F$_3$	J3101 +J1	J3102	J3103 -J1	J3104	J3105	J3106 -J1	J3107	J3108	J3109	J3110	J3111	J3112	J3113	J3114	J3115	J3116	J3117	J3118	J3119 +J1	J3120	J3121 +J1	J3122	J3123	J3124 -J1	J3125	J3126	J3127	J3128	J3129	J3130	J3131 +J1	J3132	J3133	J3134 -J1	J3135	J3136	J3137 +J1	J3138
		+J1		-J1			-J1		+J1											+J1		+J1			-J1							+J1			-J1			+J1	
Xtxp37	A	A		A	A		A		A											A		A			A							A			A			A	
Xtxp75	A	A		A	A		A		A											A		A			A							A			A			A	
Xisp10258	H	H		A	H		H		B											H		B			A							A			A			B	
*Xtxp65**	H	H		A	H		H		B											H		B			A							A			A			B	
*Xtxp15**	A	A		A	A		A		A											A		A			A							A			A			A	

Target A+J	BC$_4$F$_3$	J3139	J3140	J3141	J3142	J3143	J3144	J3148 +J1	J3146	J3147 +J1	J3148	J3149	J3150	J3151	J3152	J3153	J3154 -J1	J3155	J3156	J3157	J3158 +J1	2968	IS18551
				MS				+J1		+J1				MS			-J1				+J1		
Xtxp37	A	A	A		A	A	A	A	A	A	A	A	A		A	A	A	A	A	A	A	A	B
Xtxp75	A	A	A		A	A	A	A	A	A	A	A	A		A	A	A	A	A	A	A	A	B
Xisp10258	H	H	A		H	H	H	B	H	B	H	H	H		B	H	H	A	H	H	B	A	B
*Xtxp65**	H	H	A		H	H	H	B	H	B	H	H	H		B	H	H	A	H	H	B	A	B
*Xtxp15**	A	A	A		A	A	A	A	A	A	A	A	A		A	A	A	A	A	A	A	A	B

Contd.....

Target J1

BC$_4$F$_3$	J3159	J3160	J3161	J3162	J3163	J3164	J3165	J3166	J3167	**J3168**	J3169	J3170	**J3171**	**J3172**	J3173	J3174	**J3175**	J3176	J3177
Target J1								MS	MS	**-J1-J2**			**-J1-J2**	**-J1-J2**		MS	**+J1**	MS	
Xisp10258	H	A	B	H	H	H	H	A	A	**A**	H	H	**A**	**A**	H	A	**B**	B	H
*Xtxp65**	H	A	B	B	A	B	A	B	A	**A**	H	H	**A**	**A**	B	A	**B**	B	A
*Xtxp15**	H	H	H	A	A	A	A	A	A	**A**	A	H	**A**	**A**	A	A	**A**	A	A

BC$_4$F$_3$	J3178	J3179	**J3180**	J3181	J3182	J3183	J3184	J3185	**J3186**	J3187	J3188	J3189	J3190	J3191	J3192	J3193	J3194	J3195	J3196
Target J1		MS	**-J1?J2?**						**-J1?J2?**										
Xisp10258	H	B	**A**	H	B	H	H	H	**A**	H	B	B	H	H	B	H	H	H	H
*Xtxp65**	A	B	**A**	H	B	H	B	H	**A**	B	B	B	H	H	B	H	A	H	H
*Xtxp15**	B	A	**B**	A	H	H	A	H	**B**	H	H	H	H	H	H	H	B	B	A

BC$_4$F$_3$	**J3197**	J3198	J3199	**J3200**	J3201	**J3202**	J3203	J3204	J3205	J3206	296B	IS18551
	-J1-J2			**-J1?J2?**		**+J1**						
Xisp10258	**A**	H	H	**A**	H	**B**	H	B	H	B	A	B
*Xtxp65**	**A**	B	H	**A**	H	**B**	H	B	H	H	A	B
*Xtxp15**	**A**	H	H	**B**	H	**A**	H	H	H	H	A	B

*MS Male sterile

Unexpected genotype data for second progeny (due to an apparent tracking mistake), so can only select from first

Target J

BC$_4$F$_3$	J3207	J3208	J3209	J3210	J3211	J3212	**J3213**	J3214	**J3215**	J3216	J3217	J3218	J3219	J3220	J3221	**J3222**	J3223	J3224	J3225
Target J							**+J1+J2**		**-J1-J2**							**-J1-J2**			
Xisp10258	H	H	A	H	A	A	**B**	B	**A**	A	B	A	H	H	H	**A**	A	H	B
*Xtxp65**	H	B	A	A	H	A	**B**	H	**A**	B	B	A	H	H	B	**A**	A	B	B
*Xtxp15**	B	A	H	H	H	H	**B**	A	**A**	H	H	H	H	A	A	**A**	H	B	H

BC$_4$F$_3$	J3226	J3227	J3228	J3229	J3230	**J3231**	J3232	**J3233**	J3234	**J3235**	J3236	J3237	J3238	**J3239**	J3240	J3241	**J3242**	**J3243**	**J3244**
Target J						**-J1-J2**		**+J1+J2**		**+J1+J2**				**+J1?-J2**			**-J1?J2?**	**+J1+J2**	**-J1-J2**
Xisp10258	H	B	A	H	H	**A**	H	**B**	H	**B**	B	A	B	**B**	H	A	**A**	**B**	**A**
*Xtxp65**	H	B	H	B	B	**A**	H	**B**	H	**B**	B	A	B	**A**	H	A	**A**	**B**	**A**
*Xtxp15**	A	H	B	A	H	**A**	A	**B**	B	**B**	H	H	H	**A**	H	H	**B**	**B**	**A**

BC$_4$F$_3$	J3245	J3246	J3247	J3248	J3249	J3250	J3251	J3252	J3253	J3254	J3255	J3256	J3258	J3259	J3260	J3261	J3262
Xisp10258	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
*Xtxp65**	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
*Xtxp15**	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

BC$_4$F$_3$	J3263	J3264	J3267	J3268	J3269	J3270	J3271	J3272	J3273	J3274	J3275	J3276	J3277	J3278	J3279	296B	IS18551
Xisp10258	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B
*Xtxp65**	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B
*Xtxp15**	A	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	B

Table, 46. Identified four RIL parents derived from BTx623 x IS18551 mapping population each RIL parent having the following QTLs

Target QTL	Marker	(A+E+G+J)	(A+G+J)	(A+E+J)	(A+G+J)
SBI-01 = A					
	Xtxp32	B	B	B	B
	Xtxp37	B	B	B	B
	Xtxp75	B	B	B	B
	Xtxp229	B	B	B	B
SBI-07 = E					
	Xgap342	A	**A**	A	**A**
	Xtxp159	B	**A**	B	**A**
	Xtxp312	B	**A**	B	**A**
	Xtxp40	B	**A**	B	**A**
SBI-10 = G					
	Xcup07	B	A	**A**	A
	Xtxp141	B	B	**A**	B
	Xgap1	B	B	**A**	B
	Xisp263	A	A	**A**	B
SBI-05 = J1+J2					
	Xisp258	B	B	B	B
	Xtxp65	B	B	B	B
	Xtxp94	B	B	B	B
	Xisp257	B	B	B	B
	Xtxp15	B	B	B	B
	Xtxp283b	B	B	B	B
	Xtxp225	A	B	B	B

Table 47. Marker verification of QTL composition of selected RILs from mapping population chosen for use as donor parents

	Self highlighted true-to-type plants																															
	RIL 166										RIL 189										RIL 252										RIL 153									
Marker/Sample	1	2	3	4	5	6	7	8	9	10	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**1**	**2**	**3**	**4**	**5**	6	**7**	**8**	**9**	**10**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	10
Xtxp37	B	H	B	B	B	B	O	O	B	O	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	H	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	B
Xtxp75	A	H	A	A	A	A	A	A	A	A	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	H	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	B
Marker/Sample																																								
Xtxp65	B	B	B	B	B	B	B	B	B	B	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	A	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	B
Xtxp94	B	B	B	B	B	B	B	B	B	B	**B**	-	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	-	**B**	A	**B**	**B**	**B**	**B**	**B**	**B**	-	**B**	**B**	-	**B**	**B**	**B**	H
Xtxp15	B	B	A	A	A	A	A	A	A	A	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	A	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	-	**B**	**B**	B
Marker/Sample																																								
Xtxp159	A	H	A	A	A	A	A	A	A	A	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	A	**A**	**A**	**A**	**A**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	B
Xtxp40	A	A	A	A	A	A	A	A	A	A	**B**	**B**	**B**	**B**	**B**	-	**B**	**B**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	A	**A**	**A**	**A**	**A**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	B
Marker/Sample																																								
Xtxp141	O	O	O	O	O	-	O	O	O	O	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**B**	**B**	**B**	**B**	**B**	A	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	B
Xgap1	B	B	B	B	B	B	B	B	B	B	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	A	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	B

Table 48. Parental screening of four RIL donor parents screened along with RIL BC_3F_1 generation progeny; *Xtxp159* and *Xtxp40* were not screened

Locus	A,G,J RIL153-1	A,G,J RIL153-2	RIL 166-1	RIL 166-2	A,J RIL189-1	A,J RIL189-2	A,G,J RIL252-1	A,G,J RIL252-2
Xtxp37	B	B	H	B	B	B	B	B
Xtxp37	B	B	H	-	B	B	B	B
Xtxp75	B	B	A	A	B	B	B	B
Xtxp75	B	B	A	A	B	B	B	B
Xtxp141	B	B	H	B	A	A	B	B
Xtxp141	B	B	H	B	A	A	B	B
Xtxp141	B	B	H	B	A	A	B	B
Xgap1	B	B	H	-	A	A	B	B
Xgap1	B	B	H	-	A	A	B	B
Xgap1	B	B	H	-	A	A	B	B
Xtxp65	B	B	H	B	B	B	B	B
Xtxp65	B	B	H	B	B	B	B	B
Xtxp65	B	B	H	B	B	B	B	B
Xtxp65	B	B	H	B	B	B	B	B
Xtxp65	B	B	H	B	B	B	B	B
Xtxp94	B	B	H	B	B	B	B	B
Xtxp94	B	B	H	B	B	B	B	B
Xtxp94	B	B	H	B	B	B	B	B
Xtxp94	B	B	H	B	B	B	B	B
Xtxp94	B	B	H	B	B	B	B	B
Xtxp15	B	B	A	B	B	B	B	B
Xtxp15	B	B	B	B	B	B	B	B
Xtxp15	B	B	B	B	B	B	B	B
Xtxp15	B	B	B	B	B	B	B	B

Table 49. Marker genotyping of RIL F_1 hybrids of backcross parents BTx623 and IS 18551

RIL153 A+E?+G+J Marker/ Sample		A+G	A+G+J1	A+G	A+G+J1	A+G+J1
	J1358	**J1359 x J1387**	**J1360 x J1380**	**J1361 x J1391**	**J1362 x J1391**	J1363 x J1385
Xtxp37	-	**H**	**H**	**H**	**H**	H
Xtxp75	-	**H**	**H**	**H**	**H**	H
Xtxp141	B	**H**	**H**	**H**	**H**	H
Xgap1	B	**H**	**H**	**H**	**H**	H
Xtxp159	A	A	-	A	A	A
Xtxp40	A/H?	A	A	A	A	A/H?
Xtxp65	B	A	**H**	H	**H**	H
Xtxp94	B	A	**H**	A	**H**	H
Xtxp15	A	A	A	-	A	A

RIL166 A?+G+J Marker/ Sample	G+J	G+J1	G+J	G+J	G+J1	J	G+J1	G+J
	J1364 x J1388	**J1365 x J1399**	**J1366 x J1393**	**J1367 x J1379**	**J1368 x J1392**	**J1369 x J1396**	**J1370 x J1379**	**J1371 x J1390**
Xtxp37	**H**	H	H	H	H	H	H	H
Xtxp75	-	A	A	A	A	A	A	A
Xtxp141	**H**	**H**	**H**	**H**	**H**	H	**H**	**H**
Xgap1	**H**	**H**	**H**	**H**	**H**	-	**H**	**H**
Xtxp65	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**
Xtxp94	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**
Xtxp15	**H**	-	**H**	**H**	-	**H**	A	**H**

RIL 189 A+E?+J Marker/ Sample	A+J1	A+J	A+J	A+J	A+J	A+J
	J1372 x J1388	**J1373**	**J1374 x J1389**	**J1375 x J1390**	**J1376 x J1386**	J1377 x J1378
Xtxp37	H	**H**	**H**	**H**	**H**	H
Xtxp75	H	**H**	**H**	**H**	**H**	H
Xtxp159	A	A	A	A	A	A
Xtxp40	A/H?	A	A/H?	A/H?	A/H?	A/H?
Xtxp65	H	**H**	**H**	**H**	**H**	H
Xtxp94	H	**H**	**H**	**H**	**H**	H
Xtxp15	-	**H**	**H**	**H**	**H**	H

Contd.....

RIL 252 A+G+J Marker/ Sample	**A+G+J**	**A+G+J**	**A+G+J1**		**A+G+J?**	**A+G+J**	**A+G+J**
	J1351 x J1382	**J1352 x J1377**	**J1353 x J1389**	J1354 x J1374	**J1355 x J1383**	**J1356 x J1381**	**J1357 x J1391**
Xtxp37	**A+G+J**	**H**	**H**	H	**H**	**H**	**H**
Xtxp75	**H**	**H**	**H**	A	**H**	**H**	**H**
Xtxp141	**H**	**H**	**H**	A	**H**	**H**	**H**
Xgap1	**H**	**H**	**H**	A	**H**	**H**	**H**
Xtxp65	**H**	**H**	**H**	A	**H**	**H**	**H**
Xtxp94	**H**	**H**	**H**	A	-	**H**	**H**
Xtxp15	**H**	**H**	A	H	**H**	**H**	**H**

Highlighted plant numbers are selected for advance by backcross to recurrent parent BTx623

Table 50. RIL F_1 Plants selected for advance to second backcross

RIL donor	Target QTLs	RIL F_1 crosses	RILBC_1F_1 Progeny
RIL 252	A+G+J	J1351 x J1382	J1801 - J1812
RIL 252	A+G+J	J1352 x J1311	J1813 - J1824
RIL 252	A+G+J	J1356 x J1381	J1825 - J1836
RIL 252	A+G+J	J1357 x J1391	J1837 - J1848
RIL 153	A+G	J1359 x J1380	J1849 - J1860
RIL 153	A+G	J1361 x J1391	J1861 - J1872
RIL 166	G+J	J1364 x J1388	J1873 - J1878
RIL 166	G+J	J1366 x J1393	J1879 - J1884
RIL 166	G+J	J1367 x J1379	J1885 - J1890
RIL 166	G+J	J1371 x J1390	J1891 - J1896
RIL 189	A+J	J1373 x J1389	J1897 - J1902
RIL 189	A+J	J1374 x J1390	J1903 - J1908
RIL 189	A+J	J1375 x J1388	J1909 - J1914
RIL 189	A+J	J1376 x J1378	J1915 - J1920

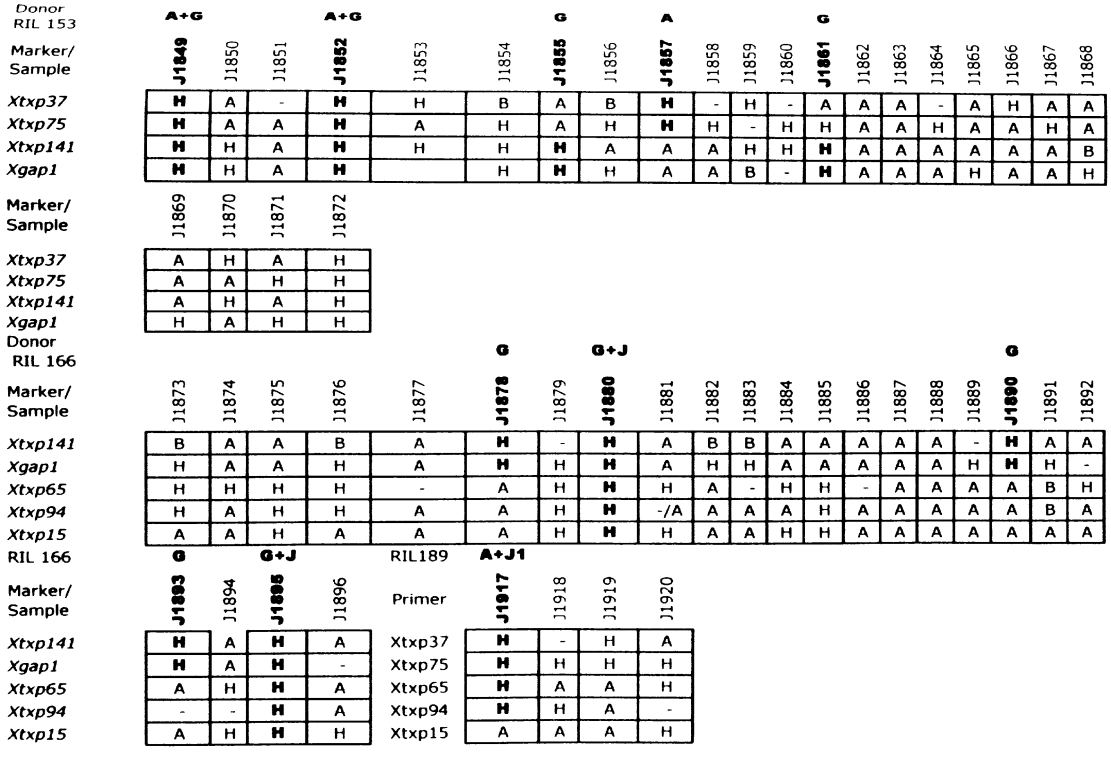
Table 51. Foreground marker genotyping of RIL BC_1F_1 backcross generation progenies

RIL 252		A+G+J	A+G		J2		A+J		G							A+J1			
Marker/ Sample	J1801	**J1802**	J1803	**J1804**	J1805	**J1806**	J1807	**J1808**	J1809	J1810	J1811	J1812	J1813	J1814	J1815	J1816	**J1817**	J1818	J1819	J1820
Xtxp37	B	**H**	H	A	A	**H**	B	A	A	H	H	B	A	A	A	A	**H**	H	H	B
Xtxp75	B	**H**	H	A	A	**H**	H	A	H	H	A	B	A	A	A	H	**H**	H	H	-
Xtxp141	A	**H**	B	A	B	A	H	**H**	H	B	H	H	H	H	H	H	H	H	H	H
Xgap1	A	**H**	B	A	H	A	H	**H**	A	H	A	H	H	A	A	-	A	-	A	H
Xtxp65	B	H	-	A	A	**H**	H	H	H	H	A	H	B	H	A	A	**H**	H	H	B
Xtxp94	B	A/ -	-	**H**	A	**H**	H	-	H	B	A	H?	A	H/A?	A	A	**H**	H	H	B
Xtxp15	B	H	A	**H**	B	**H**	B	H	-	B	A	B	-	A	-	A	A	A	A	H

RIL 252										
Marker/ Sample	J1821	J1822	J1823	J1824	J1825	J1826	J1827	J1828	J1829	J1830
Xtxp37	H	A	A	A	-	A	H	-	A	B
Xtxp75	A	A	H	A	H	A	H	H	-	B
Xtxp141	H	H	A	H	H	A	H	H	A	H
Xgap1	A	B	A	A	H	H	B	B	H	A
Xtxp65	H	B	H	H	H	H	A	B	H	H
Xtxp94	H	B	H	H	A	A	A	B	H	H
Xtxp15	A	H	H	H	A	H	A	H	A	H

RIL 252	A+G																	
Marker/ Sample	**J1831**	J1832	J1833	J1834	J1835	J1836	J1837	J1838	J1839	J1840	J1841	J1842	J1843	J1844	J1845	J1846	J1847	J1848
Xtxp37	**H**	H	H	B	-	-	A	-	A	-	A	A	A	A	A	A	A	A
Xtxp75	**H**	H	A	B	B	H	A	A	A	A	A	-	A	A	A	A	A	A
Xtxp141	**H**	B	A	B	H	H	A	A	A	A	A	A	A	A	A	A	A	A
Xgap1	**H**	B	H	H	H	H	A	A	A	A	A	A	A	A	A	A	-	A
Xtxp65	A	A	B	A	H	H	-	A	A	A	A	A	H	A	A	A	-	-
Xtxp15	A	A	B	H	H	H	A	A	A	A	A	A	A	A	A	A	A	A

Contd.....


Donor RIL 153
A+G
A+G
G
A
G
Marker/ Sample
J1849
J1850
J1851
J1852
J1853
J1854
J1855
J1856
J1857
J1858
J1859
J1860
J1861
J1862
J1863
J1864
J1865
J1866
J1867
J1868
Xtxp37
Xtxp75
Xtxp141
Xgap1
Marker/ Sample
J1869
J1870
J1871
J1872
Xtxp37
Xtxp75
Xtxp141
Xgap1
Donor RIL 166
G
G+J
G
Marker/ Sample
J1873
J1874
J1875
J1876
J1877
J1878
J1879
J1880
J1881
J1882
J1883
J1884
J1885
J1886
J1887
J1888
J1889
J1890
J1891
J1892
Xtxp141
Xgap1
Xtxp65
Xtxp94
Xtxp15
RIL 166
G
G+J
RIL189
A+J1
Marker/ Sample
J1893
J1894
J1895
J1896
Primer
J1917
J1918
J1919
J1920
Xtxp141
Xgap1
Xtxp65
Xtxp94
Xtxp15
Xtxp37
Xtxp75
Xtxp65
Xtxp94
Xtxp15

Contd.....

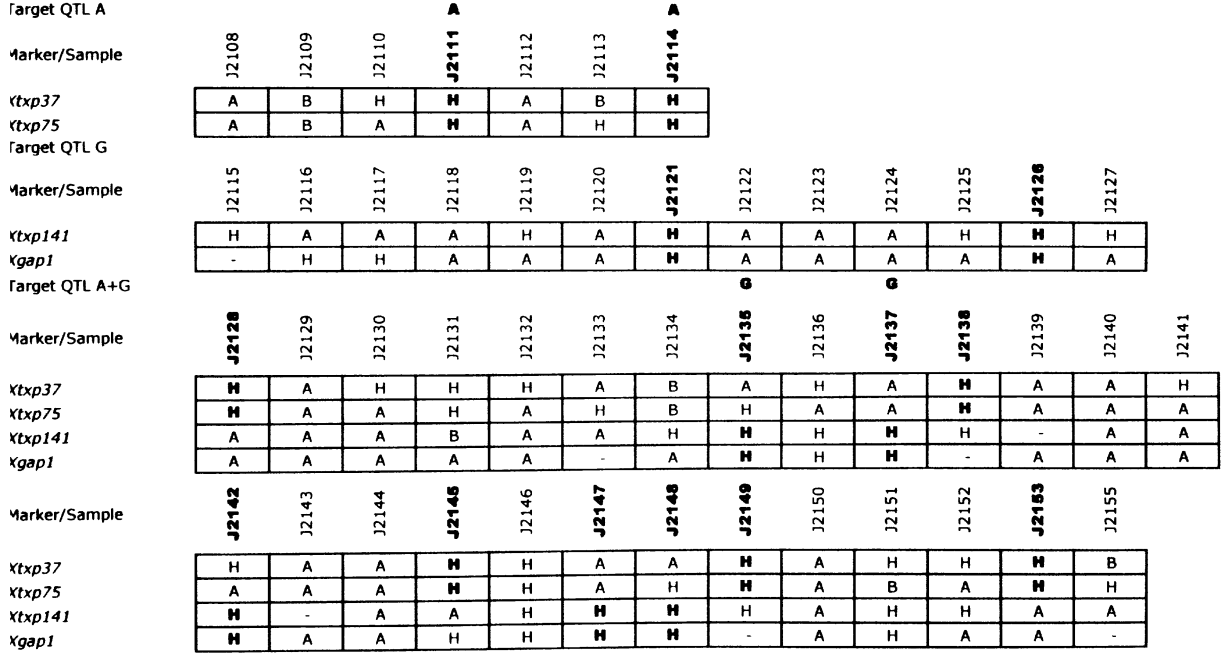
Donor HDL 189	A																			
Marker/ Sample	**J1897**	J1898	J1899	J1900	J1901	J1902	J1903	J1904	J1905	J1906	J1907	J1908	J1909	J1910	J1911	J1912	J1913	J1914	J1915	J1916
Vvp37	**H**	A	H	H	A	B	A	A	H	-	A	H	A	A	A	A	H	H	A	A
Vvp75	**H**	A	H	H	A	H	H	A	H	H	A	H	H	-	A	A	H	A	-	A
Vvp65	A	A	B	B	H	B	H	H	H	H	H	-	-	A	H	A	A	A	A	A
Vvp94	A	H	B	B	B	B	H	H	H	H	H	A	H	A	A	A	A	A	H	A
Vvp15	A	H	B	B	H	H	H	A	H	A	A	H	H	H	A	H	A	A	H	A

J1802, J1806, J1808, J1897, J1917 are F2's from background marker data in Table 51

Table 52. Background screening of foreground-selected RIL BC_1F_1 plants. Those advanced after background screening: J1857(A), J1878 (G), J1893 (G), J1849 (A+G), J1831 (A+G), J1817 (A+J1), J1917 (A+J1), J1895 (G+J), and J1880 (G+J).

	+J	Best J2		+J2	**Best A+J1**	**2nd best A+G**	**Best A+G**			**Best A**		**Best G**	**2nd best G+J**	3rd best G	**2nd best G**	**Best G+J**		**2nd best A+J1**		
	A+G	J2	A+J	G				A+G	G		G			G			A			
Marker/Sampl	J1802	J1804	J1806	J1808	**J1817**	**J1831**	**J1849**	J1852	J1855	**J1857**	J1861	**J1878**	**J1880**	J1890	**J1893**	**J1895**	J1897	**J1917**	BTx623	IS 18551
Xtxp316	H	H	H	B	**A**	**A**	**A**	A	H	**A**	H	**A**	**A**	A	**A**	**A**	B	**H**	A	B
Xtxp319	H	A	H	H	**A**	**A**	**A**	H	A	**A**	H	**A**	**H**	A	**A**	**H**	B	**H**	A	B
Xtxp32	H	A	A	A	**A**	**H**	**H**	H	A	**H**	A	**A**	**A**	A	**A**	**A**	B	**H**	A	B
Xtxp302	A	A	A	B	-	**A**	**A**	A	A	**A**	A	**A**	**A**	A	**A**	**A**	A	**A**	A	B
Xtxp211	A	A	A	A	**A**	**A**	**A**	A	A	**A**	A	**A**	**A**	A	**A**	**A**	A	**A**	A	B
Xtxp96	A	A	A	A	**A**	**A**	**A**	A	A	**A**	A	**A**	**A**	A	**A**	**A**	A	**A**	A	B
Xtxp4	A	A	A	A	**A**	**A**	**A**	A	A	**H**	H	**A**	**H**	H	**A**	**H**	A	**A**	A	B
Xtxp1	H	A	B	A	**A**	**A**	**A**	H	A	**A**	H	**A**	**H/O?**	H/O?	**A**	**H/O?**	B	**H**	A	B
Xtxp286	A	H	B	A	**A**	**A**	**A**	H	A	**A**	H	**A**	**O**	O	**A**	**O**	B	**B**	A	B
Xtxp9	H	H	A	H	**A**	**A**	**A**	A	A	**A**	A	**H**	**A**	A	**A**	**A**	A	**H**	A	B
Xtxp285	A	A	A	A	**A**	**A**	**A**	H	A	**A**	A	**A**	**A**	A	**A**	**A**	A	**A**	A	B
Xcup11	B	H	A	A	**A**	**A**	**A**	A	H	**H**	A	**A**	**A**	A	**A**	**A**	H	**A**	A	B
Xtxp21	H	H	A	A	**A**	**A**	**A**	A	A	**A**	A	**A**	**A**	A	**A**	**A**	A	**H**	A	B
Xtxp41	A	A	A	A	**A**	**A**	**A**	A	A	**A**	A	**A**	**A**	A	**A**	**A**	A	**A**	A	-
Xtxp27	A	H?	H	H	**A**	**A**	**A**	A	A	-	A	**A**	**A**	A	-	**A**	A	**H**	A	B
Xtxp159	-	-	-	A	**A**	**A**	**A**	A	A	**A**	A	-	**A**	-	-	**A**	A	-	A	B
Xisp344	A	A	A	A	**A**	**A**	**H**	H	H	**A**	A	**A**	**H**	H	**A**	**A**	A	**A**	A	B
Xtxp295	A	A	A	A	**A**	**A**	**H**	H	H	**A**	A	**A**	**A**	A	**A**	**A**	A	**A**	A	B
Xtxp258	A	H	H	H	**H**	**A**	**A**	H	A	**A**	H	**A**	**H**	A	**A**	**A**	H	**A**	A	B
Xtxp289	B	H	H	H	**A**	**A**	**H**	A	A	**H**	H	**H**	**A**	A	**A**	**A**	A	**H**	-	B
Xisp263	H	A	A	B	**A**	**A**	**A**	A	A	**A**	A	**H**	**A**	H	**H**	**H**	B	**H**	A	B
Xtxp18	H	A	H	A	**A**	**A**	**A**	A	H	**A**	H	**H**	**H**	H	**H**	**A**	H	**A**	A	B
Xisp198	H	A	H	H	**A**	-	**A**	-	H	**H**	H	**A**	**H**	H	**H**	**A**	B	**H**	A	B
Xtxp105	H	H	B	A	**A**	**A**	**A**	H	H	**A**	H	**H**	**A**	H	**H**	**A**	H	**A**	A	B
Xisp258	H	A	H	H	**H**	**A**	**A**	A	H	**H**	H	**A**	**H**	A	**A**	**H**	A	**H**	A	B
Xtxp6	B	H	H	H	**A**	**A**	**A**	A	A	**A**	A	**A**	**A**	A	**A**	**A**	A	**H**	A	B
Xtxp317	B	H	A	A	**H**	**H**	**A**	A	A	**A**	A	**A**	**A**	A	**A**	**A**	A	**H**	A	B
Xtxp265	B	H	B	A	**H**	**H**	**A**	A	A	**A**	A	**A**	-	A	**A**	**A**	A	**H**	A	B
Xtxp274	B	H	B	A	**H**	-	**A**	-	A	**A**	A	**A**	**A**	A	**A**	**A**	A	**H**	A	B
Xisp10328	H	-	B	A	**H**	**H**	**A**	A	A	**A**	A	**H**	**A**	H	**H**	**O**	H	**H**	A	B
Xisp10264	H	A	B	A	**H**	**H**	**A**	A	A	**A**	A	**A**	**A**	A	**A**	**A**	H	**H**	A	B
Xtxp57	H	A	B	A	**H**	**H**	**A**	A	A	**A**	A	**A**	**A**	A	**A**	**A**	H	**H**	A	B
Xisp10347	O	A	O	A	**A**	**O**	**A**	A	O	**A**	A	**H**	**A**	H	**H**	**H**	A	**A**	A	B
A	11	18	14	22	**24**	**24**	**29**	22	24	**26**	22	**25**	**23**	22	**25**	**25**	19	**13**	32	0
H	14	12	9	8	**8**	**6**	**4**	9	8	**6**	11	**7**	**7**	8	**6**	**5**	7	**18**	0	0
B	6	0	8	3	**0**	**0**	**0**	0	0	**0**	0	**0**	**0**	0	**0**	**0**	7	**1**	0	32
-	1	2	1	0	**1**	**2**	**0**	2	0	**1**	0	**1**	**1**	1	**2**	**0**	0	**1**	1	1
O	1	0	1	0	**0**	**1**	**0**	0	1	**0**	0	**0**	**1**	1	**0**	**2**	0	**0**	0	0
Others	0	1	0	0	**0**	**0**	**0**	0	0	**0**	0	**0**	**1**	1	**0**	**1**	0	**0**	0	0
Grand total	33	33	33	33	**33**	**33**	**33**	33	33	**33**	33	**33**	**33**	33	**33**	**33**	33	**33**	33	33
Comment	F2 not BC1F1	True BC1F1	F2 not BC1F1	F2 not BC1F1	**True BC1F1**	**True BC1F1**	**True BC1F1**	True BC1F1	True BC1F1	**True BC1F1**	True BC1F1	**True BC1F1**	**True BC1F1**	True BC1F1	**True BC1F1**	**True BC1F1**	F2 not BC1F1	**F2 not BC1F1**		
%A	**33%**	**55%**	**42%**	**67%**	**73%**	**73%**	**88%**	67%	73%	**79%**	67%	**76%**	**70%**	67%	**76%**	**76%**	58%	**39%**	97%	0%

Table 53. Foreground marker genotype data from RIL BC_2F_1 families



Contd.....

Target QTL A+J1	**A+J1**	**J1**			BC_1F_1	BC_1F_1	BC_1F_1	BC_1F_1	BC_1F_1	BC_1F_1	BC_1F_1	BC_1F_1	BC_1F_1	BC_1F_1
Marker/Sample	**J2156**	**J2157**	J2158	J2159	J2160	J2161	J2162	J2163	J2164	J2165	J2166	J2167	J2168	J2169
Xtxp37	**H**	A	H	A	A	A	H	B	A	H	H	H	H	B
Xtxp75	**H**	H	A	A	A	A	H	B	A	B	A	H	A	B
Xtxp65	**H**	**H**	-	A	-	B	-	H	A	H	B	B	B	H
Xtxp94	**H**	**H**	A	H	A	B	A	H	H	A	H	B	B	H
Xtxp15	A	A	A	A	A	A	A	A	A	A	A	A	A	A

	BC_1F_1	BC_1F_1	BC_1F_1	BC_1F_1
Marker/Sample	J2170	**J2171**	J2172	**J2173**
Xtxp37	B	**H**	H	**H**
Xtxp75	H	**H**	B	**H**
Xtxp65	B	A	H	**H**
Xtxp94	B	H	A	**H**
Xtxp15	A	A	A	A

Target QTL G+J				**J2**							**G+J1**	**G+J1**		
Marker/Sample	J2174	J2175	**J2176**	**J2177**	**J2178**	J2179	J2180	J2181	J2182	J2183	**J2184**	**J2185**	**J2186**	J2187
Xtxp141	H	B	**H**	H	**H**	A	B	H	H	H	**H**	**H**	H	H
Xgap1	A	A	**H**	A	**H**	A	A	-	A	A	**H**	**H**	A	A
Xtxp65	A	B	-	A	A	B	-	A	A	B	**H**	**H**	**H**	B
Xtxp94	A	B	A	**H**	A	B	B	A	A	B	**H**	**H**	**H**	B
Xtxp15	H	H	H	**H**	H	B	H	H	H	B	A	A	A	B

Table 54. BC_2F_1 Crosses selected for advance to BC_3F_1 progenies

Target QTL	$RILBC_2F_1$ Cross	A% in BC_1F_1	$RILBC_3F_1$ progenies	No. of seed germinated
SBI-01 = A	J2111 x BTx623-3	79%	J2651-J2675	25
SBI-01 = A	J2114 x BTx623-25	79%	J2676-J2700	25
SBI-10 = G	J2135 x BTx623-13	88%	J2701-J2719	19
SBI-10 = G	J2137 x BTx623-6	88%	J2720-J2725	6
A + J1	J2156 x BTx623-20	73%	J2726-J2747	22
SBI-05 = J1	J2157 x BTx623-15	73%	J2748-J2776	29
SBI-05 = J2	J2177 x BTx623-4	76%	J2777-J2784	8
G + J1	J2184 x BTx623-23	70%	J2785-J2789	5
G + J1	J2185 x BTx623-11	70%	J2790	1

Table 55. Foreground marker genotype data of RIL BC_3F_1 families

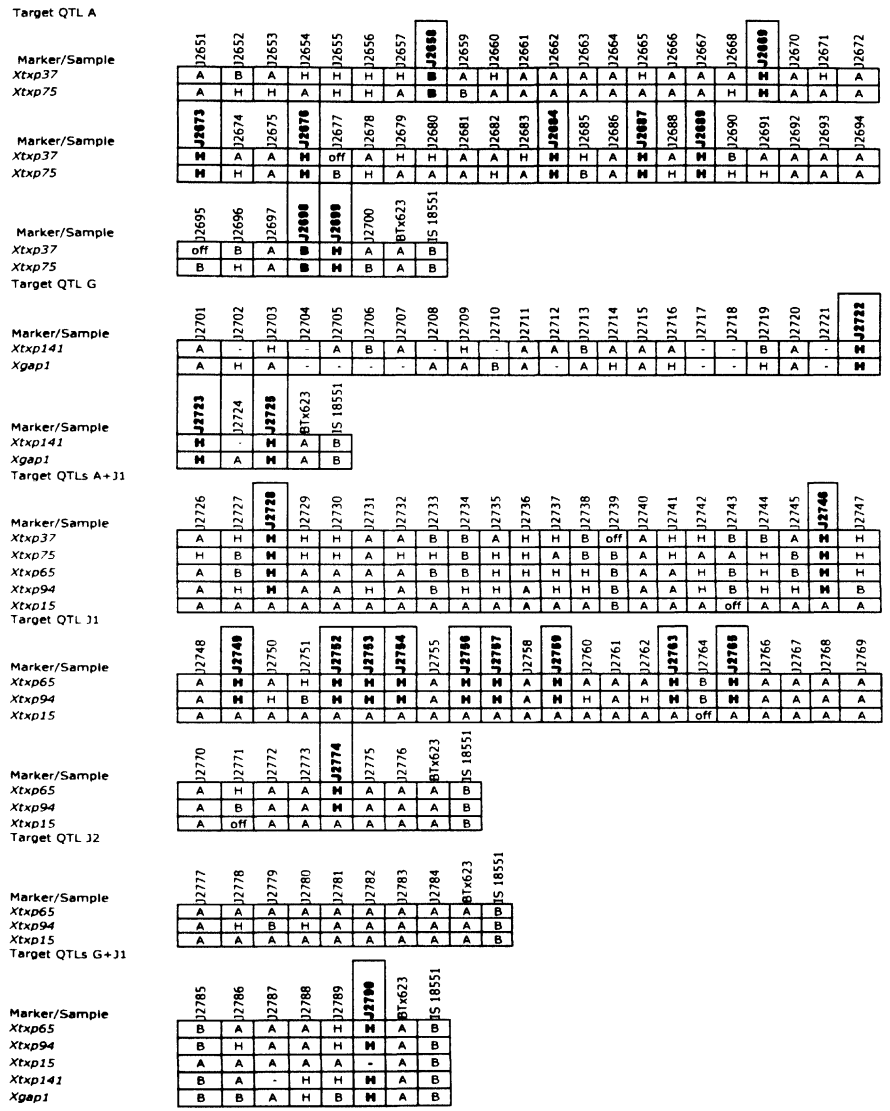


Table 56. Harvest list of RIL BC_4F_1 and BC_3F_2 seed from selected BC_3F_1 plants, *rabi* 2005-06

S.No.	BC3F1 Cross	BC4F1 seed	BC3F2 seed weight	Plant height	QTL complement
RIL 153 donor					
1	J2658 (BC2F2)	-	24(BC2F3)		**+QTL A homozygote**
3	J2669 x BTx623	123	10	4-dwarf height	QTL A heterozygote
4	J2673 x BTx623	104	10		QTL A heterozygote
5	J2676 x BTx623	108	26		QTL A heterozygote
6	J2684 x BTx623	115	10		QTL A heterozygote
7	J2687 x BTx623	140	6		QTL A heterozygote
8	J 2689 x BTx623	97	15		QTL A heterozygote
9	J2698 (BC2F2)	-	16(BC2F3)		**+QTL A homozygote**
10	J2699 x BTx623	168	18		QTL A heterozygotes
11	J2722 x BTx623	115	15	2-dwarf height	QTL G heterozygote
12	J2723 x BTx623	78	11		QTL G heterozygote
13	J2725 x BTx623	164	14	4-dwarf height	QTL G heterozygote
RIL 252 donor					
14	J2728 x BTx623	166	18	4-dwarf height	QTL A+J1 heterozygote
15	J2746 x BTx623	31	9	4-dwarf height	QTL A+J1 heterozygote
16	J 2749 x BTx623	42	12	1-dwarf height	QTL J1 heterozygote
17	J 2752 x BTx623	138	7	3-dwarf height	QTL J1 heterozygote
18	J 2753 x BTx623	110	4	4-dwarf height	QTL J1 heterozygote
19	J 2754 x BTx623	24	11	3-dwarf height	QTL J1 heterozygote
20	J 2756 x BTx623	19	5		QTL J1 heterozygote
21	J 2757 x BTx623	62	5	1-dwarf height	QTL J1 heterozygote
22	J 2759 x BTx623	63	13		QTL J1 heterozygote
23	J 2763 x BTx623	90	9	3-dwarf height	QTL J1 heterozygote
24	J 2765 x BTx623	74	17	2-dwarf height	QTL J1 heterozygote
25	J 2774 x BTx623	109	13	2-dwarf height	QTL J1 heterozygote
RIL 166 donor					
26	J2788 x BTx623	53	10	1-dwarf height	QTL G heterozygote
27	J 2790 x BTx623	11	12	3-dwarf height	QTL G+J1 heterozygote

Table 57. *Kharif* 2006 field screen results for near-isogenic line sets in BTx623 background, differing in presence (+QTL) or absence (-QTL) for IS 18551 marker alleles flanking specific target QTLs for shoot fly resistance located on linkage groups SBI-01 (A), SBI-07 (E), SBI-05 (J) and SBI-10 (G).

Mean phenotypic values in near-isogenic lines derived from the cross BTx623 (susceptible) x IS 18551 (resistant) for different components of resistance to shoot fly and other traits in *kharif* season 2006 at ICRISAT, Patancheru.

	Near-Isogenic Lines									RIL Parents			Parental Controls			Checks	
Trait	Isoline+A	Isoline-A	Isoline+E	Isoline-E	Isoline+J1	Isoline-J1	Isoline+J	Isoline-J	Isoline +G	RIL 153	RIL 189	RIL 252	BTx623	IS 18551	Swarna	IS 1054	IS 2312
Glossiness score	4.58*	4.88	4.63	4.71	3.79**	4.79	3.25**	4.58	3.00**	2.46**	2.00**	2.042**	4.79	1.75**	4.83**	2.63**	1.29**
Seedling vigor score	2.54**	2.96	2.75**	3.00	2.96	2.58	2.92	2.50	1.83*	2.667*	2.13**	1.92**	2.92	2.13**	1.21	1.25	1.25
Oviposition I	78.28	65.16*	76.34	54.57**	51.2**	75.60	56.65**	84.77	81.52*	47.66**	59.45**	69.23**	91.53	51.92**	91.40**	72.07**	56.01**
Eggs/100 plants I	111.17	84.64*	91.55	70.08**	65.31**	108.90	66.91**	114.67	97.80*	57.61**	72.2**	93.30**	128.77	62.36**	139.20**	91.8**	70.6**
Oviposition II	95.12	92.73	93.16	86.72*	83.75*	92.33	85.40*	97.62	88.24**	84.71**	90.77**	95.56	98.21	85.25**	98.52**	94.52*	89.67**
Eggs/100 plants II	142.90	120.6*	111.90	120.00	118.70	130.60	107.80	125.00	105.00*	114.00*	125.60	143.60	141.60	122.90	141.50	142.80	131.30
Deadhearts incidence I	48.21	38.39	49.40	28.16**	28.84**	46.32	36.26*	53.67	51.91*	22.78**	31.43**	40.20**	69.48	19.07**	72.22**	45.13**	24.82**
Deadhearts incidence II	81.73	76.25	85.82	69.45**	62.05**	83.26	69.72**	90.77	71.14**	62.16**	69.46**	78.54**	92.61	57.46**	93.27**	73.96*	66.24**

RIL 153 = A,E,G,J RIL 189 = A,E,J RIL 252 = A,G,J

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

Seedling vigor (1-5 scale): 1 = high vigor, 5 = low vigor; * significant at P=0.05, ** significant at P=0.01

Table 58. *Rabi* 2006-07 field screen results for near-isogenic line sets in BTx623 background (81-entry trial), differing in presence (+QTL) or absence (-QTL) for IS 18551 marker alleles flanking specific target QTLs for shoot fly resistance located on linkage groups SBI-01 (A), SBI-07 (E), SBI-05 (J) and SBI-10 (G).

Mean phenotypic values in near-isogenic lines derived from the cross BTx623 (susceptible) x IS 18551 (resistant) for different components of resistance to shoot fly and other traits in rabi season 2006-07 at ICRISAT, Patancheru.

	Near-Isogenic Lines											RIL Parents			Parental Controls			Checks	
Trait	Isoline+A	Isoline-A	Isoline+E	Isoline-E	Isoline+J1	Isoline-J1	Isoline+J	Isoline-J	Isoline+G	+J1-J2? (BBBA)	-J1+J2? (AAAB)	RIL 153	RIL 189	RIL 252	BTx623	IS 18551	Swarna	IS 1054	IS 2312
Glossiness score	5.00	5.00	5.00	5.00	4.04**	5.00	3.96**	5.00	3.417**	4.33**	5.00	2.88**	3.13**	2.17**	5.00	1.79**	5.00**	3.05**	1.65**
Seedling vigor score I	3.00	3.18	3.27**	4.60	4.08	3.46*	2.43	1.58**	1.75	4.83	1.91**	3.00	2.06	2.73**	2.17	2.88**	2.35	2.23**	1.48**
Seedling vigor score II	3.04	3.38	2.79**	3.60	3.15	3.04	1.98**	2.89	2.00**	4.00	2.41**	2.17**	2.08**	2.60	2.88	1.88**	2.87**	1.53	1.30**
Oviposition I	93.13	87.16	84.23	75.83	75.14*	88.59	89.84**	96.60	86.72*	78.05*	92.17	73.17**	80.31**	74.02**	95.13	55.03**	94.66**	69.21*	57.05**
Eggs/100 plants I	199.9	187.4	169.5	132.0*	151.7	177.1	169.5**	211.8	153.9**	115.5**	193.1	112.6**	141.6**	123.3**	220.5	80.1**	202.1**	101.8*	80.4**
Oviposition II	40.11	43.52	43.58	42.07	37.75	42.63	39.59	38.37	40.11	96.30	36.28**	37.42	39.55	42.12	37.44	38.47	36.87**	42.07**	36.48
Eggs/100 plants II	172.6	162.1	159.4	153.0	132.5*	158.9	139.3**	173.7	105.0**	129.4	139.6	88.6**	125.9**	110.0**	188.3	68.8**	170.7**	81.1	66.2**
Deadhearts incidence I	27.75	24.10	20.01	6.71**	10.32*	20.92	17.97**	44.17	23.61	5.00*	29.72	4.21**	11.66**	9.85**	25.72	2.31**	35.04**	9.53	7.59**
Deadhearts incidence II	89.48	86.86	80.30	82.43	65.06**	87.66	68.32**	90.98	58.48*	64.95	85.22	43.71**	63.76**	52.18**	92.32	31.47**	90.74**	37.41	32.5**
Deadhearts incidence	93.98	93.82	88.82*	93.23	78.62**	93.85	77.96**	93.93	63.71*	85.52	89.33	55.47**	74.86**	61.13**	95.74	43.69**	95.63**	50.77	45.71**

Isoline J plants were screened with markers *Xisp258*, *Xtxp65*, *Xtxp94* and *Xtxp15*

RIL 153 = A,E,G,J RIL 189 = A,E,J RIL 252 = A,G,J

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

Seedling vigor (1-5 scale): 1 = high vigor, 5 = low vigor; * significant at P=0.05, ** significant at P=0.01

Table 59. *Rabi* 2006-07 field screen results for near-isogenic line sets in 296B background (110-entry trial), differing in presence (+QTL) or absence (-QTL) for IS 18551 marker alleles flanking specific target QTLs for shoot fly resistance located on linkage groups SBI-01 (A), SBI-07 (E), SBI-05 (J) and SBI-10 (G).

Mean phenotypic values in near-isogenic lines derived from the cross 296B (susceptible) x IS 18551 (resistant) for different components of resistance to shoot fly and other traits in rabi season 2006-07 at ICRISAT, Patancheru.

	Near-Isogenic Lines												Parental Controls			Checks	
Trait	Isoline+A	Isoline-A	Isoline+E	Isoline-E	Isoline+J1	Isoline-J1	Isoline+J	Isoline-J	+J1?-J2 (BAA)	-J1?+J2? (AAB)	+J (BBA)	-J (AAA)	296B	IS 18551	Swarna	IS 1054	IS 2312
Glossiness score	4.67	4.89	4.86	4.77	3.06**	5.00	3.25**	4.89	3.08**	4.83	2.67**	5.00	5.00	1.56**	5.00**	2.50**	1.18**
Seedling vigor score I	2.44	2.31	3.13*	3.59	2.94	2.67*	2.64	2.71	2.75	2.60	3.17	3.08	2.77	2.64	2.35**	1.68	1.43**
Seedling vigor score II	2.83	2.47	3.17	3.37	2.81	2.50	2.44	2.77	2.67	2.60	2.75	3.08	2.93	1.71**	2.74**	1.33	1.25**
Oviposition II	89.59	93.57	85.50	80.78	83.08**	92.09	87.88	91.76	82.31	93.30	83.10	96.21	91.16	45.57**	93.22**	62.00**	45.01**
Eggs/100 plants II	153.1	175.7	127.6	165.6	127.8*	154.1	134.7	149.1	135.9	151.5	127.6*	152.6	149.9	60.7**	158.7**	88.1**	57.5**
Deadhearts incidence I	15.46	16.73	11.67	26.69	5.82*	11.61	7.86*	13.54	2.89**	15.28	5.42	17.14	26.69	11.89	25.59**	7.72**	3.40**
Deadhearts incidence II	82.05	82.18	73.40	68.56	66.16**	83.81	69.09**	82.25	68.66*	80.57	72.30*	88.95	84.31	25.11**	87.87**	37.09	26.60**
Deadhearts incidence III	87.09	91.56	82.25	79.07	76.22**	88.64	76.75*	88.19	78.28*	88.06	79.86*	92.62	88.48	35.34**	91.23**	44.23	34.46**
											From A+J samples						

Note: +J1?-J2, -J2?J2?,+J lines were screened with markers *Xisp258*, *Xtxp65*, and *Xtxp15*

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

Seedling vigor (1-5 scale): 1 = high vigor, 5 = low vigor; * significant at P=0.05, ** significant at P=0.01

Table 60. *Kharif* 2007 field screen results for near-isogenic line sets in BTx623 background (64-entry trial), differing in presence (+QTL) or absence (-QTL) for IS 18551 marker alleles flanking specific target QTLs for shoot fly resistance located on linkage groups SBI-01 (A), SBI-07 (E), SBI-05 (J) and SBI-10 (G).

Mean phenotypic values in near-isogenic lines derived from the cross BTx623 (susceptible) x IS 18551 (resistant) for different components of resistance to shoot fly and other traits in *kharif* season 2007 at ICRISAT, Patancheru.

	Near Isogenic Lines									RIL Parents			Parental Controls			Checks	
	Isoline+A	Isoline-A	Isoline+E	Isoline-E	Isoline+G	Isoline+J1	Isoline-J1	Isoline+J	Isoline-J	RIL 153	RIL 189	RIL 252	BTx623	IS 18551	Swarna	IS 1054	IS 2312
Glossiness score	5.00	5.00	5.00	5.00	3.56**	4.13**	5.00	3.50**	5.00	1.97**	3.56**	1.56**	5.00	1.00**	5.00**	3.00**	1.72**
Oviposition 14 dae	99.37	99.35	99.77	99.18	95.77**	98.25*	99.55	99.05	99.60	96.79**	96.36**	95.56**	99.64	92.07**	99.62**	94.97	93.39**
Deadhearts incidence 14 dae	93.90	93.84	94.69	93.30	81.90**	89.04**	94.83	90.61*	95.66	74.07**	81.24**	78.97**	94.09	65.99**	94.61**	63.91	64.10**
Deadhearts incidence 21 dae	99.81	99.93	99.41	99.54	96.08*	98.59*	99.62	98.3**	100.00	95.72**	96.21**	94.84**	99.59	88.61**	99.81**	89.81	87.95**
Eggs/100 plants 14 dae	166.3	166.7	166.2	185.2	142.6	164.2	176.5	184.6	205.2	171.2	153.1	156.7*	185.2	124.2**	176.4	137.1	152.8

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

Counts of eggs/100 plants taken at 14 DAE from a single replication

* significant at P=0.05, ** significant at P=0.01

Table 61. *Kharif* 2007 field screen results for near-isogenic line sets in 296B background (20-entry trial), differing in presence (+QTL) or absence (-QTL) for IS 18551 marker alleles flanking specific target QTLs for shoot fly resistance located on linkage groups SBI-01 (A), SBI-07 (E), SBI-05 (J) and SBI-10 (G).

Mean phenotypic values in near-isogenic lines derived from the cross 296B (susceptible) x IS 18551 (resistant) for different components of resistance to shoot fly and other traits in 20-entry trial, kharif season 2007 at ICRISAT, Patancheru.

Trait	Near Isogenic Lines							Parental Controls		
	Isoline+A	Isoline-A	Isoline+E	Isoline-E	Isoline+J1	Isoline+J	Isoline-J	BTx623	296B	IS 18551
Glossiness score	5.00	4.50	5.00	5.00	2.33**	2.83**	5.00	5.00	5.00	1.50**
Oviposition 14 dae	88.42	90.80	92.02	95.28	92.83	93.60	95.80	92.02	85.75	86.18
Eggs/100plants 14 dae	162.40	200.80	199.10	177.90	225.00	244.20	232.70	193.80	167.90	144.10
Oviposition 21 dae	97.95	98.82	97.82	97.98	95.70**	96.43	100.00	96.98*	97.68**	88.80
Deadhearts incidence 14 dae	63.38	70.70	68.25	76.43	61.82	67.45	83.27	61.35	63.52	53.55
Deadhearts incidence 21 dae	97.58	94.90	98.28*	100.00	97.68	95.60	95.58	93.98	95.82	95.78

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

* significant at P=0.05, ** significant at P=0.01

Table 62. *Kharif* 2007 field screen results for near-isogenic line sets in 296B background (84-entry trial), differing in presence (+QTL) or absence (-QTL) for IS 18551 marker alleles flanking specific target QTLs for shoot fly resistance located on linkage groups SBI-01 (A), SBI-07 (E), SBI-05 (J) and SBI-10 (G).

Mean phenotypic values in near-isogenic lines derived from the cross 296B (susceptible) x IS 18551 (resistant) for different components of resistance to shoot fly and other traits in 84-entry trial, kharif season 2007 at ICRISAT, Patancheru.

Trait	Near Isogenic Lines										Parental Controls		
	Isoline+A	Isoline-A	Isoline+E	Isoline-E	Isoline+J1	Isoline-J1	Isoline+J	Isoline-J	Isoline+J1?	Isoline+J2?	BTx623	296B	IS 18551
Glossiness score	4.83	4.33	4.86	4.75	3.34*	5.00	3.67**	4.78	4.00	5.00	4.88	5.00	1.00**
Oviposition 14 dae	93.73	96.28	87.34	91.48	90.77	92.53	89.58	92.54	97.05	89.07	92.55**	91.75**	64.11
Eggs/100plants, 14 dae	219.6	203.8	162.7	167.4	181.6	197.0	218.3	184.0	365.0	206.5	189.4**	191.7**	99.1
Deadhearts incidence 14 dae	87.45	76.85	66.34	67.49	66.73	84.88	64.10	77.69	91.20	76.81	80.19**	84.26**	38.30
Deadhearts incidence 21 dae	97.35	100.00	92.94	92.74	95.75	100.00	92.37	98.45	100.00	99.09	100*	97.08	88.69

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

* significant at P=0.05, ** significant at P=0.01

DISCUSSION

DISCUSSION

Sorghum [*S. bicolor* (L.) Moench] is the fifth major cereal crop of the world cultivated in around 96 countries (FAO, 2004). Sorghum grain is produced as dietary staple for millions of people in semi-arid areas of Asia (mainly India and China) and Africa where drought stress causes frequent failures of other cereal crops. Stems and foliage of sorghum can be used as green chop, hay, silage and pasture for feeding ruminant livestock. Sorghum shoot fly is one of the most important biotic constraints found in all sorghum growing areas of India. This insect pest resembles a housefly; its total life cycle is completed in 18-25 days and involves egg, larva, pupa and adult stages. The insect attacks the sorghum at seedling stage (7-30 days after emergence). The adult female lays white, cigar shaped eggs on the undersurface of the sorghum seedling leaf blade. The larvae crawl towards the plant whorl, move downwards between the leaf folds and cuts the growing point to feed on the juice causing central portion of the whorl to wilt and dry resulting in deadheart formation.

Shoot fly is a major insect pest of sorghum. Although genetic studies have been made on host plant resistance to shoot fly by a number of workers using different genetic backgrounds, the genetic information available is limited and available in piece meal. Shoot fly resistance is quantitative in nature and influenced by G x E interaction. Therefore, direct phenotypic trait selection for this trait will be difficult. Despite efforts made over the last two decades utilizing the existing cultivated sources of shoot fly resistance, the level of resistance achieved so far is limited in agronomically elite genetic backgrounds. Marker-assisted selection is expected to increase the efficiency of breeding for such traits.

Attempts to study the genetic architecture of shoot fly resistance and its component traits in appropriate breeding material (derived from crosses of donor parent IS 18551 with elite, susceptible hybrid parental lines) were made by Gowri Sajjanar (2002) and Santhosh Deshpande (2005) in two different RIL populations. Gowri Sajjanar (2002) used 252 RILs (constructed from parents BTx623 and IS 18551), for phenotyping along with parents and checks under three environments, *viz.*, *kharif* and *rabi* seasons at ICRISAT-Patancheru (E1 and E2) and early *rabi* at Dharwad (E3). 93 RILs forming subset of this mapping population were genotyped with 44 SSR primer pairs. She mapped 2 major QTLs associated with shoot fly resistance (glossiness, J1; on SBI-05) and seedling leaf

blade trichome density (trichomes, G; on SBI-10). In addition to these, 14 other minor QTLs were detected in at least two of the three screening environments. Some of these minor QTLs, including oviposition II, deadhearts I and deadhearts II mapped in the vicinity of the two major QTLs. Further, the heritability estimates of glossiness (>0.92) was consistently high in individual screens and across environments. The trichome density on lower surface of leaf blades also recorded consistently high heritability estimates in each of the individual screens and across environments (>0.90), while that on the upper leaf blade surface also recorded consistently high heritability (>0.8) in each of the individual screens and across test environments.

Santhosh Deshpande (2005) used 259 RILs (constructed from parents 296B and IS 18551) for phenotyping under two environments, *viz.*, late *kharif* (E1) and *rabi* (E2) at Parbhani. These RILs were genotyped with 114 SSR primer pairs. He detected a major QTL for glossiness on linkage group J (SBI-05) along with one QTL on linkage group G (SBI-10) accounting for 11.3% (*kharif*), 9.4% (*rabi*) and 19.5% (across environments) of the phenotypic variation for trichome density on the adaxial leaf surface. This QTL co-localized with a major QTL for trichome density on the abaxial leaf surface, explaining 25% of the variation across the two screening environments, pointing to similarities in genetic control of trichome densities on either surface of a sorghum seedling leaf blade. He concluded that a single major QTL on LG G is involved in the control of trichome density on both sides of the sorghum seedling leaf blade. These QTL mapping results corresponded to the QTLs mapped in the (BTx623 x IS 18551)-derived RIL population (Gowri Sajjanar, 2002). The heritability estimates for glossiness observed by Deshpande were consistently high in individual *kharif* (0.9) and *rabi* (>0.8) environments, and across these environments (>0.8). Trichome density recorded consistently high heritability estimates (>0.97) in both *kharif* and *rabi* environments for both adaxial and abaxial leaf surfaces. However, the across-environment analysis revealed lower estimates of heritability. For adaxial leaf surface, heritability was recorded as 0.64 and for abaxial leaf surface 0.55, in the across-environment analysis, indicating the prominent role of environment, and genotype x environment interaction, in expression of these traits.

Plate 11: Life cycle of the sorghum shoot fly, *Atherigona soccata*

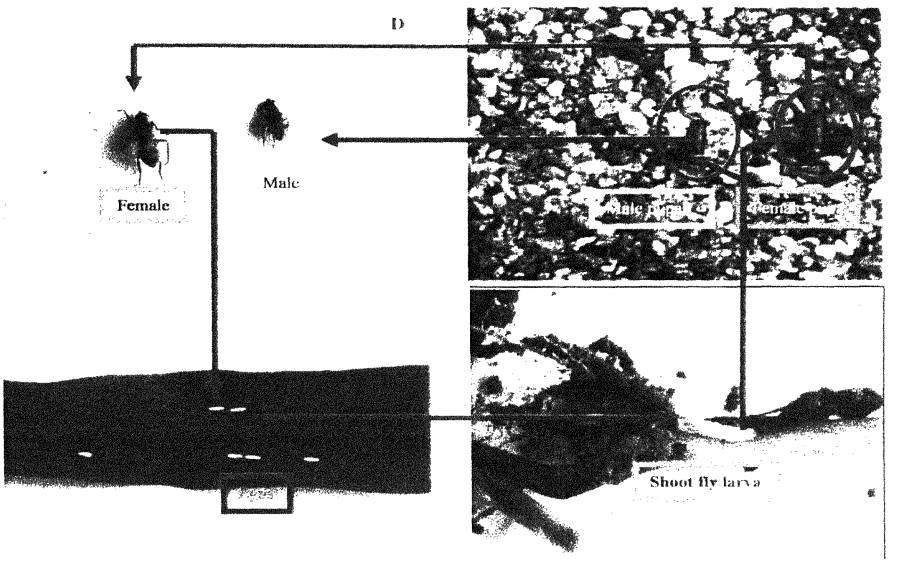


Plate 1. Various life stages of the sorghum shoot fly, *Atherigona soccata*. A = Eggs, B = Larva, C = Pupae, D = Adults

The current project work was initiated with the objective of introgressing four consensus QTLs, for shoot fly resistance component traits from donor parent IS 18551, identified on linkage groups SBI-01, SBI-07, SBI-10, and SBI-05 with flanking markers pairs, *Xtxp37* and *Xtxp75*; *Xtxp159* and *Xtxp40*; *Xtxp141* and *Xgap1*; and *Xtxp65*, *Xtxp94*, and *Xtxp15*, respectively, from the combined QTL mapping studies of Gowri Sajjanar (2002) and Santosh Deshpande (2005). BC_1F_1 to BC_4F_3 generations were grown and the DNA of parents and segregating progenies was extracted on a single-plant basis by CTAB-miniprep (Mace *et al.*, 2003). Each generation, progeny were analyzed with microsatellite markers flanking the target shoot fly resistance QTLs (identified in earlier studies as mentioned above). Individuals heterozygous for donor alleles at the above-listed foreground SSR marker loci immediately flanking a particular shoot fly QTL were advanced by further selfing and backcrossing. The SSR markers used were highly polymorphic and co-dominantly inherited. Background screening was performed in the BC_2F_1 generation seeking recurrent parent alleles at more distant flanking markers surrounding the foreground loci (among individuals heterozygous for donor alleles at the foreground loci). Individual segregants heterozygous or homozygous for recurrent parent alleles at these background marker loci

surrounding the target QTL region were selected and advanced further. Secondly, in the BC_3F_1 generation, background screening was performed on the target QTL linkage groups with all available markers on that linkage group. Individual segregants having shorter donor introgressions (but containing donor alleles in the QTL target region) were expected to show less linkage drag of undesirable traits from the agronomically poor donor parent, so were selected and then further background screened extensively on non-target linkage groups with three primers on each linkage group to further reduce the sample number to be advanced and while reducing the level of undesirable contributions from the donor parent. When there were small numbers of choices to select from, such segregants were advanced right a way without further background screening. BC_4F_1 and BC_4F_2 generations were advanced by selfing to produce paired near-isogenic families homozygous for individual QTL introgressions [that is homozygous for donor alleles ('plus-QTL' isoline) or recurrent parent alleles ('minus-QTL: isoline)] for field evaluation. A final round of background screening was performed on the BC_4F_1 segregants, to determine the amount of donor parent genome remaining.

3.1 MAS as a potential tool for genetic improvement

Marker-assisted selection (MAS) is a complementary technology, for use in conjunction with more established conventional methods of genetic selection for plant improvement. Molecular markers located far from the target gene, increase the probability of recombination taking place between them resulting in false positive marker-gene associations and thus reducing the efficiency of MAS. Therefore it is very important to opt for tight marker-gene linkage to minimize losses through recombination. MAS is best used in applied breeding programs when there is very tight linkage between the markers and the gene or genomic region of interest, to avoid wasting precious resources. Single-gene controlled traits had received most attention, but little progress had been made with multiple-gene traits. Many MAS studies had adopted a single trait approach, pointing out that with a multi-trait breeding objective, response for one trait often goes at the expense of another.

MAS is typically recommended when conventional approaches to selection are difficult or impossible, for example for traits where it is difficult to get good quality phenotypic data on a regular basis. Introgression of genes from wild or unadapted sources into elite cultivated germplasm has also been proposed to be a good use of MAS. Traits that are highly influenced by the environment or

production system, including crop yield, have not been considered easily amenable to MAS. A major problem associated with MAS has been lack of polymorphism at the DNA level between the trait donor and elite germplasm that is to be improved for the target trait--this would render a trait not amenable to MAS as a result of inadequate coverage of the genetic map with polymorphic molecular markers. Nature of the trait should also be considered for MAS to be efficient: single versus multi-gene, additive versus dominant inheritance, expressivity and penetrance.

3.2 *Kharif* field evaluation (2006) of NILs (BTx623 recurrent parent background)

The test material consisted of 64 entries, primarily of BTx623-background introgression materials. These were assessed in six replications laid out in an alpha-lattice design (8x8) sown on the second week of July 2006 at ICRISAT-Patancheru. Each entry was sown in a single-row plot of 2 meter length with inter-row spacing of 0.75 meter, 1 meter path between plots within rows, and plant to plant distance within plots maintained at 10 cm. Observations were noted on plant characters such as glossiness, seedling vigor, oviposition I, oviposition II, deadhearts I and deadhearts II. The field data were subjected to general t-paired tests to assess statistical significance of differences between groups of entry means.

3.3 *Rabi* field evaluation (2006) of NILs (both recurrent backgrounds, BTx623 and 296B)

The *rabi* evaluation of shoot fly introgression near isogenic lines was conducted at ICRISAT-Patancheru, with sowings in the first week of November 2006. These *rabi* trials consisted two sets of materials, one set was that used in the kharif screen described above, but with some 20 additional entries. There were six replications laid out in 9x9 alpha lattice design for these BTx623-background introgression materials. The second set was comprised of 110 entries, primarily of 296B-background near-isogenic introgression lines. These were assessed in an 11x10 alpha lattice design. Each genotype was sown in single-row plots of 2 m length with inter-row spacing of 0.75 meter, 1 meter path between plots within rows, and plant-to-plant spacing within plots of 10 cm. All other required conditions were maintained at the same levels used in the *kharif* 2006 screening environment. Data were recorded on glossiness, seedling vigor I, seedling vigor II, oviposition II, deadhearts I, deadhearts II, and deadhearts III for the BTx623-background trial set. For the 296B-background trial set glossiness, seedling vigor

I, seedling vigor II, oviposition II, deadhearts I, deadhearts II, and deadhearts III were recorded on all plots. The field data were subjected to general t-paired tests to assess statistical significance of differences between groups of entry means.

3.4 Kharif field evaluation (2007) of NILs (both recurrent backgrounds, BTx623 and 296B)

The second *kharif* season field evaluation of shoot fly QTL introgression near-isogenic lines in this study was conducted at ICRISAT-Patancheru, with sowings in the first week of August 2007. The experimental materials used were nearly two identical sets of materials used in earlier experiments. BTx623-background introgession lines (64 entries) were evaluated in 2-row plots of 4 meters with 4 replications laid out in an 8x8 alpha-lattice design. The 296B-background introgression material was subdivided into an 84-entry set with 2 replications in single-row plots of 2 meter length arranged in a 12x7 alpha-lattice design, and a second set of 20 entries with 3 replications in 2 row plots of 4 meter length arranged in a 5x4 alpha-lattice design. All other field conditions were maintained at the same levels as that of 2006 *kharif* season trial. Data on glossiness, number of eggs per 100 plant (from a single replication) and numbers of plants with eggs (oviposition) 14 days after seedling emergence, and plants with deadhearts on 14 and 21 days after emergence were recorded on all plots in the BTx623-background trial material. For the 84-entry 296B-background trial set, plant glossiness, number of eggs per 100 plants, number of plants with eggs (oviposition) on 14 DAE, and number of plants with deadhearts on 14 and 21 DAE were noted. For the 20-entry 296B-background trial set, glossiness, number of eggs per 100 plants on 14 DAE, number of plants with eggs (oviposition) on 14 and 21 DAE, and number of plants with deadhearts on 14 and 21 DAE were recorded. The data on number of eggs was expressed as number of eggs per 100 plants, and plants with eggs and deadhearts in terms of percentage of the total number of plants in the plot. The field data were subjected to general t-paired tests to assess statistical significance of differences between groups of entry means.

3.5 Performance of parents and checks

Highly significant differences were obtained between pairs of recurrent and donor parents BTx623 and IS 18551, and 296B and IS 18551 in each of the screening environments for almost all the shoot fly resistance parameters observed. Thus, seedlings of donor parent IS 18551 were more glossy but initially less vigorous,

with low oviposition and low deadheart formation in comparison to BTx623 and 296B recurrent parent lines. Highly resistant check, IS 2312 and moderately resistant check IS 1054 were both highly glossy in all three screening environments. The susceptible check Swarna was non-glossy in all three environments. The highly resistant check had significantly lower numbers of eggs laid and deadhearts formed than moderately resistant check, which in turn had lower oviposition and deadheart formation than the highly susceptible check. Thus there was some amount of variation in performance between the highly resistant check and moderately resistant check in terms of glossiness, seedling vigor, oviposition and deadhearts incidence. Further, multiple QTL recombinant inbred line (RIL) checks *viz.*, RIL 153, RIL 189, and RIL 252, were highly glossy in all three screening environments and exhibited resistance (compared to their susceptible parent BTx623) for almost all of the observed resistance parameters. These RIL checks were significantly better than BTx623 for seedling vigor as well as more direct measures of resistance to shoot fly.

Shoot fly resistance is mainly the result of combined effect of seedling traits like glossiness intensity, seedling vigor and trichome density (Omori *et al.*, 1983). Deadhearts incidence (%) was negatively correlated with glossiness score (Jadhav *et al.*, 1986; Sajjanar, 2002), seedling vigor score (Sharma *et al.*, 1977, Jadhav *et al.*, 1986; Sajjanar, 2002), trichome length (Jadhav *et al.*, 1986), trichome density (Halalli *et al.*, 1982; Jadhav *et al.*, 1986; Sajjanar, 2002) and number of effective tillers (Sharma *et al.*, 1977). Oviposition was previously found to be negatively correlated with glossiness score, seedling vigor score (Sajjanar, 2002) and trichome density (Halalli *et al.*, 1982; Karanjkar *et al.*, 1992; Sajjanar, 2002). Deadhearts incidence and oviposition recorded a significant positive correlation (Sharma *et al.*, 1977; Halalli *et al.*, 1982; Sajjanar, 2002). While correlation between plant recovery and oviposition was observed to be negative (Halalli *et al.*, 1982), positive correlation was observed between plant recovery and trichome density. The observations for field trial performance of the parental lines and check entries in the current study are generally in agreement with these prior studies of sorghum shoot fly resistance and its component traits.

3.6 Glossiness as a component trait of shoot fly resistance

3.6.1 Glossiness factor for 'Plus-G' introgression line

The 'plus-G' introgression line (a single line, only available in the BTx623-background) experienced lower levels of shoot fly infestation in comparison to all

other introgression lines. Compared to its recurrent parent BTx623 (as no 'minus-G' isoline was available for testing), the seedling leaves of this line were highly significantly more glossy in all three screening environments and were densely trichomed. Maiti and Bidinger (1979) observed higher levels of resistance to shoot fly when both glossiness and trichomes occurred together than when these traits were available individually. The glossy trait alone (mean of 71% deadhearts) seemed to be more effective than trichomes alone (84% deadhearts) in reducing deadheart formation. The combination of both the characters, however (61% deadhearts) was significantly superior to the means of either of the two traits taken singly. Similarly, Agarwal and House (1982) reported that the level of resistance was greater when both the glossy and trichome traits occurred together. Two component characters, *viz.*, trichome intensity (abaxial surface) and glossiness intensity showed negative significant correlations with shoot fly resistance, ($-0.73<r<-0.82$) and ($-0.81<r<-0.94$), respectively (Omori *et al.*, 1983), which suggested that glossiness was more important than trichomes for shoot fly resistance in sorghum. However, in most cases glossiness and trichomes exist together (Omori *et al.*, 1983) and there was a highly significant positive correlation between glossiness intensity and trichome intensity ($0.85<r<0.83$). Further, shoot fly egg laying was highly significantly and negatively associated with trichomes ($-0.697<r<-0.752$) and glossiness ($-0.747<r<-0.825$) indicating that these traits are deterring ovipositional preference of the shoot in sorghum varieties (Omori *et al.*, 1983). Although the correlation of glossiness intensity and trichome intensity is high, these don't play any direct role in building up the total variability in shoot fly resistance (Omori *et al.*, 1983). In the current study, the superior field performance for direct measures of shoot fly resistance (*i.e.*, oviposition and deadhearts incidence) of the 'plus-G' isoline was consistent with the earlier studies mentioned above, confirming that the combination of higher levels of glossiness and trichome density contributed by the target genomic region on linkage group G (SBI-10) make this the best single target for marker-assisted or conventional trait-based phenotypic selection aimed at improving shoot fly resistance using donor parent IS 18551.

3.6.2 Glossiness factor for 'plus-J1' and 'plus-J' introgression lines

The 'plus-J1' and 'plus-J' introgression lines showed highly significantly increased degree of glossiness (*i.e.*, lower glossiness scores) compared to their near-isogenic counterparts (and their recurrent parents) in both BTx623 and 296B genetic backgrounds in all field trials conducted in this study. A major gene for

glossiness appears to be associated within the J1 region as this character is detected for both 'plus-J' and 'plus-J1' lines. This is consistent with QTL mapping results reported for this trait by Sajjanar (2002) and Deshpande (2005). This is further supported by performance of the BTx623-background introgression lines '+J1-J2?' (BBBA) and '-J1+J2?' (AAAB) and the 296B-background introgression lines '+J1' (BBA) and '+J2?' (AAB), which showed highly significant differences for the glossiness trait in the *rabi* 2006/07 screen. Further, 296B-background introgression lines '+J1?-J2?' (BAA) and '-J1?J2?' (AAB) differed highly significantly for glossiness, suggesting that the glossiness gene is located in the vicinity of *Xisp258*, above *Xtxp65* at the top of SBI-05. Thus, the marker-assisted backcrossing program for the major glossiness QTL on linkage groups J (SBI-05) has permitted identification of a more closely linked marker, meeting the fine-mapping objective of this thesis research program.

3.7 Oviposition non - preference

3.7.1 Contribution of the 'plus-G' allele to reducing oviposition and deadhearts

The 'plus-G' introgression line was highly significantly better than its BTx623 recurrent parent for oviposition II, deadhearts II and significantly better for oviposition I, eggs per 100 plants I, eggs per 100 plants II, and deadhearts I in the *kharif* 2006 field screen (Table 57). Thus, this QTL introgression line showed improved shoot fly resistance by all of the direct measures of this trait screened in this first of screening environment, in which pest pressure on the test materials was nearly optimum for discrimination of resistance and susceptibility to shoot fly. In the *rabi* 2006-07 field screen (Table 58), this 'plus-G' isoline was highly significantly better than BTx623 for eggs per 100 plants I and eggs per 100 plants II, and was significantly better for oviposition I, deadhearts II, and deadhearts III. In this testing environment also, conditions were favorable for discrimination of shoot fly resistant and susceptible material and the 'plus G' QTL introgression line clearly expressed an improved level of resistance compared to its recurrent parent. Finally, in the *kharif* 2007 field screen (Table 60), the 'plus-G' QTL introgression line was highly significantly better than its recurrent parent for oviposition I and deadhearts I, and significantly better for deadhearts II. Only in case of eggs per 100 plants, was the difference between the 'plus G' isoline and BTx623 not significant. This non-significant difference can perhaps be explained by the higher level of insect pressure observed in this screening environment. Overall, the results of these three screens validate existence of the major shoot

fly resistance from donor parent IS 18551, associated with glossiness score and trichome density, that was previously reported (Sajjanar, 2002) on sorghum linkage group G (SBI-10).

3.7.2 Contribution of the 'plus-J1' and 'plus-J' alleles to reducing oviposition and deadhearts

3.7.2.1.1 BTx623 – background near-isogenic lines 'plus-J1' and 'minus-J1'

- In the *kharif* 2006 field screen (Table 57), the 'plus-J1' QTL introgression lines were highly significantly better than their near-isogenic 'minus-J1' counterparts for level of shoot fly infestation as indicated by oviposition I, eggs per 100 plants I, deadhearts I, and deadhearts II, and significantly better for oviposition II. Thus the 'plus-J1' introgression lines showed improved shoot fly resistance by nearly all of the direct measurements of this trait in this near-optimal screening environment.
- In the *rabi* 2006-07 field screen (Table 58), the 'plus-J1' QTL introgression lines were highly significantly better than their 'minus-J1' near-isogenic counterparts for deadhearts II and deadhearts III, and significantly better for oviposition I, eggs per 100 plants II, and deadhearts I. Differences between these groups of near-isogenic lines were non-significant for eggs per 100 plants I and oviposition II, but numerically favored the 'plus-J1' isolines. Thus in this testing environment also, the 'plus-J1' QTL introgression line clearly expressed an improved level of resistance compared to its near-isogenic 'minus-J1' counterpart.
- In the *rabi* 2006-07 field screen (Table 58), the '+J1-J2?' (BBBA) QTL introgression lines were highly significantly better than than the recurrent parent BTx623 or the minus '-J1+J2?'(AAAB) introgression line for eggs per 100 plants I, and significantly better for oviposition I and deadhearts I. Differences were non-significant, but numerically favorable for eggs per 100 plants II, deadhearts II and deadhearts III. While not as clear as in the comparison of the 'plus-J1' and 'minus-J1' isolines in this environment, the '+J1-J2?' isoline also expressed improved shoot fly resistance compared to recurrent parent BTx623 for most of the direct measurements of this trait.
- In the *kharif* 2007 field screen (Table 60), the 'plus-J1' QTL introgression lines in BTx623-background were highly significantly better than their

near-isogenic 'minus-J1' counterparts for deadhearts I and significantly better for oviposition I and deadhearts I.

Overall, the results of these three screens validate the existence of a major shoot fly resistance QTL from donor parent IS 18551, associated with glossiness score, that was previously reported (Sajjanar, 2002; Deshpande 2005) at the top of sorghum linkage group J (SBI-5).

3.7.2.1.2 296B - background near-isogenic lines 'plus-J1' and 'minus-J1'

- In the *rabi* 2006-07 field screen (Table 59), the 296B-background 'plus-J1' QTL introgression lines were highly significantly better than their 'minus-J1' near-isogenic counterparts for oviposition II, deadhearts II, and deadhearts III, and significantly better for eggs per 100 plants II and dead hearts I. Thus the 'plus-J1' introgression lines in 296B-background showed improved shoot fly resistance by nearly all of the direct measurements of this trait in this screening environment, which was favorable for discriminating between shoot fly resistant and shoot fly susceptible genotypes.
- Similarly, in the *rabi* 2006-07 field screen (Table 59), the 296B-background '+J1?-J2' (BAA) QTL introgression lines were highly significantly better than recurrent parent 296B or the 'minus-J1' introgression line for deadhearts I, and significantly better for deadhearts II and deadhearts III. Differences were non-significant, but numerically favorable for oviposition II and eggs per 100 plants II. Thus the '+J1?-J2' introgression lines in 296B-background showed improved shoot fly resistance by many of the direct measurements of this trait in this screening environment.
- Further, in the *rabi* 2006-07 field screen (Table 59), the 296B-background '+J1' (BBA) QTL introgression line was significantly better than recurrent parent 296B or the 'minus-J1' introgression lines for eggs per 100 plants II, deadhearts II, and dead hearts III. Here too, the '+J1' (BBA) QTL introgression line in 296B-background showed improved shoot fly resistance by several direct measurements of this trait.
- In the 20-entry *kharif* 2007 field trial (Table 61), the 296B-background 'plus-J1' QTL introgression lines were highly significantly better than their near-isogenic 'minus-J' counterparts for oviposition II. The high level of insect pressure in this environment, probably contributed to this failure to

detect significant differences in any direct measure of shoot fly resistance between these sets of near-isogenic lines.

- No significant differences were detected between the 'plus-J1' and 'minus-J1' isolines in 296B-background in the 84-entry *kharif* 2007 field trial (Table 62). The smaller plot size and reduced replication number of this trial, combined with the high level of insect pressure in this environment, probably contributed to this failure to detect significant differences in any direct measure of shoot fly resistance between these sets of near-isogenic lines.
- Overall, the results of two of the three screening environments of 296B-background introgression lines (*i.e.*, the *kharif* 2006 and *rabi* 2006-07 screening environments, but not those of *kharif* 2007) validate the existence of a major shoot fly resistance from donor parent IS 18551, associated with glossiness score, that was previously reported (Sajjanar, 2002; Deshpande 2005) at the top of sorghum linkage group J (SBI-5).

3.7.2.2.1 BTx623 - background near-isogenic lines 'plus-J' and 'minus-J'

- In the *kharif* 2006 field screen (Table 57), the 'plus-J' introgression lines were highly significantly better than their near-isogenic 'minus-J' counterparts for level of shoot fly infestation as indicated by oviposition I, eggs per 100 plants I and deadhearts II; and significantly better for oviposition II and deadhearts I. Non-significant, but numerically favorable differences were also observed for eggs per 100 plants II. Thus the 'plus-J' introgression lines showed improved shoot fly resistance by nearly all of the direct measurements of this trait in this near-optimal screening environment.
- In the *rabi* 2006-07 field screen (Table 58), the 'plus-J' introgression lines were highly significantly better than their near-isogenic 'minus-J' counterparts for oviposition I, eggs per 100 plants I, eggs per 100 plants II, deadhearts I, deadhearts II and deadhearts III. Thus in this favorable screening environment the 'plus-J' introgression lines showed improved shoot fly resistance by nearly all observed direct measurements.
- In the *kharif* 2007 field screen (Table 60), the 'plus-J' QTL introgression lines in BTx623-background were highly significantly better than their near-isogenic 'minus-J1' counterparts for deadhearts II and significantly better for deadhearts I.

- The results of these three field screens validate the existence of one or more major shoot fly resistance QTLs from donor parent IS 18551, that were previously reported (Sajjanar, 2002; Deshpande 2005) on sorghum linkage group J (SBI-5).

3.7.2.2.2 296B – background near-isogenic lines 'plus-J' and 'minus-J'

- In the *rabi* 2006-07 field screen (Table 59), the 296B-background 'plus-J' QTL introgression lines were highly significantly better than their 'minus-J' near-isogenic counterparts for deadhearts II, and significantly better for deadhearts I and deadhearts III. Further, although non significant, the observed differences between means of these near-isogenic lines for oviposition II and eggs per 100 plants II favored the 'plus-J' QTL introgression lines. Thus the 'plus-J' introgression lines in 296B-background showed improved shoot fly resistance several direct measurements of this trait in this favorable screening environment.

Ovipositional non-preference is reported to be a primary mechanism of shoot fly resistance (Blum, 1967; Krishnanada *et al.*, 1970; Rangadang *et al.*, 1970; Jotwani *et al.*, 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, 1984; Rana *et al.*, 1984; Unnithan and Reddy, 1985). However, the efficiency of the ovipositional non-preference mechanism of shoot fly resistance is not stable and it is ineffective at heavy levels of shoot fly pressure (Singh and Jotwani, 1980a; Borikar *et al.*, 1982a and Sharma *et al.*, 1997a). The results obtained in the present study from the favorable screening environments in *kharif* 2006 and *rabi* 2006-07, and the less favorable (due to higher levels of shoot fly pressure) screening environment of *kharif* 2007, are compatible with these previous reports that the non-preference mechanism can be rendered ineffective when shoot fly pressure is very high.

3.8 Trichomes as a component of resistance to shoot fly larval penetration into the whorl ('plus-G' contribution to trichome density)

Recurrent parents BTx623 and 296B lacked trichomes whereas the shoot fly resistance donor parent IS 18551 showed trichome densities of 145.2 (adaxial) and 131.1 (abaxial) per microscopic field under 10X magnification in *kharif* season 2006 observations (Table 63). In *rabi* season observations (Table 64), IS 18551 showed similar trichome densities as in the *kharif* season, *viz.* 145.9

(adaxial) and 141.6 (abaxial). In the *kharif* 2006 season field screen, the BTx623-background near-isogenic line 'plus-G' recorded trichome density values of 146.1 (adaxial) and 129.8 (abaxial), comparable to those of the donor parent. Further, in the *rabi* 2006-07 screening environment, this QTL introgression line recorded trichome density values greater than those of its donor parent: 199.2 (adaxial) and 203.4 (abaxial) suggesting the strong environmental influence, and potential for genotype x environment interaction, on this trait. Thus the genetic control of trichome density from donor parent IS 18551 appears to be largely confined to linkage group SBI-10, in the region flanked by SSR marker loci *Xtxp141* and *Xgap1*, in agreement with the QTL mapping results of Sajjanar (2002) and Folkertsma *et al.* (2004). Near-isogenic QTL introgression lines 'plus-A', 'plus-E' and 'plus-J' had no trichomes in either of these two screening environments (not shown). RIL 252 recorded the highest trichome density among BTx623-background near-isogenic lines, RILs, parents, controls and checks in the field trials conducted in these two seasons. Trichomes were absent on seedling leaf blades of RIL 189. Among the check entries, highly resistant check IS 2312 had more trichomes followed by moderately resistant check IS 1054, whereas highly susceptible check Swarna had very few if any trichomes. In general, trichome density was moderately greater in the *rabi* season. Maiti and Gibson (1983) also indicated that expression of trichomes is comparatively lower in *kharif* than in *rabi*. Further, they reported that in *kharif* season, trichome density on the upper leaf surface was lower than that on the lower leaf surface. Whereas, in *rabi* season, both surfaces bore similar trichome densities.

The role of trichomes as a deterring factor was suggested by Maiti and Bidinger (1979). Total egg count per plant was negatively correlated (-0.28) with number of trichomes per unit leaf area (-0.19) (Halalli *et al.*, 1982). Trichomes reportedly have a high correlation with ovipositional non-preference (r_g = -0.75, r_p = -0.63) and shoot fly damage (r_g = -0.78, r_p = -0.72) (Agarwal and Abraham, 1984). Positive correlation between trichome density and resistance to shoot fly was also observed by Omori *et al.* (1983) and Patel and Sukhani (1990). Highly significant negative correlation between trichome intensity and shoot fly infestation (deadhearts formation) was observed by Karanjkar *et al.* (1992). Trichomes don't play any direct role in reducing deadhearts incidence, but help indirectly in reducing oviposition (Karanjkar *et al.*, 1992). Trichomes may be less effective as a deterrent to shoot fly infestation during the *kharif* season than in the *rabi* season (Maiti and Gibson, 1983). Observations on trichome density variation in the present study were generally in agreement with these earlier reports.

Table 63. *Kharif* 2006 trichome count

Lines	Adaxial Trichomes/ Microscopic Field	SE(±)	Abaxial Trichomes/ Microscopic Field	SE(±)
Isoline 'plus-G'	129.8	9.0	146.1	9.3
296B	0.0	0.0	0.0	0.0
BTx623	0.0	0.0	0.0	0.0
IS 18551	131.1	3.3	145.2	3.9
Swarna	0.0	0.0	0.0	0.0
IS 1054	20.7	6.0	36.5	9.1
IS 2312	44.1	3.8	87.3	3.5
RIL 252	149.6	4.6	168.7	3.1
RIL 153	32.3	3.4	36.0	2.3
RIL 189	0.0	0.0	0.0	0.0

Table 64 *Rabi* 2006-07 trichome count

Lines	Adaxial Trichomes/ Microscopic Field	SE(±)	Abaxial Trichomes/ Microscopic Field	SE(±)
Isoline 'plus-G'	199.2	3.2	203.4	3.8
296B	0.0	0.0	0.0	0.0
BTx623	0.0	0.0	0.0	0.0
IS 18551	145.9	4.1	141.6	3.5
Swarna	2.7	0.4	1.3	0.2
IS 1054	109.5	2.0	105.4	2.4
IS 2312	151.6	5.6	143.6	5.1
RIL 252	223.6	12.2	213.6	8.7
RIL 153	26.5	2.4	25.8	3.3
RIL 189	0.0	0.0	0.0	0.0

3.9 Role of seedling vigor in shoot fly resistance

Jadhav *et al.* (1986) studied morphological plant characters in 158 sorghum entries for shoot fly interaction measured in terms of deadhearts and concluded that apart from glossy trait and presence of trichomes, an initially faster plant growth rate contributes to shoot fly resistance in sorghum. There was a highly significant and positive correlation between height of the plant (r=-0.56**) and initial faster plant growth (r=-0.41*) with the percentage of deadhearts caused by shoot fly (Jadhav *et al.*, 1986). Fast seedling growth might prevent the first instar larva from reaching the growing tip although leaf margins may be cut without causing deadheart symptoms. Studies by Kurana and Verma (1985) indicated a positive correlation between plant height and resistance to shoot fly. Leaf trichome density and plant height showed significant negative correlations with shoot fly deadhearts. Hence it has been suggested that trichome density and

seedling vigor can be used as selection criteria for shoot fly resistance (Karanjkar *et al.*, 1992).

3.9.1 Contribution of the 'plus-G' allele to seedling vigor

In *kharif* 2006, the 'plus-G' QTL introgression line was significantly better than its BTx623 recurrent parent for seedling vigor, and the introgression line was significantly more resistant to shoot fly infestation (Table 57) suggesting that greater seedling vigor in combination with glossiness and trichomes could reduce the shoot fly infestation. An alternative explanation is that the better seedling vigor score of this introgression line was a result of its lower degree of shoot fly infestation. In *rabi* 2006-07, seedling vigor score I of the 'plus G' introgression line was numerically less (higher vigor) than its recurrent parent although the difference was not significant, whereas the difference for seedling vigor score II was highly significant (Table 58).

3.9.2 'Plus-J' allele contribution to seedling vigor

In the *kharif* 2006 field screen (Table 57), no significant differences in seedling vigor were noted between the 'plus-J1' and 'plus-J' QTL introgression lines in BTx623-background and their near-isogenic counterparts, despite the detection of significantly better seedling vigor score for donor parent IS 18551 than for recurrent parent BTx623. However, in the *rabi* 2006-07 field screen (Table 58) it was observed that recurrent parent alleles (BTx623) in the region *Xisp258* to *Xtxp94* (J1) appeared to code for seedling vigor genes. 'Minus-J1' and 'minus-J' QTL introgression line plants were significantly more vigorous than their 'plus-J1' and 'plus-J' introgression line counterparts having donor parent alleles in this genomic region, but only for the initial seedling vigor observation (seedling vigor I). Similar significant differences in seedling vigor I were observed between donor parent IS 18551 and BTx623. However, by the time of the second observation of seedling vigor in this field screen, these differences had reversed direction and higher seedling vigor II was associated with markedly lower deadhearts incidence for the 'plus-J' QTL introgression line, as well as donor parent IS 18551. Moreover, in this same field screen, the '-J1+J2?' (AAAB) introgression lines were highly significantly more vigorous than the '+J1-J2?' (BBBA) introgression lines for both seedling vigor I and seedling vigor II suggesting the fact that the genes coding for seedling vigor II QTL is associated in *Xtxp15* region (combined with

glossiness QTL in the *Xisp258-Xtxp94* region) explained the variation in reduced shoot fly damage to the 'plus-J' introgression line.

Likewise, 296B alleles in the region from *Xisp258* to *Xtxp65* appeared to be associated with better initial seedling vigor as the 'minus-J1' isolines in 296B-background had a significantly lower mean seedling vigor score I than its 'plus-J1' counterparts (Table 59). However, by the time of the second seedling vigor observation the differences between these sets of near-isogenic lines were no longer significant. Further, the differences between the 'plus-J' and 'minus-J' isolines in 296B-background were not significant in this screening environment and the shoot fly resistance donor IS 18551 expressed significantly better seedling vigor II than recurrent parent 296B. In all of the cases in this field screen, the 296B-background lines homozygous for donor parent alleles in the *Xisp258* to *Xtxp65* region had significantly lower deadhearts incidence I, II and III than their counterparts homozygous for recurrent parent alleles. Thus, the results from the *rabi* 2006-07 field screen are not consistent with a direct role of seedling vigor in shoot fly resistance.

Likewise, (Khurana and Verma, 1985) suggested that faster growing plants remain in the favorable height (susceptible stage) for a relatively shorter period than the slower growing susceptible plants. Singh and Jotwani (1980d) indicated that longer and narrow leaves and faster seedling growth as indicated by length of leaf sheath (8.4 cm in CSH 1 compared to 12.4 cm in IS5469) and seedling height (29.1 cm in CSH1 compared to 39.3 cm) coupled with some hardness of the leaf sheaths may be contributing towards the resistance to shoot fly. Blum (1972) too found that shoot fly resistant sorghum lines grew faster than susceptible ones. Finally, Deshpande (2005) reported significant positive correlation between glossiness intensity and seedling vigor I and II in both *kharif* and rabi screening environments (except for seedling vigor I in *rabi*). Strong positive association between glossiness intensity and seedling vigor was also reported by Sharma *et al.* (1997) and Borikar *et al.* (1981b).

4.1 Effects of minor QTLs for shoot fly resistance

4.1.1. Effects of the 'plus-A' allele

In the *kharif* 2006 field screen, the 'plus-A' QTL introgression lines in BTx623 background were significantly better than their 'minus-A' near-isogenic counterparts for glossiness and highly significantly better for seedling vigor (Table 57). However, in contrast to expectations, the 'minus-A' line had significantly

better mean values for oviposition I, and eggs per 100 plants I and II. Further, differences for other observed measures of shoot fly resistance were non-significant between these near-isogenic materials, but numerically favored the 'minus-A' lines. In the *rabi* 2006-07 field screens (Tables 58), differences between means of the BTx623-background 'plus-A' and 'minus-A' near-isogenic lines were non-significant for all observed traits. Results for the 296B-background plus-A' and 'minus-A' near-isogenic lines were also non-significant for all observed traits in this screening environment (Table 59). These results indicate that the putative shoot fly resistance QTL on sorghum linkage group A (SBI-01) makes little if any contribution by itself under conditions that are favorable for assessment of differences in shoot fly resistance. Further, under the more severe conditions of the *kharif* 2007 field screens (Tables 60-62), there were no significant differences observed between near-isogenic 'plus-A' and 'minus-A' lines in either BTx623 or 296B backgrounds. Thus the current study has failed to validate the existence alleles from donor parent IS 18551 that contribute to shoot fly resistance in the genomic region flanked by SSR markers *Xtxp37* and *Xtxp75* on linkage group A (SBI-10).

4.1.2 Effects of the 'plus-E' allele

In the *kharif* 2006 field screen of BTx623-background materials, the 'plus-E' isolines were highly significantly better than their 'minus-E' counterparts for mean seedling vigor score. However, the 'minus-E' isolines were significantly better than their 'plus-E' counterparts for most direct measures of shoot fly resistance in this screening environment (Table 57). In the *rabi* 2006-07 field screen, the BTx623-background 'plus-E' near-isogenic lines were highly significantly better than their 'minus-E' counterpart for seedling vigor I and seedling vigor II, and significantly for dead hearts III; whereas the 'minus-E' lines were significantly better for eggs per 100 plants I and deadhearts incidence I (Table 58). Similarly, the rabi 2006-07 screen of 296B-background 'plus-E' lines were significantly better than their near-isogenic 'minus-E' counterparts for seedling vigor I, but differences better these materials for other observed traits were non significant. Further, under the more severe conditions of the *kharif* 2007 field screens (Tables 60-62), there were no significant differences observed between near-isogenic 'plus-E' and 'minus-E' lines in either BTx623 or 296B backgrounds. Thus the current study has failed to validate the existence alleles from donor parent IS 18551 that contribute to shoot fly resistance in the genomic region flanked by SSR markers *Xtxp40* and *Xtxp159* on linkage group E (SBI-07).

Although the 'plus-A' and 'plus-E' introgression lines were often more vigorous than their near-isogenic counterparts, these putative shoot fly introgression lines generally did not have lower shoot fly infestation levels or shoot fly damage than their near-isogenic recurrent parent allele counterparts in the field trials conducted in the present study. These unexpected results can be explained in two ways. First, double crossovers between the widely spaced flanking markers *Xtxp37* and *Xtxp75* for the putative QTL on linkage group A (SBI-01), and *Xtxp159* and *Xtxp40* for that on linkage group E (SBI-07) might have resulted in the loss of the IS 18551 alleles at these QTLs in the course of the marker-assisted backcrossing program. An alternative explanation is that favorable alleles from donor parent IS 18551 at these QTLs have small effects that are easily overcome if they are deployed in isolation in otherwise shoot fly susceptible genetic backgrounds.

Regardless of the cause, the results from this study fail to support the existence of shoot fly resistance QTLs on linkage groups A (SBI-01) and E (SBI-07) of donor parent IS 18551 that could be recommended as targets for applied marker-assisted selection to improve shoot fly resistance. If in fact there are favorable alleles from IS 18551 in these genomic regions, it appears that they have minor effects that are readily overcome under moderate to high levels of shoot fly infection if they are deployed in isolation in otherwise shoot fly susceptible genetic backgrounds.

4.2. Inheritance of factors involved in shootfly 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 progenies were evaluated under three levels of shoot fly infestation. From the data of F_2 progenies it was indicated that resistance was partially dominant when evaluated under low shoot fly population pressure. Under high shoot fly population, susceptibility appeared to be dominant. Balakotaiah *et al.* (1975) observed exotics to be the most susceptible. The Indian parents and their derivatives being the least susceptible. Amongst F_2 progenies, exotic x exotic crosses had the highest mortality counts followed by exotic x derivative, exotic x Indian, derived x derived, derived x Indian and Indian x Indian. The characteristic way in which the mortalities gradually decreased from 65 to 23% in that order further confirmed that resistance was due to gradual accumulation of desirable alleles rather than due to one or two major genes. Rana *et al.* (1981) studied the

behavior of shoot fly resistance over the F_1, F_2, F_3 and advance generations. The F_1 was almost intermediate between the parents with an added heterotic advantage of lower deadheart percentage. Resistance showed partial dominance under low to moderate shoot fly population but this may shift under heavy shoot fly infestation. They concluded that resistance is polygenic in nature and governed by additive genes. Halalli *et al.* (1983) evaluated advance generations to estimate the extent of variability, heritability and genetic advance for shoot fly resistance. Five (BC) F_3 progenies, one F_3 progeny and 3 F_4 progenies were found to be more resistant than the highly resistant parent, IS 1084, suggesting transgressive inheritance of the character.

4.2.1 Inheritance of glossiness

Deshpande (2005) observed in his RIL population, a continuous distribution from high intensity of glossiness (score 1) to non-glossiness (score 5) with an apparent valley between scores 3 to 3.5 in frequency distribution graph that suggested the involvement of major genes controlling glossiness. The presence of two major QTLs (on SBI-10 and SBI-05) that largely control glossiness from donor parent IS 18551 indicates that glossiness is inherited oligogenically. According to Menendez and Hall (1995), the absence of discrete segregating classes for a trait suggested 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. Tarumato (1980) indicated that the presence of glossiness is controlled by a single recessive gene. However its intensity is quantitatively governed and is controlled by both additive and non additive genes (Agarwal and Abraham, 1984). The results of the current study, which confirmed the existence of QTLs of large effect for glossiness score on SBI-05 and SBI-10, are in agreement with the prior QTL mapping studies of Sajjanar (2002) and Deshpande (2005), as well as the earlier Mendelian genetic studies of this trait.

4.2.2 Inheritance of seedling vigor

Observations by Sajjanar (2002) and Deshpande (2005) of continuous variation for seedling vigor at both stages (1 and 2) of observation indicated that this trait is quantitative in nature. The equality of the means of the RIL population and their mid-parent value for seedling vigor 1 indicated that the trait was mainly controlled by additive gene action and the genes involved are in linkage equilibrium (they are not linked) (Deshpande, 2005). The current study detected

the effects of putative QTLs contributing to seedling vigor located on SBI-01, SBI-05, SBI-07 and SBI-10, but these effects were not all detected consistently across screening environments or genetic backgrounds. These findings are in agreement with earlier reports of the quantitative nature of this trait. Under conditions of moderate shoot fly pressure (*e.g.*, *kharif* 2006 and *rabi* 2006-07 screens of BTx623-background materials), in some cases the favorable alleles (BTx623 or IS 18551) for seedling vigor were reversed between the first and second observation for this trait, suggesting that values for the later observation (where the IS 18551 allele was favorable) were a result of shoot fly resistance differences and not a direct effect of alleles for seedling vigor *per se*.

4.2.3 Inheritance of trichome density

Deshpande (2005) observed continuous distribution of progenies in his RIL population for trichome density on the lower leaf surface, which was skewed towards trichomelessness. Deviation of the RIL population mean from the mid-parental value indicated the presence of epistasis (Jinks and Pooni, 1976). The expression of trichomes per unit leaf area was previously found to be regulated by both additive and dominance effects but the former were of greater importance than the latter (Halalli *et al.*, 1982). The appearance of transgressive segregation in among the RILs studied by Sajjanar et al. (2002) and included as control entries in the present study (*i.e.*, RIL 252) is likely due to recombination of favorable alleles received from both parents. The presence of slightly fewer trichomes in the resistant parent IS 18551 can be explained in terms of this line having a preponderance of favorable alleles and the presence of a few unfavorable alleles and their interaction. Due to the recombination of positive and negative alleles in the F_2 and subsequent inbreeding generations events, individual RILs with a higher proportion of favorable alleles than the best parent have been observed as transgressive segregants. The degree of transgressive segregation depends on the degree of dispersion of the interacting alleles in the parental lines. Gibson and Maiti (1983) evaluated 85 F_3 lines derived from the BC_1F_1 of (IS 1054 x CK 60B) x CK 60B showed that those derived from trichomed plants were always trichomed and that some lines derived from trichomeless plants were trichomesless while others segregated. This indicated that trichome presence was recessive and controlled by a single locus in this genetic background. No explanation was offered for the absence of homogeneous trichomeless F_3 lines derived from the cross A2219 x IS 2312. However, density of trichomes per unit area of leaf lamina surface was genetically controlled in this

cross (Maiti and Gibson, 1983). Results of the current study suggest the predominant role of a single genomic region on linkage group G (SBI-10) in control of the presence of trichomes on upper and lower surfaces of sorghum seedling leaf blades (Tables 63 and 64), with genetic background and the environment contributing to variation in trichome density among lines homozygous for the allele associated with presence of trichomes. This is in agreement with the QTL analysis reported by Sajjanar (2002) and Folkertsma *et al.* (2005).

4.3 Pleiotropism on SBI-10

Expression of seedling vigor, glossiness and trichomes conferred by IS 18551 alleles in the interval *Xtxp141-Xgap1* on linkage group SBI-10 can be explained as the presence of pleiotropism or closely linked genes in this region. In the absence of recombinants in the current study (in part due the absence of polymorphic markers between those flanking this interval), it is not possible to say clearly if this is the result of a single gene that is truly pleiotropic or whether it is the result of several genes that are closely linked. A large number of crosses may have to be made, or a very large number of segregants generated, before a recombinant genotype is formed to confirm if the genes involved are closely linked. The presence of a single recombinant genotype however, would be sufficient to establish that two closely linked genes are involved and not a single pleotropic gene.

4.4 QTL Mapping

4.4.1 Seedling vigor QTL mapping

Deshpande (2005) mapped one QTL on SBI-07 across two seasons spanning an interval from *Xtxp159* to *Xtxp312* on SBI-07 explaining both seedling vigor I and II. This was deemed as a minor QTL that explained a very low portion of the observed phenotypic variance and exhibited no Q x E interaction. In individual environments, in *kharif* season, seedling vigor I mapped to the same region whereas in *rabi* it was mapped to the slightly broader interval *Xtxp40-Xtxp159*. Seedling vigor II was mapped to adjacent interval *Xtxp312-Xisp233* in *kharif*, but could not be mapped to this region in the *rabi* screen. These individual and across-season analyses pinpoint to the location of seedling vigor genes

somewhere in the interval *Xtxp40-Xtxp159-Xtxp312-Xisp233*. Since the last two markers in this series are closely associated with *Xtxp159*, there are more chances of these to be carried along with the prior one. Folkertsma *et al.* (2005) also mapped seedling vigor II on this linkage group between *Xtxp159* and *Xtxp312*. The present studies also showed high significance for the association of improved seedling vigor with IS 18551 alleles in the *Xtxp40*-Xtxp159 region, but failed to confirm the role of this genomic region in more direct measures of shoot fly resistance.

Further, Deshpande (2005) mapped another QTL for seedling vigor I in his across-season analysis. This QTL spanned *Xisp258-Xtxp23* on linkage group SBI-05. This same QTL was mapped for seedling vigor I and seedling vigor II in *kharif* at Parbhani. This region (*Xisp258-Xtxp23*) also was associated with a QTL for shoot height I in *kharif* at Parbhani. The present studies confirmed the map location of this QTL (*Xtxp94-Xtxp15*) and it was this QTL (Seedling vigor I and II) that has been successfully transferred in the '-J1+J2?' (AAAB) and 'plus-J' introgression lines in BTx623 background (Table 58).

Deshpande (2005) also mapped one QTL to interval *Xcup67-Xcup73* on linkage group SBI-10 for shoot height I and shoot height II, in rabi at Parbhani. However, *Xcup73* was located at the distal end of linkage group SBI-01 in the map reported by Folkertsma *et al.* (2005). Folkertzma *et al.* (2005) reported that the *Xtxp141-Xgap1* region (towards *Xcup*67) on SBI-10 included QTLs for glossiness, trichomes, seedling vigor II, shoot height I, oviposition I, oviposition II, deadhearts I and deadhearts II. The present study successfully demonstrated improved expression of glossiness, seedling vigor, and trichomes, accompanied by reductions in oviposition and deadhearts, associated with introgression of IS 18551 alleles in this region, and this validates the shoot fly resistance QTL(s) mapping to this genomic region. As indicated in section 9.6 above, in the absence of recombinants in the current study (in part due the absence of polymorphic markers between those flanking this interval), it is not possible to say clearly if this is the result of a single gene that is truly pleiotropic or whether it is the result of several genes that are closely linked.

4.4.2 Glossiness QTL

Deshpande (2005) mapped four QTLs for glossiness across seasons out of which two QTLs were common in individual screening environments. These consistent QTLs for glossiness were located on SBI-05 in interval *Xisp215-Xisp258* and on SBI-07 in interval *Xtxp40-Xtxp159*. Gowri Sajjanar (2002) mapped a major

glossiness gene on SBI-05 in interval *Xtxp65-Xtxp94*. Folkertzma *et al.* (2005) mapped consistent glossiness QTLs on linkage group SBI-05 in interval *Xisp258-Xtxp65* and on SBI-10 in interval *Xgap1-Xtxp141*. The present studies confirmed the earlier observations regarding the presence of glossiness QTLs on SBI-05 and SBI-10, donor parent IS 18551 providing alleles for higher glossiness intensity. Further, the current study more clearly refined the map position of the glossiness gene on SBI-05, indicating that this is confined to or tightly linked with *Xisp258*. Finally, it indicated that there is no glossiness gene from in interval *Xtxp40-Xtxp159* on SBI-07, or at least that any glossiness gene from IS 18551 mapping to this region is not expressed in isolation.

4.4.3 Trichomes QTL

Sajjanar (2002) and Folkertsma *et al.* (2005) mapped one QTL for trichomes on SBI-10 in interval *Xtxp141-Xgap1*. This QTL co-localized with QTLs for glossiness, seedling vigor II, shoot height I, oviposition I, oviposition II, deadhearts I and deadhearts II. Deshpande (2005) too mapped trichomes on SBI-10 in interval *Xgap1-Xcup67*. Results of the current study suggest the predominant role of a single genomic region (interval *Xtxp141-Xgap1*) on linkage group G (SBI-10) in control of the presence of trichomes on upper and lower surfaces of sorghum seedling leaf blades (Tables 63 and 64), with genetic background and the environment contributing to variation in trichome density among lines homozygous for the allele associated with presence of trichomes. This is in agreement with the QTL analyses reported by Sajjanar (2002) and Folkertsma *et al.* (2005). Further, the current study suggests that this genomic region represents the best single target for applied marker-assisted selection to improve shoot fly resistance of agronomically elite, shoot fly susceptible sorghum genotypes.

4.4.4 QTLs for oviposition and deadhearts

Folkertsma *et al.* (2005), mapped QTLs for seedling vigor II, shoot height I, trichomes, oviposition I, oviposition II, deadhearts I and deadhearts II on SBI-10, and these co-localized with a glossiness QTL on SBI-10. Similarly, QTLs for oviposition I, deadhearts I, deadhearts II, and seedling vigor II, co-localized with a glossiness QTL on SBI-05. However, Deshpande (2005) did not find co-localization of oviposition or deadhearts QTL on these linkage groups across seasons. In *kharif* at Parbhani, Deshpande (2005) mapped two QTLs one for oviposition I, across interval *Xtxp23-Xtxp15* on SBI-05 and for oviposition II, the

second across interval *Xtxp40-Xtxp159* on SBI-07. Results from the current study validated the two QTLs detected by Folkertsma *et al.* (2005) and suggest that they represent the two best targets for applied marker-assisted selection to improve shoot fly resistance of agronomically elite, shoot fly susceptible sorghum genotypes.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The present study entitled "SSR marker-assisted backcross introgression of QTL for host plant resistance to *Atherigona soccata* in *Sorghum bicolor*" was aimed at the transfer and validation of putative QTL shoot fly resistance from donor parent IS 18551, which were mapped in previous studies, into the genetic backgrounds of elite hybrid parental lines BTx623 and 296B. Single F1 plants from each of the two crosses of BTx623 and IS 18551, 296B and IS 18551 were backcrossed to their respective recurrent parents BTx623 and 296B. Four RIL parents *viz*. RIL 166, RIL 154, RIL 189 and RIL 252 identified from the Recombinant Inbred Line mapping population developed from BTx623 and IS 18551, were also used as donors in crosses to elite recurrent parents in an attempt to , reduce the number of cycles of crossing and selection required to efficiently combine elite agronomic features of the elite lines with shoot fly resistance. The recurrent backcrossing resulted in the development of near-isogenic lines, Plus A, Plus E, Plus G, Plus J1 and Plus J with individual shoot fly resistance QTLs A, E, G, J1 and J = J1+J2 respectively, in the genetic backgrounds of BTx623 and 296B. Starting from the BC_1F_1 onward, polymerase chain reaction (PCR)-based molecular markers *Xtxp37* and *Xtxp75* (linked to QTL A), *Xtxp141* and *Xgap1* (linked to QTL G), *Xtxp159* and *Xtxp40* (linked to QTL E), *Xtxp94, Xtxp65* and *Xtxp15* (linked to QTL J1 and J2) were used to select individual segregants expected to carry the resistance alleles in heterozygous or homozygous form. A similar strategy was used till the generation of BC_4F_3. The BC_4F_3 lines expected (on the basis of their QTL-flanking marker genotypes) to be homozygous for individual shoot fly resistance QTL were identified on the basis of marker analysis and then field screened in replicated multi-season trials.

The field experiments were conducted in single-row plots of 2-m length spaced 75 cm apart (rainy season 2006 and postrainy season 2006/07). In the case of rainy season 2007 trials (84-entry set in BTx623 background and 20-entry set in 296B background) two-row plots of 4-meter length were used. The seed was sown with a four-cone planter at a depth of 5 cm below the soil surface. The field was irrigated immediately after sowing. Ten days after seedling emergence, thinning was carried out to maintain a spacing of 10 cm between the plants. Shoot fly infestation was optimized through the use of interlard fishmeal technique (Soto, 1974). Normal agronomic practices were followed for raising the sorghum crop and no insecticide was applied in the experimental plots. Infester rows were chopped off 30 days after emergence in the main plots to avoid shading of the test plots. Data were recorded on all plots for glossiness score (all three seasons), seedling vigor score (rainy season 2006 and postrainy season 2006/07), trichome density (rainy season 2006 and postrainy season 2006/07) number of eggs and numbers of plants with eggs at 14 and 21 days after seedling emergence (DAE) (all three seasons), and plants with deadhearts at 14 and 21 days after emergence (all three seasons). The data on number of eggs was expressed as number of eggs per 10 plants, and plants with eggs and deadhearts in terms of the percentage of the total number of plants in the plot.

The results of these field experiments can be summarized briefly as follows:

- Parental lines and near-isogenic pairs revealed wide variation in phenotypic values for shoot fly resistance in all three screening environments. Wide variation was observed for shoot fly resistance component traits like seedling glossiness intensity, seedling vigor, oviposition (%), deadheart incidence (%) and seedling leaf blade trichome density. These traits can be used as simple criteria for selection of resistant genotypes.

- The glossiness character is quantitative (oligogenic) and shows pleiotropic effects. Glossiness is associated with J1, J and G QTL introgression lines. Introgression lines expected to carry these QTL exhibited significantly lower shoot fly damage than their recurrent parents in one or more of the three screening environments. Thus seedling glossiness is an inherited trait and has a definite role in contributing to resistance to *Atherigona soccata*.
- Present studies validated the existence of, and fine-mapped the location of, a major glossiness gene (corresponding to shoot fly resistance QTL J1) on sorghum chromosome SBI-05 in the interval *Xisp258-Xtxp65* and found it maps near *Xisp258*.
- The introgression lines for the QTL G region on SBI-10 also exhibited superior seedling vigor and trichome density compared to their recurrent parent BTx623 (transfer of this QTL to the background of recurrent parent 206B was not successful), therefore the QTL G introgression lines performed significantly better than their recurrent parent for all observed shoot fly resistance traits.
- Trichomes are absent in the introgression lines for QTL A, E, J1 and J. One major QTL controlling both glossiness and trichomes was transferred during QTL G introgression..
- Introgression of QTL J2 (*Xtxp15*) located on donor chromosome SBI-05 was associated with improved seedling vigor, and thus contributed to faster seedling growth in QTL J introgression lines compared to their recurrent parents.
- The SBI-05 glossiness QTL and the SBI-10 trichome QTL had large effects on shoot fly resistance and are good targets for applied marker-assisted selection.

- Resistance to shoot fly increased when both glossiness and trichomes occurred together.
- Trichome density was highly influenced by the environment. The density of trichomes is expressed to be less in the *Kharif* (rainy season) than in the *Rabi* (postrainy season). The trichome density (both upper and lower leaf surface) has previously been reported to exhibit both high estimates of broad-sense heritability and large G x E interaction (Deshpande, 2005).
- Previously detected minor putative shoot fly resistance QTL on SBI-01 (QTL A) and SBI-07 (QTL E) do not appear to be good targets for applied marker-assisted selection. Introgression lines for these QTL exhibited little if any improvement in shoot fly resistance.
- RIL 252 recorded high trichome density, RIL 189 lacks trichomes and RIL 153 recorded moderate trichome density. Highly resistant check IS 2312 recorded high trichome density and moderately resistant check IS 1054 recorded moderate trichome density.

Future Prospects

Transfer of shoot fly resistance into CMS lines ATx623 and 296A

The discovery of cytoplasmic male-sterility (CMS) in sorghum by Stephens and Holland (1954) made development of commercial hybrid cultivars possible in this species (House, 1985). Because more than 75% of the area under rainy season sorghum cultivation in India is now planted to high-yielding hybrids, it is important to transfer genes conferring resistance to sorghum shoot fly into cytoplasmic male-sterile (A-lines), maintainer (B-lines), and restorer (R-lines) lines that can be used develop hybrids with high grain yield and resistance to this pest. The current study transferred several putative shoot fly resistance QTL from donor parent IS 18551 into the genetic backgrounds of two elite maintainer lines, BTx623 and 296B.

Recurrent backcrossing of these validated QTL introgression lines in BTx623 and 296B backgrounds, to male-sterile lines ATx623 and 296A, respectively, will produce more shoot fly resistant versions of these elite A-lines that can then be used in breeding hybrids with improved resistance levels.

Gene Pyramiding

Advances in development of DNA marker-based genetic linkage maps and their use in identifying genomic regions contributing to economically important traits (Paterson *et al.*, 1991; Gale and Witcombe, 1992) have made possible the routine use of marker-assisted selection (MAS) to produce near-isogenic lines (NILs) and gene pyramids for crop disease and pest resistance. For example, in pearl millet, Jones *et al.* (1995) have identified a number of quantitative trait loci (QTL) that confer strain-specific, host-plant resistance to pearl millet downy mildew. Similar results for pests and pathogens in other crops include rice blast disease caused by *Magnaporthe grisea* (Herbert) Barr (Yu *et al.*, 1991). Pyramiding of validated shoot fly resistance QTL on SBI-05 and SBI-10 into the genetic backgrounds of BTx623 and 296B and comparing the pyramids with individual introgression lines now needs to be undertaken to determine the cumulative effect of introgressing multiple QTL for resistance to this pest. Pyramiding can be performed by a single generation of crossing of the available single-QTL introgression lines in a given genetic background followed by two generations of self-pollination. This pyramiding is required to accurately assess the epistatic effects, if any, of these shoot fly resistance QTL.

Large Scale Field Evaluation

The effect of a single QTL as well as interactions between that QTL and others in one or more genetic backgrounds can be efficiently studied in multi-environment field screens of near-isogenic lines. In the case of QTL for shoot fly resistance, it is

essential that these field screens be conducted across a wide range of shoot fly pressure in order to identify individual QTL or combinations of QTL that confer effective resistance only at moderate levels of shoot fly pressure. This is because the levels of resistance available from cultivated sorghum donors are modest and readily overcome when shoot fly pressure is very high. The results from such field trials can be used to determine the effects of individual QTL and combinations of QTL in multiple genetic backgrounds. This information will assist sorghum breeders to decide which among the putative QTL are good candidates for used in applied marker-assisted selection for the target trait.

Hybrid Testing and Release

Hybrid testing programs first test in ideal conditions and against the known major specific constraints before engaging in extensive multi-locational testing, which is required to adequately expose new hybrids to the range of environmental variations expected in the target domain. In private-sector breeding programs these extensive multi-locational trials are known as strip tests and almost all are placed in farmers fields and managed by farmers, permitting a good estimation of the G x E interaction of the new hybrids. Performance data (including grain quality), extensive visual evaluations, farmers' opinions and seed production are all considered in the decision to commercialize a new hybrid combination. In addition, government-imposed regulatory requirements must also be met before commercialization of a hybrid based on an improved hybrid parent can be undertaken. In case of essentially-derived genotypes such as products of marker-assisted backcrossing programs, it may be practical to relax these regulatory requirements to speed delivery to farmers of improved versions of already popular hybrids.

Fine Mapping Approaches

High resolution mapping of NILs to characterize allelic variation at each locus of interest by using 'chromosome haplotyping' is essential to determine a precise position for the loci of interest. Introgression libraries provide perfect starting material for this purpose. Each line containing a locus of interest can be backcrossed to the recurrent parent and if necessary selfed to create a large segregating population. This population can be used to identify recombinants within the introgression segment using flanking markers. Phenotyping these recombinants, combined with further saturation of the introgression segment with additional marker loci enables the locus to be mapped at high resolution. Thus segregating populations, on the order of thousands of individuals, derived from crossing such NILs, can be used to narrow down the position of a major gene contributing to a QTL to a small genomic region in which candidate genes can be found (IParan, 2003). Finally, the identity of a QTL can be validated by complementation tests by genetic transformation. In the current study, in the course of introgressing the major QTL J1 for seedling glossiness, the position of the major glossiness gene was identified as being very closely linked to SSR marker *Xisp258* on SBI-05. As additional markers are developed for this genomic region, and for the genomic region associated with QTL for trichome density and seedling glossiness in the interval between *Xgap1* and *Xtxp141* on SBI-10, it will be possible to use the introgression lines developed in the present study to further refine the map positions of these QTL and ultimately identify the underlying genes responsible for these important components of host plant resistance to the sorghum shoot fly.

REFERENCES

REFERENCES

Anonymous, 1965-1967; 1968-1969 - Progress report of the All India Sorghum Improvement Project.

Agrawal, B.L., and L.R. House, 1982. Breeding for pest resistance in sorghum. In Sorghum in Eighties, Proceedings of the International Symposium on Sorghum, 2-7 November 1981, International Crops Research Institute for the Semi-Arid Tropics, Andhra Pradesh, India. Pp. 435-446

Agarwal, B.L., and C.V. Abraham, 1984. Breeding sorghum for resistance to shoot fly (*Atherigona soccata*) and midge (Contarinia sorghicola). Proceedings of the International Sorghum Entomology Workshop, 15-21, July 1984, Texas A & M University, College Station Texas, USA. ICRISAT, Andhra Pradesh, India. Pp. 371-383

Ainsworth, C.C., and M.D. Gale, 1987. Enzyme structural genes and their exploitation in wheat genetics and breeding. In Enzymes and their role in cereal technology. Eds. Kruger, J.E., Lineback, D.R., and Stuffer, C.E., St. Paul, USA: American Association of Cereal Chemists pp.53-82

Akkaya, M.S., A.A. Bhagwat, P.B. Cregan, 1992. Length polymorphisms of simple sequence repeat DNA in Soybean. Genetics 132: 1131-1139

Akkaya, M.S., R.C. Shoemaker, J.E. Specht, A.A. Bhagwat, P.B. Cregan, 1995. Integration of simple sequence repeat DNA markers into a soybean linkage map. Crop Science 35: 1439-1445

Allard, R.W., 1960. Principles of plant breeding. Wiley, New York.

Anderson, J.A., G.A. Churchill, J.E. Antrique, S.D. Tanksley, M.E. Sorrells, 1993b. Optimizing parental selection for genetic linkage maps. Genome 31: 181-186

Amasino, R.M., and T. Osborn, 2002. Brassica genetics, mutant library-glossy, University of Wisconsin. Available online at
http://www.biochem.wisc.edu/brassicacla
ss roomgenetics/glossy (Verified 8 June 2005)

Ballard, B.A., and Y. Ramachandra Rao, 1924. A preliminary note on the life history of certain anthomyiid flies. *Atherigona spp* and *Acritochaeta excisa* Thomson. Report of proceedings of the 5 th Entomology Meeting. Pusa, 1923, pp. 275-277

Balasubramanian, G., M. Balasubramanian, M. Gopalan, and N. Sivaprakasam, 1987. Seed treatment of sorghum for the control of shoot fly (*Atherigona soccata* Rondan). Madras Agricultural Journal 74: 237-239

Blum, A., 1967. Varietal resistance of sorghum to the sorghum shoot fly [*Atherigona varia soccata*]. Crop Science 7: 461-462

Beck, S.D., 1965. Resistance of plants to insects. Annual review of entomology 10: 207-232

Blum, A., 1971. Sorghum breeding for shoot fly resistance in Israel. In: Control of Sorghum shoot fly (Ed. Jotwani, M.G. and W.R. Young). Oxford and IBH Publising Co., New Delhi. Pp. 180-191

Bapat, D.R. and U.N. Mote, 1982. Sources of shoot fly resistance in sorghum. Journal of Maharashtra Agricultural University 7: 238-240

Bapat, D.R., K.A. Nayeem, and H.M. Markande, 1975. Correlation studies of HCN content and shoot fly attack in sorghum. Sorghum Newsletter 18: 50

Blum, A., 1963. The penetration and development of the sorghum shoot fly in susceptible sorghum plants. Hassadeh 44: 23-25

Birader, S.G., S.T. Borikar and R.D. Chundurwar, 1986. Trichome density in some progeny of sorghum. Sorghum Newsletter 29:107

Blum, A., 1969. Factors associated with tiller survival in sorghum varieties resistant to sorghum shoot fly (*Atherigona varia soccata*). Crop Science 9: 508-510.

Blum, A., 1968. Anatomical phenomena in seedlings of sorghum varieties resistant to sorghum shoot fly (*Atherigona soccata*). Crop Science 8: 388-391

Burton, G.W., W.W. Hanna, J.C. Johnson, JR D.B. Jeuck, W.G. Monson, J.B. Prwell, H.D. Wells and N.W. Wildstorm, 1977. Pleiotropic effects of the trichomeless gene in pearl millet on transpiration forage quality and pest resistance. Crop Science 17: 613-616

Borikar, S.T., and P.R. Chopde, 1982. Stability for shoot fly resistance in sorghum. Indian Journal of Genetics and plant breeding 42: 155-158

Borikar, S.T., and R.R. Chopde, 1981. Shoot fly resistance in sorghum. Indian Journal of Genetics and Plant breeding 41: 191-199

Bhise, H.T., B.B. Desai, and H.D. Chavan, 1996. Effect of chemical constituents on resistance of shoot fly in sorghum. Journal of Maharashtra Agricultural University 21: 293-294

Brown, S.M., M.S. Hopkins, S.E. Mitchell, M.L. Senior, T.Y. Wang, R.R. Duncan, F. Gonzalez-Candelas, S. Kresovich, 1996. Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum [*Sorghum bicolor* (L.) Moench]. Theoretical and Applied Genetics 93: 190-198

Burrow, M.D., and T.K. Blake, 1998. Molecular tools for study of complex traits. In: Molecular Dissection of Complex Traits, (Ed. Paterson, A.H.), CRC Press, Boca Raton, New York, pp: 13-30

Beckmann, J.S., and M. Soller, 1990. Toward a unified approach to genetic mapping of eukaryotes based on sequence tagged microsatellite sites. Biotechnology 8: 930–932

Bell, C.J., and J.R. Ecker, 1994. Assignment of 30 microsatellite loci to the linkage map of Arabidopsis. Genomics 19: 137-144

Burr, B., 2001. Some concepts and new methods for molecular mapping in plants. In DNA based markers in plants. Eds. Phillips, R. L. and Vasil, I.K., Kluwer. Academic Publishers, The Netherlands pp. 1-8

Buscot, F., D. Wipf, C. DI Battista, J.D. Munch, B. Botton, and F. Martin, 1996. DNA Polymorphism in morels: PCR/RFLP analysis of the ribosomal DNA spacers and microsatellite primed PCR. Mycological Research 100: 63-71

Bowers, J.E., C. Abbey, S. Anderson, 2003. A high-density genetic recombination map of sequence-tagged sites for sorghum, as a framework for comparative structural and evolutionary genomics of tropical grains and grasses. Genetics 165: 367–386

Bhattramakki, D., J. Dong, A.K. Chhabra, and G. Hart, 2000. An integrated SSR and RFLP linkage map of *Sorghum bicolor* (L.) Moench. Genome 43: 988–1002

Beavis, W.D., 1998. QTL analysis: power, precision, and accuracy. Pp. 145-162. In Paterson, A.H.(ed.) Molecular dissection of complex traits. CRC Press, Boca Raton/ New York

Clearwater, J.R., and S.M. Othieno, 1977. Population dynamics of *Atherigona soccata* in the field. Fifth Annual Report, International Centre of Insect Physiology and Ecology (ICIPE), pp. 14-16, Nairobi, Kenya.

Chand, P., M.P. Sinha, and A. Kumar, 1979. Nitrogen fertilizer reduces shoot fly incidence in sorghum. Sci. Cult. 45: 61-62

Channabasavanna G.P., B.V. Venkata Rao, and G.K. Rajagopal, 1969. Preliminary studies on the effect of incremental levels of phosphatic fertilizer on the incidence of jowar shoot fly. Mysore Journal of Agricultural Science 3: 253-255

Collard B.C.Y., M.Z.Z. Jahufer, J.B. Brouwer, and E.C.K. Pang, 2005. An introduction to markers, quantitative trait loci mapping and marker assisted selection for crop improvement: The basic concepts. Euphytica 142: 169-196

Chin, E.C.L., M.L. Senior, H. Shu, and J.H.C. Smith, 1996. Maize simple repetitive DNA sequences: abundance and allele variation. Genome 39: 866-873

Charlesworth, B., P. Sniegowski, and W. Stephen, 1994. The evolutionary dynamics of repetitive DNA elements in Eukaryotes. Nature 371: 215-220

Condit, R., and S.P. Hubbell, 1991. Abundance and DNA sequence of two base regions in tropical tree genomes. Genome 34: 66-71

Chen, S., XH Lin, C.G. Xu, Q. Zhang, 2000. Improvement of bacterial blight resistance 'Minghui 63', an elite restorer line of hybrid rice, by molecular marker-assisted selection. Crop Science 40: 239-244

Chittenden, L.M., K.F. Schertz, Y-R Lin, R.A. Wing, and A.H. Paterson, 1994. A detailed RFLP map of *Sorghum bicolor* × *S. propinquum*, suitable for high-density mapping, suggests ancestral duplication of Sorghum chromosomes or chromosomal
segments. Theoretical and Applied Genetics 87: 925–933

Cregan, P.B., J. Mudge, E.W. Fickus, L.F. Marek, D. Danesh, R. Denny, R.C. Shoemaker, B.E. Mathews, T. Jarvik, and N.D. Young, 1999. Targeted isolation of simple sequence repeat markers through the use of bacterial artificial chromosomes. Theoretical and Applied Genetics 98: 919-928

Cifarelli, R.A., M. Gallitelli and F. Celliini, 1995. Random amplified hybridization microsatellites (RAHM): isolation of a new class of microsatellite-containing DNA clones. Nucleic Acids Research 23: 3802–3803

Dogett, H., 1988. Sorghum, Second Edition, Longman, London 688 pp.

Deeming, J.C., 1971. Some species of *Atherigona Rondani* (Diptera: Muscidae) from Nothern Nigeria, with special reference to those injurious to cereal crops. Bulletin of Entomological Research (Bull. ent. Res.) 61: 133-190

Davies, J.C., and K.V. Seshu Reddy, 1980. Shoot fly species and their graminaceous hosts in Andhra Pradesh, India. Presented at the International Study Workshop on the Sorghum Shoot fly, 5-8 May 1980, ICIPE, Nairobi, Kenya

Delobel, A.G.L., and G.C. Unnithan, 1981. The status of *Sorghum arundinaceum* as host of *Atherigona soccata* Rondani (Diptera: Muscidae) in Kenya. Insect Science Application 2: 67-71

Delobel, A.G.L., 1981. The distribution of the eggs of the sorghum shoot fly, *Atherigona Soccata* Rondani (diptera: muscidae). Insect Science Application 2: 63-66

Delobel, A.G.L., 1982. Oviposition and larval survival of the sorghum shoot fly, *Atherigona soccata* Rond., as influenced by the size of its host plant (Diptera, Muscidae). Z. ang. Ent. 93: 31-38

Davis J.C., and D. Jewett, 1966. Increases in the incidence of *Atherigona indica* infuscate Van Emden (Diptera: Anthomyiidae) on sorghum due to spraying. Nature, Lond. 209, 104

Davies, J.C., and K.V. Seshu Reddy, 1981. Observations on oviposition of Shoot fly, *Atherigona soccata* Rond. (Diptera: Muscidae). Sorghum Entomology Progress Report 4, ICRISAT, Patancheru.

Dhams, R.G., 1943. Insect resistance in sorghum and cotton. American Society of Agronomy Journal 35: 704-715

Davies J.C., 1981. Pest losses and control of damage on sorghum in developing countries – the realities and myths. In: Sorghum in the Eighties. Proc. Int. Symp. Sorghum, 2-7 Nov. 1981, pp. 215-223, ICRISAT, Patancheru, Andhra Pradesh, India.

Dhillon, M.K., 2004. Effects of cytoplasmic male-sterility on expression of resistance to sorghum shoot fly, *Atherigona soccata* (Rondani). Ph.D. Thesis, 382 pp. Department of Entomology, Chaudhary Charan Singh Haryana Agricultural University, Hisar 125004, Haryana, India.

Dogget, H., K.J. Starks, and S.A. Eberhart, 1970. Breeding for resistance to the Sorghum shoot fly. Crop Science 10: 528-531

Dogget, H., 1971. Breeding for resistance to sorghum shoot fly in Uganda. In: Control of Sorghum Shoot Fly. (Eds.Jotwani, M.G. and W.R.Yound). Oxford and IBH publishing Co.,New Delhi, pp.1925-201

Dhawan, P.K., S.P. Singh, A.N. Verma, and D.R. Arya, 1993. Antibiosis mechanism of resistance to shoot fly, *Atherigona soccata* (Rondani) in sorghum. Crop Research 6: 306-310

Dhillon, M.K., H.C. Sharma, Ram Singh, and J.S. Naresh, 2005. Mechanisms of resistance to shoot fly, *Atherigona soccata* in sorghum. Euphytica 144: 301–312

Darvasi, A., A. Weinreb, V. Minke, J.I. Weller, and M. Soller, 1993. Detecting marker QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. Genetics 134: 943-951

Emerson, R.A., G.W. Beadle, and A.C. Fraser, 1935. A summary of linkage studies in maize. Cornell University and Agric. Exp. Sta. Memoir 180.

Edwards, M.D., T. Helentjaris, S. Wright, and C.W. Stuber, 1992. Molecular marker facilitated investigations of quantitative trait loci in maize. Theoretical and Applied Genetics 83: 765-774

Eshed, Y., and D. Zamir, 1995. An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141: 1147-1162

Ender, A., K. Schwenk, T. Stadler, B. Streit and B. Schierwater, 1996. RAPD identification of microsatellites in Daphina. Molecular Ecology 5: 437–441

Edwards, A., H. Civitello, H.A. Hammond and C.T. Caskey, 1991. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. American Journal of Human Genetics 49: 746–756

Fletcher, T.B., 1914. Some south Indian Insects and other Animals of Importance Considered specially From an Economic Point of View. Government Press, Madras.

Farrell, J.A.K., 1977. Plant resistance to insects and the selection of resistant lines. The New Zealand Entomologist 6: 244–261

Frisch, M., and A.E. Bohn Mand Melchinger, 1999a. Minimum sample size and optimum positioning of flanking markers in marker-assisted backcrossing for transfer of a target gene. Crop Science 39: 967-975

Frisch, M., M. Bohn, and A.E. Melchinger, 1998. Markerdichte und Anzahl beno¨ tigter Markeranalysen in markergestu¨ tzten Ru¨ ckkreu zungs-programmen. Vortra¨ge fr Pflanzenzu¨ chtung 42: 1–3

Frisch, M., and A.E. Bohn Mand Melchinger, 1999b. Comparison of selection strategies for marker-assisted backcrossing of a gene. Crop Science 39: 1295-1301

Granados, Y.R., 1972. The role of wild hosts on the population dynamics of the sorghum shoot fly in Thailand. Pages: 112-118 in Control of Sorghum Shoot fly, eds. M.G. Jotwani and W.R. Yound, New Delhi, India: Oxford and IBH publishing Co.

Gibson, P.T. and R.K. Maiti, 1983. Trichomes in segregating generations of sorghum matings. I. Inheritance of presence and density. Crop Science 23: 73-75

Goud, J.V., K.H. Anahosur, and K.A. kulkarni, 1983. Breeding for multiple resistance in sorghum. Proceedings of National Seminar on Breeding Crop Plants for resistance pests and diseases, 25-27th May, 1983, Tamilnadu Agricultural University, Coimbatore, pp. 3

Gupta, P.K., H.S. Balyan, M. Prasad, R.K. Varshney, J.K. Roy, Harjit Singh, and H.S. Dhaliwal, 1999. Molecular markers for some quality traits in wheat. In national symposium on Frontiers of research in plant sciences. P14, Dec2-4, 1999, Calcutta, India

Gupta, P.K. and R.K. Varshney, 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113: 163-185

Gupta, M., Y.S. Chyi, J. Romero-severson, and J.L. Owen, 1994. Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple sequence repeats. Theoretical and Applied Genetics 89: 998-1006

Gowda, P.S.B., G.W. Xu, R.A. Frederiksen, and C.W. Magill, 1995. DNA markers for
downey mildew resistance genes in sorghum. Genome 38: 823-826

Gimelfarb, A., and R. lande, 1995. Marker assisted selection and marker QTL associations in hybrid populations. Theoretical and Applied Genetics 91: 522-528

Hiremath, I.G., S.G. Bhuti, and S. Lingappa, 1995. Imidacloprid, a new promising seed dress for the management of sorghum shoot fly, Karnataka Journal Agricultural Sciences 8: 163-167

Halalli, M.S., B.T.S. Gowda, K.A. Kulkarni, and J.V. Goud, 1983. Evaluation of advanced generation progenies for resistance to shoot fly in sorghum. Indian Journal of Genetics and Plant Breeding 43: 291-293

Halalli, M.S., B.T.S. Gowda, K.A. Kulkarni, and J.V. Goud, 1982. Inheritance of resistance to shoot fly (*Atherigona soccata* Rond.) in sorghum [*Sorghum bicolor* (L.) Moench]. SABRAO Journal 14: 165-170

Halalli, M.S., B.T.S. Gowda, K.A. Kulkarni, and J.V. Goud, 1982. Inheritance of resistance to shoot fly (*Atherigona soccata* Rond) in Sorghum (*Sorghum bicolor* (L.) Moench). SABRAO J. 14(2): 165-170

Hillel, J., T. Schaap, A. Haberfeld, J. Jeffreys, Y. Plotzky, A. Cahaner, and U. Lavi, 1990. DNA fingerprints applied to gene introgression in breeding programs. Genetics 124: 783-789

Hospital, F., C. Chevalet, and P. Mulsant, 1992. Using markers in gene introgression breeding programs. Genetics 132: 1199-1210

Hospital, F., and A. Charcosset, 1997. Marker-assisted introgression of quantitative trait loci. Genetics 147: 1469-1485

Haley, C.S., and S.A. Knott, 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69: 315-324

Hallauer, A.R., and J.B. Miranda, 1988. Quantitative genetics in maize breeding. Iowa state University Press, Ames, Iowa.

Hui Liu, Ben., 1998. Statistical genomics. Boca Raton, FL: CRC Press LLC.

Harshbarger, R.J., and J.J. Reynolds, 1993. Calculus: with applications. Lexington, Heath.

Haussmann, B.I.G., V. Mahalakshmi, B.V.S. Reddy, N. Seetharama, C.T. Hash, and H.H. Geiger, 2002. QTL mapping of stay-green in two sorghum recombinant inbred populations. Theoretical and Applied Genetics 106: 133–142.

Hearne, C.M., S. Ghosh and J.A. Todd, 1992. Microsatellites for linkage analysis of genetic traits. Trends in Genetics 8: 288–294

Hash, C.T., and P. Bramel-Cox, 2000. Survey of molecular marker applications. In Application of molecular markers in Plant breeding. Eds. Haussmann, B.I.G., Geiger, H.H., Hess, D.E., Hash, C.T., and P. Bramel-Cox., Training manual for seminar held at IITA, Ibadan, Nigeria, from 16-17, August 1999. International Crops Research Institute for Semi-Arid Tropics, Patancheru, India.

Huang, N., E.R. Angeles, J. Domingo, G. Magpantay, S. Singh, G. Zhang, N. Kumaravadivel, J. Bennett, and G.S. Khush, 1997. Marker-assisted selection using RFLP and PCR. Theoretical and Applied Genetics 95: 313-320

Hash, C.T., J.R. Witcombe, R.P. Thakur, S.K. Bhatnagar, S.D. Singh, and J.P. Wilson, 1997. Breeding for pearl millet disease resistance. In: Proceedings of the International conference on genetic improvement of sorghum and pearl millet, September 22-27, 1996. INTSORMIL, Publication No. 97-5. INTSORMIL and ICRISAT. Pp. 337-372

Hash, C.T., S.D. Singh, R.P. Thakur, and B.S. Talukdar, 1999. Breeding for disease resistance. In: Pearl millet breeding. Khairwal, I.S., Rai, K.N., Andrews, D.J. and Harinarayana, G. (eds.). Oxford and IBH Publishing Co. Pvt. Ltd: New Delhi, India. Pp. 337-379

Hash, C.T., and J.R. Witcombe, 2002. Gene management and breeding for downy mildew resistance. In: John F.Leslie (ed.). Sorghum and millets diseases. Pp. 27-36.

Helentjaris, T., King, G., Slocum, M., Siedenstrang, C., Wegman, S., 1985. Restriction fragment length polymorphisms as probes for plant diversity and their development as tools for applied plant breeding. Plant Molecular Biology 5: 109-118

International Crops Research Institute for the Semi Arid Tropics, 1978, ICRISAT Annual Report, 1977-78, Patancheru, India.

Jotwani, M.G., K.K. Marwaha, K.N. Srivastava, and W.R. Young, 1970. Seasonal incidence of sorghum shoot fly (*Atherigona varia soccata* Rond.) in jowar hybrids at Delhi. Indian Journal of Entomology 32: 7-15

Jotwani, M.G., W.R. Young, and G.L. Teetes, 1980. Elements of integrated control of sorghum pests. FAO Plant Production and Protection Paper, FAO, Rome, Italy, 159pp.

Jotwani, M. G., 1982. Factors reducing sorghum yields- Insect pests. Pp. 251-255. In Sorghum in the Eighties. Proceedings of the International Symposium on sorghum, 2-7 November 1981. Patancheru, A. P., India: ICRISAT.

Jotwani, M.G., T.R. Sukhani, and S. Singh, 1971. Seed treatment of sorghum with carbofuran for the control of shoot fly (*Atherigona varia soccata* Rond.). Pesticides 5(4): 13-14

Jotwani, M.G., and T.R. Sukhani, 1968. Seed treatment for the control of shoot fly, *Atherigona varia soccata*. Pesticides 5(10): 40-41

Jotwani, M.G., 1978. Investigations on insect pests of sorghum and millets. Special reference to host plant resistance. Final Tech. Report (1972-1979). Division of Entomology, I.A.R.I., New Delhi. pp. 114

Jotwani M.G., G.C. Sharma, B.G. Srivastava, and K.K. Marwaha, 1971. Ovipositional response of shoot fly, *Atherigona varia soccata* (Rondani) on some promising resistant lines of sorghum. In Investigations on Insect Pests of Sorghum and Millets (1965-70) (Edited by Pradhan S.), pp. 119-122. Final Technical Report, Division of Entomology, IARI, New Delhi.

Jadhav, S.S., U.N. Mote, and D.R. Bapat, 1986. Biophysical plant characters contributing to shoot fly resistance. Sorghum Newsletter 29: 70

Jain K.K., and M.P. Bhatnagar, 1962. Studies on varietal resistance to jowar shoot fly. Indian Journal of Genetics and Plant Breeding 22: 224-229

Jayanthi, P.D.K., B.V.S. Reddy, D.D.R. Reddy, T.B. Gour, and K.F. Nwanze, 1996. Genetics of shoot fly resistance in sorghum hybrids of cytoplasmic male sterile lines. Proceedings of 2nd International Crop Science Congress: Crop Productivity and Sustainability- Shaping the Future, 17-24 November 1996, New Delhi, pp. 152

Jayanthi, P.D.K., B.V.S. Reddy, T.B. Gour, and D.D.R. Reddy, 1999. Genetics of glossy and trichome characters in sorghum hybrids of cytoplaplasmic male sterile lines. Journal of Maharashtra Agricultural University 24: 251-256

Jotwani, M.G., and K.P. Srivatstava, 1970. Studies on sorghum lines resistant against shoot fly *Atherigona varia soccata* Rond. Indian Journal of Entomology 32: 1-3

Jansen, J.P.A., 1996. Aphid resistance in composites. International application published under the patent cooperation treaty (PCT) No. WO 97/46080.

Joshi, S., P. Ranjekar, and V. Gupta, 1999. Molecular markers in plant genome analysis. Current Science 43: 350-357

Jones Neil, Helen Ougham and Howard Thomas, 1997. Markers and mapping: we are all geneticists now. New Phytology 137: 165-177

Jorboe, S.G., W.D. Beavis, and Openshaw, 1994. Prediction of responses to selection in marker-assisted backcross programs by computer simulation. Abstracts of the Second International Conference on Plant Genome, Scherago International Inc., pp 38.

Kundu, G.G., P. Kishore, and M.G. Jotwani, 1971. Seasonal incidence of sorghum shoot fly, Atherigon varia soccata Rond. at Udaipur (Rajasthan). In Investigations on Insect Pests of Sorghum and Millets (1965-70) (Edited by Pradhan S) pp. 130-137. Final technical Report, PL 480 Project grant No. FG-In-227, Project no. A7-Ent-31, Division of Entomology, IARI, New Delhi.

Kundu, G.G., Prem Kishore, and M.G. Jotwani, 1971. New records of parasites of the sorghum shoot fly *Atherigona varia soccata* Rondani (Anthomyiidae: Diptera), 145-146, Investigations on Insect pests of sorghum and millets (1965-1970).

Final technical report, PL 480, Project grant no. FG-In-227, Project No. A7-Ent-31, Division of Entomology, IARI, New Delhi.

Kundu, G.G., M.G. Jotwani, and P. Kishore, 1978. Efficacy of carbofuran seed treatment for the control of sorghum shoot fly at different levels of nitrogen fertilization. Indian Journal of Entomology 40: 346-347

Kogan, M., and E.E. Ortman, 1978, Antixenosis: a new term proposed to replace Painter's 'non-preference' modality of resistance. Bulletin of Entomological Society of America 24: 175-176

Krishnananda, M., S. Jayaraj, and T.R. Subramaniam. 1970. Resistance in sorghum to stem fly, *Atherigona varia soccata* R. Madras Agriculture Journal 57: 674-679

Khurana. A.D., and A.N. Verma, 1982. Amino acid contents in sorghum plant resistant/susceptible to stem borer and shoot fly. Indian Journal of Entomology 44: 184-188

Khurana. A.D., and A.N. Verma, 1983. Some biochemical plant characters in relation to susceptibility of sorghum to stem borer and shoot fly. Indian Journal of Entomology 45: 29-37

Khurana, A.D and A.N. Verma, 1985. Some physical plant characters in relation to susceptibility of sorghum to stem borer and shoot fly. Indian Journal of Entomology 47: 14-19

Kamathar, M.Y., and P.M. Shalimath, 2003. Morphological traits of Sorghum associated with resistance to shoot fly, *Atherigona soccata* Rondani. Indian Journal of Plant Protection 31(1): 73-77

Kishore, P., B.S. Rana, and K.N. Agarwal, 1985. Selection for shoot fly *Atherigona soccata* Rondani resistance in high yielding varieties of sorghum. Journal of Entomological Research 9: 188-194

Karanjkar, R.R., R.D. Chundurwar, and S.T. Borikar, 1992. Correlations and path analysis of shoot fly resistance in sorghum. Journal of Maharashtra Agricultural University 17: 389-391

Kumar, L.S. 1999. DNA markers in plant improvement: An overview. Biotechnology Advances 17: 143-182

Kearsey, M.J., and A.G.L. Farquhar, 1998. QTL analysis in plants, where are we now? Heredity 80: 137-142

Knapp, S.J., W.C. Jr Bridges, and D. Birkes, 1990. Mapping quantitative trait loci using molecular marker linkage maps. Theoretical and Applied Genetics 79: 583-592

Kim, J.S., K.L. Childs, and M.N. Islam-Faridi, 2002. Integrated karyotyping of sorghum by in situ hybridization of landed BACs. Genome 45: 402–412

Kim, J.S., P.E. Klein, R.R. Klein, H.J. Price, J.E. Mullet, and D.M. Stelly, 2005b. Chromosome identification and nomenclature of Sorghum bicolor. Genetics 169: 1169–1173

Klein, R.R., Rodriguez-Herrera, R., Schlueter, J.A., Klein, P.E., Yu ZH, and Rooney, W.L., 2001. Identification of genomic regions that affect grain-mould incidence and other traits of agronomic importance in sorghum. Theoretical and Applied Genetics 102: 307–319

Kong, L., J. Dong, and G.E. Hart, 2000. Characteristics, linkagemap positions, and allelic differentiation of *Sorghum bicolor* (L.) Moench, DNA simple-sequence repeats (SSRs). Theoretical and Applied Genetics 101: 438–448

Langham, R.M., 1968. Unpublished M.Sc. thesis (Ahmahdu Bello university, Zaria, Nigeria, 1968)

Levin, D.A., 1973. The role of trichomes in plant defense. The Quaterly Review of Biology 48: 3-15

Lavi, U., M. Akkaya, A. Bhagwat, E. Lahav, P.B. Cregan, 1994. Methodology of generation and characteristics of simple sequence repeat DNA markers in avocado (Persea American M.). Euphytica 80: 171-177

Lamgercrantz, U., H. Ellegren, L. Andersson, 1993. The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic acids Research 21: 1111-1115

Lynch, M., and b. Walsh, 1998. Genetics and analysis of quantitative traits. Sinauer Associates Inc., Sunderland, MA, USA.

Men, U.B., H.G. Kandalkar, and S.K. Mundiwale, 1986. Effect of water logging on sorghum shoot fly (*Atherigona soccata*) infestation. Sorghum Newsletter 29: 58-59

Manorama Sharma, K.N. Kapoor, and G.S. Bharaj, 1996. Effect of seed treatment of sorghum with some new insecticides for control of shoot fly. Crop Res. 11(1): 90-92. J. N. K. V. V. College of Agriculture, Indore-452 001 (Madhya Pradesh), India.

Mote, U.N., J.R. Kadam, and D.R. Bapat, 1986. Antibiosis mechanism of resistance to sorghum shoot fly. Journal of Maharashtra Agricultural University 11 (1): 43-46

Mate, S.N., B.N. Phadnis, and Y.M. Taley, 1979. Studies on some physiological factors of shoot fly resistance in sorghum. Sorghum Newsletter 22: 66-67

Maiti, R.K., F.R. Bidinger, K.V.S. Reddy, P. Gibson and J.C. Davies, 1980. Nature and occurrence of trichomes in sorghum lines with resistance to the sorghum shoot fly. Joint Progress report of sorghum physiology/sorghum entomology, vol. 3, Icrisat, Patancheru.

Maiti, R.K., 1980. The role of glossy and trichome traits in sorghum crop improvement. Annual meeting of All India Sorghum Improvement Workshop held at Coimbatore, India, 12-14 May 1980

Maiti, R.K., K.E. Prasada Rao, P.S. Raju, and L.R. House, 1984. The glossy trait in sorghum: its characteristics and significance in crop improvement. Field Crops Research 9: 279-289

Maiti, R.K., and F.R. Bidinger, 1979. A simple approach to identification of shoot fly tolerance in sorghum. Indian Journal of Plant Protection 7: 135-140

Mate, S.N., M.R. Manjare, and R.B. Patil, 1996. Correlation studies of shoot fly attack with physiological and chemical factors in *Sorghum bicolor* (L.) Moench. Proceedings 2nd International Crop Science Congress: Crop Productivity and Sustainability-Shaping the Future, 17-24 Nov 1996, Indian Council of Agricultural Research, New Delhi, pp.154.

Mote U.N., B.N. Phadnis, and Y.M. Taley, 1979. Studies on some physiological factors of shoot fly resistance in sorghum. Sorghum Newsletter 22: 66-67

Mate, S.N., B.A. Phandanwis, and S.S. Mehetre, 1988, Studies on growth and physiological factors in relation to the shoot fly attack in sorghum. Indian Journal of Agricultural Research 22: 81-84

Maiti, R.K, and P.T. Gibson, 1983. Trichomes in segregating generations of sorghum matings. II. Association with shoot fly resistance. Crop Science 23: 76-79

Mohan Madan, Suresh Nair, A. Bhagwat, T.G. Krishna, Masahiro Yano, C.R. Bhatia and Takuji Sasaki, 1997. Genome mapping, molecular markers and marker assisted selection in crop plants. Molecular breeding 3: 87-103

Matthias Frisch, Martin Bohn, and Albrecht E. Melchinger., 1999. Comparison of selection strategies for marker-assisted backcrossing of a Gene. Crop Science 39: 1295–1301

Murray, M.G., Y. Ma, J. Romero-Severson, D.P. West, and J.H. Cramer, 1988. Restriction fragment length polymorphisms: what are they and how can breeders use them? Pages, 72-87 In: Proc. 43rd Annual Corn and Sorghum research Conference, American Seed Trade Association, eds. D. Wilkinson et al., Washington, DC.

Melchinger, A.E., M. Lee, K.R. Lamkey, A.R. Hallauer, and W.L. Woodman, 1990. Genetic diversity for restriction fragment length polymorphisms and heterosis for two diallel sets of maize inbreds. Theoretical and Applied Genetics 80: 488-496

Menz, M.A., R.R. Klein, J.E. Mullet, J.A. Obert, N.C. Unruh, and D.E. Klein, 2002. A high-density genetic map of *Sorghum bicolor* (L.) Moench based on 2926 AFLP, RFLP, and SSR markers. Plant Molecular Biology 48: 483–499

Morgante, M., and J. Vogel, 1994. Compound microsatellite primers for the detection of genetic polymorphisms. US patent application no. 08/326456.

Meyer, W., T.G. Michell, E.Z. Freedman and R. Vilgalys, 1993. Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of *Cryptococcus neoformans*. Journal of Clinical Biology 31: 2274–2280

Morgante, M., and A.M. Olivieri, 1993. PCR amplified microsatellites as markers in plant genetics. Plant Journal 3: 175-182

Mullis, K.B., F. Falo DNA, S. Scharf, R. Saiki, G. Horn, and H. Erlich, 1986. 'Specific enzymatic amplification of DNA in vitro' the polymerase chain reaction. Cold spring Harbor Symp Quanti Biol. 51. 263-273

Moreau, L., S. Lemarie, A. Charcosset, and A. Gallais, 2000. Economic efficiency on one cycle of marker-assisted selection. Crop Science 40: 329-337

Miller, J.C., and S.D. Tanksley, 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus Lycopersicon. Theoretical and Applied Genetics 80: 437-448

Nwanze, K.F., Y.V.R. Reddy, F.E. Nwilene, P. Soman, K.B. Laryea, and R. Jayachandran, 1996. Reduction of shoot fly damage in irrigated post rainy season sorghum by manipulating irrigation. Annals of applied biology 129: 391-403

Nye, I.W.B., 1960. The insect pests of graminaceous crops of East Africa. Colonial Research Studies No. 31. HM 50. London

Narayana, N.D., 1975. Characters contributing to sorghum shoot fly resistance. Sorghum Newsletter 18: 21

Nimbalkar, V.S., and D.R. Bapat, 1987. Genetic analysis of resistance in sorghum. Journal of Maharashtra Agricultural University 12: 331-334

Nwanze, K.F., Y.V.R. Reddy, and P. Soman, 1990. The role of leaf surface wetness in larval behaviour of the sorghum shoot fly, *Atherigona soccata*. Entomologia Experimentalis et. Applicata 56: 187-195

Nwanze, K.F., R.J. Pring, P.S. Sree, D.R. Butler, Y.V.R. Reddy, and P. Soman, 1992a. Resistance in sorghum to the shoot fly, *Atherigona soccata*: epicuticular wax and wetness of the central whorl leaf of young seedlings. Annals of applied biology 120: 373-382

Nwanze, K.F., P.S. Sree, D.R. Butler, D.D.R. Reddy, Y.V.R. Reddy, and P. Soman, 1992b. The dynamics of leaf surface wetness of sorghum seedlings in relation to resistance to the shoot fly, *Atherigona soccata*. Entomologia Experimentalis et. Applicata 64: 151-160

Norris, D.M., and M. Kogan, 1980. Biochemical and morphological bases of resistance. In Breeding plants resistant to insects. Eds. Maxwell, F.G. and F.R. Jennings, John Wiley and Sons, New York, pp. 22-61

Ogwaro, K., 1978 a. Observation on longevity and fecundity of the sorghum shoot fly, *Atherigona soccata*. Entomologia Experimentalis Applicata 23: 131-138

Ogwaro, K., 1978 b. Ovipositional behaviour and host plant preference of the sorghum shoot fly, *Atherigona soccata*. Entomologia experimentalis Applicata 23: 189-199

Ogwaro. K., and E.D. Kokwaro., 1981. Morphological observations on sensory structures on the ovipositor and tarsi of the female and on the head capsule of the larva of the sorghum shoot fly, *Atherigona soccata* Rondani. Insect Science Application 2: 25-32

Omori, T., B.L. Agrawal, and L.R. House, 1983. Componental analysis of the factors influencing shoot fly resistance in sorghum. JARQ 17: 215-218

Openshaw, S.J., S.G. Jarboe, and W.D. Beavis, 1994. Marker-assisted selection in backcross breeding. *In* Proceedings of the Symposium 'Analysis of Molecular Marker Data', Corvallis, OR. 5–6 Aug. age drag. 1994. Am. Soc. Hortic. Sci. and Crop Science Soc. Am.

Ponnaiya, B.W.X., 1951a. Studies on genus sorghum I. Field Observations on sorghum resistance to insect pest, *Atherigona indica*. M. Madras Univ. J. 21: 96-117

Ponnaiya, B.W.X., 1951b. Studies on genus sorghum II. The cause of resistance in sorghum to insect pest, Atherigona indica. M. Madras Univ. J. 21: 203-217

Patil, B.V., M. Bheemanna, Somsekhar, and S.C. Hiremath, 1992. Effect of seed soaking of insecticides on Sorghum shoot fly, *Atherigona soccata* Rondani. Indian Journal of Entomology 54(4): 424-427

Patel G.M., and T.R. Sukhani, 1990b. Biophysical plant characters associated with shoot fly resistance. Indian Journal of Entomology 52: 14-17

Peter, J.A., T.G. Shanower, and J. Romeis, 1995. The role of plant trichomes in insect resistance. A selective review. Phytophaga 7: 41-63

Painter, R.H., 1951. Insect resistance in plant. Macmillan, New York, USA.

Painter, R.H., 1958. Resistance of plants to insects. A. Rev. Ent. 3: 267-290

Prioul, J.L., S. Quarrie, M. Causse, and D. deVienne, 1997. Dissecting complex physiological functions through the use of molecular quantitative genetics. Journal of Experimental Botany 48(311): 1151-1163

Peleman, Johan D. and Jeroen Rouppe van der Voort, 2003. Breeding by Design TRENDS in Plant Science Vol.8 No.7

Pereira, M.G., and M. Lee, 1995. Identification of genomic regions affecting plant height in sorghum and maize. Theoretical and Applied Genetics 90: 380-388

Patterson, A.H., Y. Lin, Z. Li, K.F. Schertz, J.F. Doebley, S.R.M. Pinson, S. Liu, J.W.
Stanzel, J.E. Irvine, 1995. Convergent domestication of cereal crops by independent
mutations at corresponding genetic loci. Science 269: 1714-1718

Paterson A.H., E.S. Lander, J.D. Hewett, S. Peterson, S.E. lincoln, and S.D. Tanksley, 1998. Resolution of quantitative traits into Mendelian factors, using a complete linkage map of restriction fragment length polymorphisms. Nature (London) 335: 721-726

Pereira, M.G., M. Lee, P. Bramel-Cox, W. Woodman, J. Doebley, and R. Whitkus, 1994. Construction of an RFLP map in sorghum and comparative mapping in maize. Genome 37: 236–243

Peng, Y., Schertz, K.F., Cartinhour, S., and Hart, G.E., 1999. Comparative genome mapping of *Sorghum bicolor* (L.) Moench using a RFLP map constructed in a population of recombinant inbred lines. Plant Breeding 118: 225–235

Rondani, C., 1871. Diptera italica non vel minus cognita descripta aut annotata. Fasc. IV. Addenda Anthomyinis. Prod. Vol. VI. Boll. Soc. Ent. Ital. 2: 317-338

Rao, M., and S. Gowda, 1967. A short note on the bionomics and control of jowar fly. Sorghum News letter 10: 55–57

Rivnay, E. 1960. Field crop pests in the near East (pest of graminaceous and leguminous crops and stored products). Monograph. Biol., Hassadeh (In Hebrew)

Ram, S., D.P. Handa, and M.P. Gupta, 1976. Effects of planting dates of fodder sorghum on the incidence of shoot fly, *Atherigona soccata* Rond. Indian Journal of Entomology 38: 290-293

Reddy, K.S., and D.V. Narasimharao, 1979. Effect of nitrogen application on shoot fly incidence and grain maturity in sorghum. Sorghum Newsletter 18: 23-24

Rajashekara, B.G., G. Raghunatha, M.K. Jagannath, and K. Krishnamurthy, 1973. Control of shoot fly infesting sorghum. Indian Journal of Entomology 35: 271-273

Raina A.K., and J.G. Kibuka, 1983. Oviposition and survival of the sorghum shoot fly on intercropped maize and sorghum. Entomologia Experimentalis Applicata 34: 107-110

Rao, S.B.P., and D.V.N. Rao, 1956. Studies on the sorghum shoot fly, Atherigona indica Malloch(Anthomyiidae, Diptera) at Srirguppa. Mysore agricultural Journal 31: 158-174

Raina, A.K., H.Z. Thindwa, S.M. Othieno and L.W. Doughlass, 1984. Resistance in sorghum to sorghum shoot fly oviposition on selected cultivars. Journal of Economic Entomology 77: 648-651

Raina A.K., 1982. Fecundity and oviposition behaviour of the sorghum shoot fly, *Atherigona soccata*. Entomologia Experimentalis Applicata 31: 381-385

Raina A. K., 1981. Movement, feeding behavior and growth of larvae of the sorghum shoot fly, *Atherigona soccata*. Insect Science Application 2: 77-81

Raina, A.K., H.Z. Thindwa, S.M. Othieno and R.T. Cork-Hill, 1981. Resistance in sorghum shoot fly: larval development and adult longevity on selected cultivars. Insect Science Application 2: 99-103

Raina A.K., 1985. Mechanisms of resistance to shoot fly (*Atherigona soccata*) in sorghum:a review. Proceedings of the International Sorghum Entomology Workshop, 15-21 July 1984, Texas A & M University, College Station, TX, USA, pp. 131-136.

Rao, N.G.P., B.S. Rana, and M.G. Jotwani, 1978. Host plant resistance to major insect pests of sorghum. In: Plant Breeding for Resistance to Insect Pests: Considerations about the Use of induced Mutations, IAEA 215, Vienna, pp. 63-78

Rivnay, 1960. Field crop pests in the near east (Pests of Graminaceous and leguminous crops and stored products) Hasadeh (in Hebrew).

Ringlund K., and E.H. Eversin, 1968. Leaf pubescence in common wheat, *Triticum aestivum*, L., and resistance to the cereal leaf beetle, *Oulema melanopus* (L.). Crop Science 8: 705-710

Rogmosa, I., F. Han, S.E. Ullrich, P.M. Hayes, and D.M. Wesenberg, 1999. Verification of yield QTL through realized molecular marker assisted selection responses in a barley cross. Molecular Breeding 5: 143-152

Richardson, T., S. Cato, J. Ramser, G. Kahl and K. Weising, 1995. Hybridization of microsatellites to RAPD: a new source of polymorphic markers. Nucleic Acids Research 23: 3798–3799

Ragot, M., M. Biosiolli, M.F. Delbut, A. Dellorco, L. Malgarini, P. Thevenin, J. Vernoy, J. Vivant, R. Zimmermann, and G. Gray, 1994. Marker-assisted backcrossing: A practical example. In: Berville A and TersacM(eds) Techniques et Utilizations des Marqueurs Moleculaires. INRA Editions, Versailles, pp 29-31.

Seshu Reddy, K.V., and J.C. Davies, 1979 a. Pests of sorghum and pearl millet and their parasites and predators, recorded at ICRISAT Center, India, up to August 1979. Cereal Entomology Progress Report no. 2. Patancheru, A.P.502324, India: International Crops Research Institute for the Semi Arid Tropics. 23pp.

Sharma, H.C., 1993. Host plant resistance to insects in sorghum and its role in integrated pest management. Crop Protection 12: 11-34

Swaine, G., and G.A. Wyatt, 1954. Observations on the sorghum shoot fly. East. Afr. Agr. Forest. J. 20: 45-48

Starks K.J., 1970. Increasing infestation of the sorghum shoot fly in experimental plots. Journal of Economic Entomology 63: 1715-1716

Sharma, H.C., 1997. Plant resistance to insects: basic principles. In: Plant resistance to insects in sorghum, (Eds. Sharma, H.C., Faujdar Singh and K.F. Nwanze), ICRISAT, Patancheru 502324, Andhra Pradesh, India, pp. 24-31

Singh, B.U., and Sharma, H.C., 2002. Natural enemies of sorghum shoot fly, *Atherigona soccata* Rondani (Diptera: Muscidae). Biocontrol Science and Technology 12: 307-323

Sandhu, G.S., 1969. Chemical control of sorghum shoot fly *Atherigona varia soccata* Rond. 'PhD thesis', Indian Agricultural Research Institute, New Delhi.

Sandhu, G.S., and W.R. Young., 1974. Chemical control of sorghum shoot fly in India. Pesticide 8: 105-135

Sukhani, T.R., and M.G. Jotwani, 1982. Insecticidal seed soaking technique for the control of sorghum shoot fly, *Atherigona soccata* (Rondani). Journal of Entomological Research 6: 104-106

Shekkarappa and S.G. Bhuti, 2007. Integrated management of sorghum shoot fly *Atherigona soccata* Rondani. Karnataka Journal of Agricultural Science 20 (3): 535-536

Singh, B.U., and B.S. Rana, 1996. Emerging strategies of integrated pest management in sorghum. Proceedings 2nd International Crop Science Congress: Crop Productivity and Sustainability- shaping the future, 17-24 Nov 1996, Indian Council of Agricultural Research, New Delhi, pp.154

Sharma, H.C., S.L. Taneja, K. Leuschner, and K.F. Nwanze, 1992. Techniques to screen sorghums for resistance to insect pests. Information Bulletin no. 32. Patancheru, Andhra Pradesh 502 324.

Smith, C.M., 1989. Plant resistance to insects. New York, USA: John Wiley and Sons, 286pp.

Sharma, H.C., S.L. Taneja, N. Kameswara Rao, and K.E. Prasada Rao, 2003. Evaluation of sorghum germplasm for resistance to insect pests. Information Bulletin No. 63, 177 pp. International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Andhra Pradesh, India.

Singh, S.R., G. Vedamoorthy, V.V. Thobii, M.G. Jotwani, W.R. Young, J.S. Blan, K.P. Srivastava, G.S. Sandhu and N. Krishnananda, 1968. Resistance to stem borer, Chilo Zonellus(Swinhoe) and stem fly, Atherigona varia Rond. in the world sorghum collection in India. Memoirs Ent. Soc. India No. 7.

Soto, P.E., 1971. Mass rearing of the sorghum shoot fly and screening for host plant resistance under green house conditions. In: Control of Sorghum Shoot Fly (Eds. Jotwani, M.T. and Young W.R). Oxford & IBH, New Delhi, pp. 132-146

SAXENA, K.N., 1969. Patterns of insect-plant relationships determining susceptibility or resistance of different plants to an insect. *Entomologia Experimentalis Applicata* 12: 751-66.

Soto, P.E., 1974. Ovipositional preference and antibiosis in relation to resistance to sorghum shoot fly. Journal of Economic Entomology 67: 265-267

Singh, R., and K.L. Narayana, 1978. Influence of different varieties of sorghum on the biology of sorghum shoot fly. Indian Journal of agricultural Science 48: 8-12

Singh, S.P., and M.G. Jotwani, 1980a. Mechanism of resistance in sorghum to shoot fly. I. Ovipositional non-preference. Indian Journal of Entomology 42: 8-12

Sharma, G.C., and B.S. Rana, 1983. Resistance to sorghum shoot fly *Atherigona soccata* (Rond.) and selection for antibiosis. Journal of Entomological Research 7: 133-138

Sree, P.S., K.F. Nwanze, D.R. Butler, D.D.R. Reddy and Y.V.R Reddy, 1994. Morphological factors of the central whorl leaf associated with leaf surface wetness and resistance in sorghum to shoot fly, *Atherigona soccata*. Annals of Applied Biology 125: 467-476

Sharma, G.C., M.G. Jotwani, B.S. Rana, and N.G.P Rao, 1977. Resistance to the sorghum shoot fly, *Atherigona soccata* and its genetic analysis. Journal of Entomological Research 1: 1-12

Singh S.P., and M.G. Jotwani, 1980b. Mechanism of resistance in sorghum to shoot fly II. Antibiosis. Indian Journal of Entomology 42: 353-360

Singh, S.P., and M.G. Jotwani, 1980c. Mechanism of resistance in sorghum to shoot fly. III. Biochemical basis of resistance. Indian Journal of Entomology 42: 551-556

Soman, P., K.F. Nwanze, K.B. Laryea, D.R. Butler and Y.V.R Reddy, 1994. Leaf surface wetness in sorghum and resistance to shoot fly *Atherigona soccata*: role of soil and plant water potentials. Annals of Applied Biology 124: 97-108

Sivaramakrishnan, S., P. Soman, K.F. Nwanze, Y.V.R. Reddy, D.R. Butler, 1994. Resistance in sorghum to shoot fly *Atherigona soccata*: evidence for the source of leaf surface wetness. Annals of applied biology 125: 215-218

Singh, S.P and M.G. Jotwani, 1980d. Mechanism of resistance in sorghum to shoot fly. IV. Role of morphological characters of seedlings. Indian Journal of Entomology 42: 806-808

Schoonhoven, L.M., 1968. Chemosensory basis of host plant selection. Annual Review of Entomology 13: 115-136

Singh, B.U., and B.S. Rana, 1986. Resistance in Sorghum to the Shoot fly. *Atherigona soccata* Rondani. Insect Science Application 7: 577-587

Singh, B.U., P.G. Padmaja, and N. Seetharama, 2004. Stability of biochemical constituents and their relationships with resistance to shoot fly, *Atherigona soccata* (Rondani) in seedling sorghum. Euphytica 136: 279–289

Stuber, C.W., and M.M. Goodman, 1983. Allozyme genotypes for popular and historically important inbred lines of corn. Southern Series, No. 16, United States Department of Agriculture, Agriculture Research Service.

Subudhi, P.K., and H.T. Nguyen, 2000. Linkage group alignment of sorghum RFLP maps using a RIL mapping population. Genome 43: 240–249

Stuber, C.W., M.D. Edwards, and J.F. Wendel, 1987. Molecular marker facilitated investigations of quantitative trait loci in maize. II. Factors influencing yield and its component traits. Crop Science 27: 639-648

Southern, E.M., 1975. Detection of specific sequences of DNA fragments separated by gel electrophoresis. I. Molecular Biology 98: 503-517

Staub, J.E., F. Serquen, and M. Gupta, 1996. Genetic markers, map construction and their application in plant breeding. Hort. Science 31: 729-741

Sangai-Maroof, M.A., R.M. Biyashev, G.P. Yang, Q. Zhang, R.W. Allard, 1994. Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations and population dynamics. Proc Natl Acad Sci USA, 91: 5466-5470

Sax, K., 1923. Association of size differences with seed coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8: 552-560

Soller, M., and J.S. Beckmann, 1983. Genetic polymorphism in varietal identification and genetic improvement. Theoretical and Applied Genetics 47: 179-190

Stuber, C.W., 1994. Enhancement of grain yield in maize using marker-facilitated introgression of QTLs. roceedings of the Symposium "Analysis of Molecular Marker Data", Joint Plant Breeding Symposia Series, American Society for Horticultural Science/Crop Science of America, Corvallis, Oregon, pp 44-46.

Sanchez, A.C., D.S. Brar, N. Huang, Z. Li, and G.S. Khush, 2000. Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. Crop Science 40: 792-797

Samis, K., S. Bowley, and B. McKersie, , 2002. Pyramiding Mn-superoxide dismutase transgenes to improve persistence and biomass production in alfalfa. Journal of Experimental Biology 53(372): 1343-1350

Sharma, Hari C., Kiran K. Sharma, and Jonathan H. Crouch, 2004. Genetic transformation of Crops for insect resistance: Potential and limitations. Critical Reviews in Plant Sciences 23(1): 47-72

Taley, Y.M., and K.R. Thakare, 1979. Biology of seven new hymenopterous parasitoids of *Atherigona soccata* Rondani. Indian Journal of Agricultural sciences 49: 344-354

Taneja, S.L., and K. Leuschner, 1984. Resistance screening and mechanisms of resistance in sorghum to shoot fly. In: Proceedings of the International Sorghum Entomology Workshop, 15–21 July, 1984, Texas A&M University, College Station, **T**exas, USA, pp. 115–129. International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Andhra Pradesh, India.

Tarumoto, I., 2004. Review. Genetic studies on glossy leaves in sorghum (*Sorghum bicolor* L. Moench). JARQ 40(1): 13-20

Taneja, S.L., K.V. Seshu Reddy, and K. Leuschner, 1986. Monitoring of shoot fly population in sorghum. Indian Journal of plant Protection 14(2): 29-36

Tarumato, I., 1980. Inheritance of glossiness of leaf blades in sorghum, *Sorghum bicolor* (L.) Moench. Japanese Journal of Breeding 30: 237-240

Taramino, G., R. Tarchini, S. Ferrario, M. Lee, and M.E. Pe, 1997. Characterization and mapping of simple sequence repeats (SSRs) in Sorghum bicolor. Theoretical and Applied Genetics 95: 66-72

Tautz, D., 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research 17: 6463–6471

Tautz, D., and M. Ranz, 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Research 12: 4127-4138

Thomas, M.R., and N.S. Scott, 1993. Microsatellite repeats in grapevine reveal DNA polymorphism when analyzed as sequence-tagged sites. Theoretical and Applied Genetics 86: 985-990

Tao, Y.Z., R.G. Henzell, D.R. Jordan, D.G. Butler, A.M. Kelly, and C.L. McIntyre, 2000. Identification of genomic regions associated with stay green in sorghum by testing RILs in multiple environments. Theoretical and Applied Genetics 100: 1225–1232

Tao, Y.Z., D.R. Jordan, R.G.I. Henzell, and C.L. McIntyre, 1998. Construction of a genetic map in a sorghum RIL population using probes from different sources and its comparison with other sorghum maps. Australian Journal of Agricultural Research 49: 729-736

Tanksley, S.D., 1983. Molecular markers in plant breeding. Plant Mol. Biol. Rep. 1:3-8

Tuinstra, M.R., E.M. Grote, P.B. Goldsbrough, and G. Ejeta, 1996. Identification of quantitative trait loci associated with pre-flowering drought tolerance in sorghum. Crop Science 36: 1337-1344

Tuinstra, M.R., G. Ejeta, and P.B. Goldsbrough, 1997. Heterogeneous inbred family (HIF) analysis: An approach for developing near-isogenic lines that differ at quantitative trait loci. Theoretical and Applied Genetics 95: 1005-1011

Tuinstra, M.R., E.M. Grote, P.B. Goldsbrough, and G. Ejeta, 1997. Genetic analysis of postflowering drought tolerance and components of grain development in sorghum.
Molecular Breeding 3: 439-448

Tuinstra, M.R., G. Ejeta, and P.B. Goldsbrough, 1998. Evaluation of near-isogenic sorghum lines contrasting for QTL markers associated with drought tolerance. Crop Science 38: 835-842

Tanksley, S.D., N.D. Young, A.H. Paterson, and M. Bonierbale, 1989. RFLP mapping in plant breeding: New tools for an old science. Biotechnology 7: 257-264

Tanksley, S.D., S. Grandillo, T.M. Fulton, D. Zamir, Y. Eshed, V. Petiard, J. Lopez, and T. Beck-Bunn, 1996. Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. Theoretical and Applied Genetics 92: 213-224

Usman, S., 1968. Preliminary studies on the incidence of shoot fly on hybrid jowar under differential sowings. Mysore Journal of agricultural Science 2: 44-48

Unnithan, G.C., K.N. Saxena, M.D. Bentley, and A. Hassanali, 1987. Role of Sorghum extract in eliciting oviposition on a non-host by the sorghum shoot fly, *Atherigona soccata* (Diptera: Muscidae). Environmental Entomology 16: 967-970

Unnithan, G.C., and K.M.S. Saxena, 1990. Diversion of oviposition by *Atherigona soccata* (Diptera, Muscidae) to non-host maize with sorghum seedling extract. Environmental Entomolology 19: 1432-1437

Unnithan, G.C., and K.V.S. Reddy, 1985. Oviposition and infestation of sorghum shoot fly *Atherigona soccata* Rondani, on certain sorghum cultivar in relation to their relative resistance and susceptibility. Insect Science Application 6: 409-412

Utz, H. F., and A.E. Melchinger, 1994. Comparison of different approaches to interval mapping of quantitative loci. Pp. 195-204. In Van Oijen, J.W., and J. Jasen(eds) Biometrics in Plant Breeding: Applications of Molecular Markers. Proc. Of the 9 th meeting of the EUCARPIA Section Biometrics in Plant Breeding. Wageningen, NL.

Vedamoorthy, G., V.V. Thobbi, B.H. Mathai, and W.R. Young, 1965. Preliminary studies with seed furrow application of insecticide for the control of sorghum maggot, *Atherigona indica* Malloch (Anthomyiidae). Indian Journal of agricultural Science 35: 14-28

Vijayalashmi, K., 1993. Study of the interrelationship of important traits contributing to the resistance of sorghum shoot fly in *Sorghum bicolor* (L.) Moench. M.Sc. Thesis, Andhra Pradesh Agricultural University, Hyderabad, 161 pp.

Visscher, P.M., C.S. Haley, and R. Thompson, 1996. Marker-assisted introgression in backcross breeding programs. Genetics 144: 1923–1932

Van Oiijen, J.W. 1992. Accuracy of mapping quantitative loci in autogamous species. Theoretical and Applied Genetics 84: 803-811

Vogel, J.M., and P.A. Scolnik, 1998. Direct amplification from microsatellites: detection of simple sequence repeat based polymorphisms without cloning. In DNA markers: protocols, applications and overviews, Eds. Caetano-Anolles, G. and Gresshoff, P.M., Wileys-Liss, Inc., New York. Pp. 133-150

Wongtong, S., and S. Patanakamjorn, 1975. Entomological research during 1975. In Thailand National Corn and Sorghum Program Annual Report, 1975, pp. 301-302. Department of Agriculture, Kasetsart University, Bangkok.

Webster, J.A., 1975. Association of plant hairs and insect resistance, an annotated bibliography. USDA Miscellanious Publications, pp. 1297

Winter, P., and Kahl, 1995. Molecular marker technologies for plant improvement. World Journal of Microbiology and Biotechnology 11: 438-448

Weber, J.L., 1990. Informativeness of human (dC-dA)n.(dG-dT)n polymorphisms. Genomics 7: 524-530

Wang, Z., J.L. Weber, G. Zhong, S.D. Tanksley, 1994. Survey of plant short tandem DNA repeats. Theoretical and Applied Genetics 88: 1-6

Weber, J.L., and P.E. May, 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase reaction. American Journal of Human Genetics 44: 388-396

Weising, K., Atkinson, R.G., and Gardner, R.C., 1995. Genomic fingerprinting by microsatellite primed PCR: a critical evaluation. PCR Methods and Applications 4: 249-255.

Wolff, K., E. Zietkiewicz and H. Hofstra, 1995. Identification of chrysanthemum cultivars and stability of fingerprint patterns. Theoretical and Applied Genetics 91: 439–447

Wu, K., R. Jones, L. Dannaeberger and P.A. Scolnik, 1994. Detection of microsatellite polymorphisms without cloning. Nucleic Acids Research 22: 3257–3258

Witcombe, J.R., and C.T. Hash, 2000. Resistance gene deployment strategies in cereal hybrids using maker-assisted selection: gene pyramiding, three-way hybrids and synthetic parent populations. Euphytica 112: 175-186

Wang, X.Y., P.D. Chen, and S.Z. Zhang, 2001. Pyramiding and marker-assisted selection for powdery mildew resistance genes in common wheat. Acta Genetic Sinica 28(7): 640-646

Wang, Z.Y., Second, G., Tanksley, S.D., 1992. Polymorphism and phylogenetic relationships among species in the genus Oryzae as determined by analysis of nuclear RFLPs. Theoretical and Applied Genetics 83: 565-581

Wye, C., Rouppe van der Voort, J., and Peleman, J., 2000. Reverse QTL mapping (RQM), an efficient approach for high resolution mapping of QTL. Poster #.. Plant and Animal Genome Conference VII, San Diego.

Xu, J.C., Weerasuriya, Y.M., and Bennetzen, J.L., 2001. Construction of genetic map in sorghum and mapping of the germination stimulant production gene response to Striga asiatica. Acta Genetica Sinica 28: 870–876 (in Chinese with English abstract).

Young, W.R., and G.L. Teetes, 1977. Sorghum Entomology. Annual Review of Entomology 22: 193-281

Young, W.R., 1972. Source of resistance to the sorghum shoot fly, Atherigona varia Rond. In: Control of Sorghum Shoot Fly (Eds. Jotwani, M.T. and Young W.R). Oxford and IBH, New Delhi, pp. 168-179

Young, N.D., 2001. Constructing a plant genetic linkage map with DNA markers. In DNA based markers in plants, Eds. Phillips, R.L. and Vasil, I.K., Kluwer Academic Publishers, The Netherlands. Pp. 31-48.

Zongo, J.O., C. Vincent, and R.K. Stewart, 1993a. Biology of Trichogrammatoidea simmondsi (Hym.: Trichogrammatidae) on sorghum shoot fly, *Atherigona soccata* (Dipt.: Muscidae) eggs. Entomophaga (France) 38(2): 267-272

Zongo J.O., C. Vincent, R.K. Stewart, 1993b. Effects of neem seed kernel extracts on egg and larval survival of the sorghum shoot fly, *Atherigona soccata* Rondani (Dipt., Muscidae). Zeitschrift für Angewandte Entomologie (Z. Angew. Entomol.), 115: 363-369

Zietkiewicz, E., A. Rafalski and D. Labuda, 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20: 176–183

APPENDICES

APPENDIX I

Preparation of Stock Solutions

CTAB (Cetyl Trimethyl Ammonium Bromide) (2%) buffer

CTAB	20G
1M Tris	200ml
5M NaCL	280ml
0.5M EDTA	40ml
Na_2SO_3	2.5g
Distilled water	460 ml

Add mercaptoethanol (0.1%) fresh while using CTAB (2%) solution.

RNase (10mg/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°c.

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	70ml
Distilled water	30ml

NaCl (5M)

Dissolved 292.2g NaCl in 750ml 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°C.

Sodium Acetate (2.5M, pH5.2)

Dissolve 340.2g sodium acetate in 500ml water. Adjust pH to 5.2 with glacial acetic acid and make volume up to 1 liter and autoclave.

Tris HCl (1M, pH8.0)

Dissolve 121.1g Tris in 800 ml of water. Adjust pH to 8.0 with conc. HCl make volume up to 1 liter and autoclave.

EDTA (0.5m, pH8.0)

Dissolve 186.1 g Na_2 EDTA.$2H_20$ 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	10ml
1M EDTA pH8.0	1ml

Make volume up to 1 liter with sterile distilled water.

T50E10 Buffer

1M Tris HCl pH 8.0	50ml
0.5M EDTA pH8.0	20ml

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, 55g Boric acid and 9.3 g EDTA. Add deionised H_20 to 1 liter. The pH is 8.3 and requires no adjustment.

6X Gel Loading Buffer (0.25% Bromophenol Blue, 40% Sucrose)(10ml)

Sucrose	4g
Bromophenol blue	2.5ml
dH20	upto 10ml

Store at 4°C.

Ethidium Bromide (10mg/ml)

Dissolve 100mg ethidium bromide in 10ml of distilled water; wrap tube in aluminium foil and store at 4°C.

Caution: Ethidium bromide is extremely mutagenic.

Acrylamide / Bisacrylamide 29:1(w/w)

Acrylamide 29g

Bisacrylamide 1g

Water (deionised distilled) up to 100ml

Store at 4°C for <=1 month.

Acrylamide / Bisacrylamide 29:1(v/v)

87 ml Acrylamide

3 ml Bisacrylamide

Add deionised distilled water to 300ml. Solution can be stored up to 1 month at 4°C.

10% (W/V) Ammonium Per Sulphate

Ammonium per Sulphate 1g

Water (deionised distilled) 10ml

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)

50mM EDTA(1 ml 0.5M EDTA, Ph8.0)

50mM NaCl (100µl of 5 M NaCl)

50% (v/v) glycerol (5ml)

Make up to 9 ml with sterilized deionised water. Add 10mg fast orange G dye and adjust the volume to 10ml. If you are using bromophenol blue and cyanol then less is required.

Binding Silane

0.15 ml Bind silane

0.5ml Acetic Acid

99.35 ml Ethanol

Mix the ingredients and store at 4°C.

100 Base Pairs Ladder (50ng/ml)

100bp ladder (stock conc. 1µg/µl)	50µl
Blue(6x dye)	165µl
T10E1 buffer	785µl

Repel Silane

Ready made, store at 4°C.

Reagents used for the Silver staining for PAGE:

0.1% (w/v) CTAB

2gm CTAB in 2 liters of distilled deionised water

1M 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

8ml 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)

30g Sodium Carbonate

0.4ml Formaldehyde

Dissolved the sodium carbonate in 2 liters of distilled deionsed water. Add 0.4 ml formaldehyde.

Glycerol Solution

30ml Glycerol into 2 liters distilled deionised water

Concentrated NaOH solution

40 gram into 1 liter of water

Trial entry list of BC4F3 (BTx623 x IS18551) prog. for shoot-fly screening K06

Seed generation:	Target =LG A BC4F3/ES 06	BC4F3 seed (g)	Marker Genotype	QTL Expected	(64 entries) K2006 trial entry no.
1	J - 2650	14	AA	-QTL_A	1
2	J - 2651	38	AA	-QTL_A	2
3	J - 2662	12	AA	-QTL_A	3
4	J - 2682	15	AA	-QTL_A	4
5	J - 2668	24	BB	+QTL_A	5
6	J - 2680	24	BB	+QTL_A	6
Seed generation:	**Target =LG E**				
1	J - 2699	15	BB	+QTL_E	7
2	J - 2708	27	BB	+QTL_E	8
3	J - 2714	21	BB	+QTL_E	9
4	J - 2743	18	AA	+QTL_E	10
5	J - 2707	13	AA	-QTL_E	11
6	J - 2712	29	AA	-QTL_E	12
7	J - 2716	12	AA	-QTL_E	13
8	J - 2726	15	AA	-QTL_E	14
Seed generation:	**Target =LG J (J1)**				
1	J - 2749	24	AAA	-QTL_J1	15
2	J - 2771	12	AAA	-QTL_J1	16
3	J - 2777	28	AAA	-QTL_J1	17
4	J - 2785	20	AAA	-QTL_J1	18
5	J - 2752	27	BBB	+QTL_J1	19
6	J - 2758	13	BBB	+QTL_J1	20
7	J - 2767	31	BBB	+QTL_J1	21
8	J - 2779	15	BBB	+QTL_J1	22
Seed generation:	**Target =LG J**				
1	J - 2799	30	AAAA	-QTL_J1+J2	23
2	J - 2814	33	AAAA	-QTL_J1+J2	24
3	296B	-	-	296B	25
4	J - 2833	19	BBBB	+QTL_J1+J2	26
5	J - 2834	14	BBBB	+QTL_J1+J2	27
Seed generation:	**Target =LG G** J - 2614	24	BB	+QTL_G	28
Seed generation:	**RILBC3F2** J2658	24	AA	+QTL_A	29
2	J2698	16	AA	+QTL_A	30
	RILs				
1	296B				31
2	RIL 153-5	16			32
3	RIL 153-6	27			33
4	RIL 153-7	36			34
5	RIL 153-8	25			35
1	RIL 189-1	41			36
2	RIL 189-2	48			37
3	RIL 189-3	36			38
4	RIL 189-4	30			39

Contd..

Trial entry list of BC4F3 (BTx623 x IS18551) prog. for shoot-fly screening K06

Seed generation	Target =LG A BC4F3/ES 06	BC4F3 seed (g)	Marker Genotype	QTL Expected	(64 entries) K2006 trial entry no.
1	RIL 252-3	37			40
2	RIL 252-4	32			41
3	RIL 252-5	38			42
6	RIL 252-9	42			43
1	BTx623				44
2	BTx623				45
3	BTx623				46
4	BTx623				47
1	IS 18551				48
2	IS 18551				49
3	IS 18551				50
4	IS 18551				51
-1	Highly susceptible control				52
(2)	Highly susceptible control				53
(3)	Highly susceptible control				54
(4)	Highly susceptible control				55
(1)	Moderately susceptible control				56
(2)	Moderately susceptible control				57
(3)	Moderately susceptible control				58
(4)	Moderately susceptible control				59
(1)	Resistant control				60
(2)	Resistant control				61
(3)	Resistant control				62
(4)	Resistant control				63
(5)	296B				64

Planting list of Shoot Fly Resistantce QTL BC4F3 sel.(296B x IS 18551) in BP 2C, Rabi 06

Design : Alfha **110 x 1 x 6 reps in 2meters**

Entry	Entry name	seed quantity,	Target	Rep - I	Rep - II	Rep - III	Rep - IV	Rep -V	Rep -VI
1	J 3022	67	+A	8068	8117	8314	8376	8515	8632
2	J 3042	41	+A	8046	8203	8298	8370	8548	8610
3	J 3044	98	-A	8035	8184	8322	8333	8460	8616
4	J 3054	75	+A	8005	8214	8235	8427	8466	8596
5	J 3059	74	-A	8098	8127	8247	8406	8530	8584
6	J 3062	50	-A	8076	8137	8304	8433	8493	8552
7	J 3063	75	+J1	8106	8194	8273	8344	8509	8646
8	J 3065	25	-J	8018	8170	8289	8353	8482	8628
9	J 3066	59	-J	8030	8176	8263	8416	8474	8655
10	J 3068	55	-J	8082	8156	8257	8384	8445	8565
11	J 3071	69	-J1	8056	8143	8225	8399	8538	8579
12	J 3072	64	+J1	8063	8213	8258	8345	8528	8603
13	J 3074	47	-J	8041	8130	8224	8351	8491	8614
14	J 3075	46	+J1	8034	8135	8315	8413	8508	8595
15	J 3081	58	-J	8009	8198	8297	8385	8489	8583
16	J 3082	59	-J	8097	8161	8325	8397	8477	8560
17	J 3085	64	-J1	8079	8173	8239	8379	8446	8650
18	J 3088	64	-J	8107	8157	8245	8367	8534	8626
19	J 3089	70	+J1	8014	8147	8305	8338	8520	8657
20	J 3091	58	+J1	8027	8118	8278	8423	8549	8563
21	J 3093	51	-J	8086	8209	8281	8401	8457	8576
22	J 3097	42	+J1	8059	8186	8269	8437	8464	8638
23	J 3101	31	+J1	8069	8202	8308	8388	8452	8652
24	J 3103	94	-J	8044	8187	8277	8398	8461	8564
25	J 3106	51	-J	8036	8215	8282	8377	8527	8572
26	J 3112	44	??	8002	8126	8268	8366	8494	8640
27	J 3114	22	??	8096	8136	8260	8332	8505	8609
28	J 3119	45	+J1	8075	8192	8228	8426	8486	8619
29	J 3121	24	+J1	8110	8166	8316	8402	8479	8594
30	J 3122	49	??	8019	8178	8291	8432	8441	8585
31	J 3124	24	-J	8021	8151	8328	8342	8535	8555
32	J 3131	35	+J1	8084	8142	8240	8356	8511	8647
33	J 3132	44	??	8060	8120	8249	8419	8546	8630
34	J 3134	73	-J	8062	8190	8243	8380	8540	8653
35	J 3145	50	+J1	8045	8218	8310	8365	8519	8569
36	J 3147	32	+J1	8039	8129	8279	8335	8542	8571
37	J 3154	44	-J	8010	8140	8286	8425	8459	8631
38	J 3158	53	+J1	8100	8200	8267	8410	8463	8602
39	J 3168	49	-J	8071	8163	8252	8438	8523	8615
40	J 3171	77	-J	8108	8172	8230	8348	8500	8599
41	J 3175	44	+J1	8017	8153	8319	8360	8506	8582
42	J 3180	39	-J1?J2?	8025	8149	8300	8417	8483	8556
43	J 3186	22	-J1?J2?	8090	8111	8321	8382	8480	8641
44	J 3197	55	-J	8052	8210	8238	8392	8449	8621
45	J 3200	40	-J1?J2?	8070	8217	8248	8339	8499	8644
46	J 3202	42	+J1	8049	8122	8309	8428	8502	8622
47	J 3213	57	+J1+J2	8033	8133	8272	8403	8484	8660
48	J 3215	38	-J1-J2	8003	8196	8290	8439	8472	8570
49	J 3222	56	-J1-J2	8094	8168	8264	8350	8450	8577
50	J 3231	71	-J1-J2	8078	8174	8256	8352	8532	8639
51	J 3235	80	+J1+J2	8104	8152	8222	8411	8512	8604
52	J 3239	45	+J1?-J2	8013	8150	8311	8386	8543	8613
53	J 3242	67	-J1?J2?	8026	8116	8299	8400	8458	8600
54	J 3243	79	+J1+J2	8088	8207	8330	8373	8470	8586
55	J 3244	112	-J1-J2	8053	8181	8232	8369	8524	8554
56	J 3268	76	-J1-J2	8061	8204	8231	8440	8488	8633

Planting list of Shoot Fly Resistantce QTL BC4F3 sel.(296B x IS 18551) in BP 2C, Rabi 06

Design : Alfha **110 x 1 x 6 reps in 2meters**

Entry	Entry name	seed quan	Target	Rep - I	Rep - II	Rep - III	Rep - IV	Rep -V	Rep -VI
57	J 3271	84	-J1-J2	8050	8188	8250	8343	8475	8601
58	J 3274	95	-J1-J2	8038	8211	8302	8358	8443	8612
59	Swarna	79	-	8001	8121	8280	8418	8533	8592
60	J 3275	164	+J1	8091	8138	8283	8390	8518	8589
61	296B	70	+J1	8077	8197	8265	8391	8544	8557
62	J 3279	61	+J1	8103	8165	8254	8375	8451	8648
63	J 3282	92	-LgE	8020	8180	8223	8364	8469	8624
64	J 3283	36	-LgE	8022	8159	8317	8340	8529	8659
65	J 3284	49	-LgE	8087	8148	8292	8430	8495	8562
66	J 3289	24	+LgE	8057	8119	8327	8408	8510	8578
67	J 3295	32	-LgE	8065	8160	8293	8422	8473	8573
68	J 3296	63	+LgE	8043	8146	8329	8404	8448	8634
69	J 3300	10	-LgE	8040	8112	8234	8435	8531	8606
70	J 3301	14	-LgE	8008	8206	8246	8346	8513	8611
71	J 3307	46	+LgE	8095	8182	8301	8359	8550	8591
72	J 3308	44	+LgE	8072	8220	8275	8412	8456	8588
73	J 3315	58	-LgE	8105	8128	8284	8383	8465	8559
74	J 3317	30	-LgE	8016	8134	8262	8395	8522	8642
75	J 3319	24	+LgE	8023	8191	8255	8378	8496	8629
76	J 3323	20	+LgE	8085	8169	8226	8368	8504	8656
77	J 3324	40	+LgE	8054	8179	8312	8331	8481	8561
78	296 B			8064	8195	8326	8361	8444	8593
79	296 B			8042	8167	8233	8336	8539	8590
80	296 B			8037	8171	8244	8421	8514	8551
81	296 B			8006	8155	8306	8407	8545	8649
82	296 B			8092	8145	8274	8431	8453	8627
83	296 B			8080	8113	8285	8349	8462	8651
84	BTx623			8109	8205	8270	8354	8526	8567
85	BTx623			8012	8189	8259	8414	8497	8574
86	BTx623			8029	8219	8221	8389	8501	8635
87	BTx623			8083	8124	8313	8394	8487	8605
88	BTx623			8055	8131	8294	8374	8476	8617
89	BTx623			8067	8158	8276	8355	8471	8636
90	IS 18551			8048	8141	8288	8420	8442	8607
91	IS 18551			8031	8114	8261	8381	8536	8620
92	IS 18551			8004	8208	8253	8393	8517	8597
93	IS 18551			8093	8185	8227	8371	8547	8587
94	IS 18551			8073	8212	8320	8362	8455	8558
95	IS 18551			8101	8125	8296	8337	8467	8643
96	Swarna			8011	8132	8324	8424	8521	8623
97	Swarna			8024	8193	8237	8405	8492	8658
98	Swarna			8081	8162	8241	8436	8507	8568
99	Swarna			8058	8175	8307	8347	8490	8580
100	Swarna			8066	8139	8229	8409	8537	8566
101	IS 1054			8047	8199	8318	8434	8516	8575
102	IS 1054			8032	8164	8295	8341	8541	8637
103	IS 1054			8007	8177	8323	8357	8454	8608
104	IS 1054			8099	8154	8236	8415	8468	8618
105	IS 1054			8074	8144	8242	8387	8525	8598
106	IS 2312			8102	8115	8303	8396	8498	8581
107	IS 2312			8015	8201	8271	8372	8503	8553
108	IS 2312			8028	8183	8287	8363	8485	8645
109	IS 2312			8089	8216	8266	8334	8478	8625
110	IS 2312			8051	8123	8251	8429	8447	8654

Planting list of Shoot Fly Resistantce QTL BC4F3 sel.(BTx623 x IS 18551) in BP 2C, Rabi 06
Design : Alfha **81 x 1 x 6 reps in 2meters**

Treat.	Entry name	Target	R I	R II	R III	R IV	R V	R VI
1	J 2650	-A	1076	2003	3070	4076	5069	6050
2	J 2651	-A	1013	2007	3080	4051	5079	6004
3	J 2662	-A	1025	2002	3058	4043	5026	6032
4	J 2682	-A	1072	2001	3004	4013	5059	6015
5	J 2668	+A	1031	2009	3029	4070	5032	6026
6	J 2680	+A	1061	2006	3051	4026	5041	6055
7	J 2699	+E	1051	2005	3037	4031	5013	6070
8	J 2708	+E	1037	2004	3013	4001	5002	6040
9	J 2714	+E	1005	2008	3022	4063	5049	6081
10	J 2743	+E	1077	2027	3003	4067	5075	6030
11	J 2707	-E	1016	2026	3036	4023	5023	6051
12	J 2712	-E	1027	2023	3049	4010	5070	6006
13	J 2716	-E	1066	2021	3042	4006	5035	6057
14	J 2726	-E	1035	2022	3014	4059	5039	6012
15	J 2749	-J1	1055	2019	3025	4033	5063	6025
16	J 2771	-J1	1046	2025	3071	4052	5008	6079
17	J 2777	-J1	1040	2020	3074	4038	5051	6066
18	J 2785	-J1	1009	2024	3057	4081	5015	6044
19	J 2752	+J1	1078	2048	3041	4055	5025	6005
20	J 2758	+J1	1015	2054	3016	4028	5065	6028
21	J 2767	+J1	1026	2049	3024	4002	5074	6048
22	J 2779	+J1	1067	2047	3069	4037	5040	6021
23	J 2799	-J	1033	2046	3078	4079	5057	6060
24	J 2814	-J	1062	2053	3059	4054	5029	6016
25	J 2886	-J1+J2?(AAAB)	1048	2050	3006	4020	5046	6037
26	J 2833	+ J	1041	2051	3032	4018	5010	6074
27	J 2834	+ J	1007	2052	3046	4064	5006	6067
28	J 2614	+G	1080	2061	3011	4048	5062	6064
29	J 2658	RILBC3F2+A	1018	2062	3020	4041	5031	6045
30	J 2698	RILBC3F2+A	1021	2058	3038	4075	5045	6080
31	J 2982	+J1-J2?(BBBA)	1064	2060	3076	4065	5011	6054
32	RIL 153-5	RILS	1036	2055	3060	4021	5003	6009
33	RIL 153-6	RILS	1063	2056	3064	4017	5054	6035
34	RIL 153-7	RILS	1054	2063	3034	4008	5072	6014
35	RIL 153-8	RILS	1042	2059	3047	4057	5076	6020
36	RIL 189-1	RILS	1003	2057	3002	4030	5022	6063
37	RIL 189-2	RILS	1073	2036	3075	4019	5028	6073

Contd..

Planting list of Shoot Fly Resistantce QTL BC4F3 sel.(BTx623 x IS 18551) in BP 2C, Rabi 06
Design : Alfha **81 x 1 x 6 reps in 2meters**

Treat.	Entry name	Target	R I	R II	R III	R IV	R V	R VI
38	RIL 189-3	RILS	1014	2028	3055	4016	5037	6068
39	RIL 189-4	RILS	1020	2031	3066	4071	5056	6038
40	RIL 252-3	RILS	1068	2034	3031	4056	5009	6031
41	RIL 252-4	RILS	1032	2033	3050	4032	5047	6049
42	RIL 252-5	RILS	1060	2032	3005	4007	5014	6001
43	RIL 252-9	RILS	1052	2030	3018	4045	5078	6056
44	2801	-LgA	1045	2035	3027	4078	5020	6010
45	2808	Recomb.fc	1008	2029	3040	4046	5067	6023
46	2822	-LgA	1079	2080	3033	4029	5044	6041
47	2867	-J1-J2	1011	2079	3054	4005	5055	6078
48	2869	+J1+J2	1023	2073	3001	4062	5033	6069
49	2890	+J1+J2	1069	2077	3015	4080	5052	6008
50	2895	-J1-J2	1030	2078	3023	4049	5017	6036
51	2898	+J1+J2	1058	2076	3039	4040	5005	6046
52	2936	+J1+J2	1053	2074	3081	4011	5019	6024
53	2947	-J1-J2	1038	2081	3056	4066	5071	6062
54	2965	+J1+J2	1002	2075	3065	4027	5080	6013
55	2967	gJ (IS258-I	1074	2042	3053	4042	5016	6018
56	2990	-J1-J2	1017	2044	3008	4077	5001	6022
57	2946	1+J2?(AAA	1019	2040	3030	4050	5048	6058
58	2998	[redacted]	1071	2045	3026	4022	5064	6071
59	BTx623	Sus.Par.	1034	2039	3044	4012	5073	6043
60	BTx623	Sus.Par.	1056	2041	3012	4068	5024	6075
61	BTx623	Sus.Par.	1047	2037	3061	4060	5058	6052
62	BTx623	Sus.Par.	1039	2038	3067	4034	5034	6007
63	IS 18551	Res.Par.	1001	2043	3077	4009	5043	6034
64	IS 18551	Res.Par.	1081	2067	3019	4014	5007	6061
65	IS 18551	Res.Par.	1012	2071	3043	4069	5050	6017
66	IS 18551	Res.Par.	1024	2068	3017	4024	5012	6019
67	SWARNA	Hi.Sus.Con	1070	2066	3062	4036	5081	6076
68	SWARNA	Hi.Sus.Con	1029	2065	3068	4003	5021	6072
69	SWARNA	Hi.Sus.Con	1057	2064	3073	4058	5066	6039
70	SWARNA	Hi.Sus.Con	1050	2072	3052	4073	5036	6029
71	SWARNA	Hi.Sus.Con	1044	2069	3007	4047	5042	6053
72	IS 1054	lod.Sus.Co	1006	2070	3035	4044	5060	6002
73	IS 1054	lod.Sus.Co	1075	2013	3063	4004	5053	6027
74	IS 1054	lod.Sus.Co	1010	2017	3072	4061	5018	6059
75	IS 1054	lod.Sus.Co	1022	2014	3079	4035	5004	6011
76	IS 1054	lod.Sus.Co	1065	2016	3048	4053	5027	6042
77	IS 2312	Hi.Res.Con	1028	2010	3009	4039	5068	6077
78	IS 2312	Hi.Res.Con	1059	2012	3028	4074	5077	6065
79	IS 2312	Hi.Res.Con	1049	2011	3021	4072	5038	6003
80	IS 2312	Hi.Res.Con	1043	2018	3045	4025	5061	6033
81	IS 2312	Hi.Res.Con	1004	2015	3010	4015	5030	6047

Randomisation for Shoot fly resistance QTL introgression lines Trial RP01/K07
(296B background of BC4F3) 20ent x 2r x 3 reps

Treat	GH/S06	Target	Rep 1	Rep 2	Rep 3
1	J3022	+A	111	220	318
2	J 3054	+A	120	205	303
3	J 3044	-A	105	210	317
4	J 3059	-A	106	215	312
5	J 3296	+LgE	114	203	304
6	J 3307	+LgE	112	207	310
7	J 3282	-LgE	116	216	306
8	J 3315	-LgE	108	202	305
9	J 3063	+J1	109	214	301
10	J 3089	+J1	101	218	311
11	J 3134	-J	119	213	319
12	J 3231	-J1-J2	102	208	308
13	J 3213	+J1+J2	117	212	313
14	J 3235	+J1+J2	104	209	302
15	BTx623	BP13/R06	107	206	315
16	BTx623	BP13/R06	103	201	320
17	296B	BP13/R06	113	219	316
18	296B	BP13/R06	110	204	314
19	IS 18551	GH/S06	118	217	307
20	IS 18551	GH/S06	115	211	309

Randomisation for Shoot fly resistance QTL introgression lines Trial
(BTx623 background of BC4F3) RP 1C Kharif 2007 Patancheru
Alpha Design 64 entries x 4 replications - 8 Blocks/rep x 8 entries/block
2 rows plots of 4 meters

Treat	Plant no	Target	Rep 1	Rep 2	Rep 3	Rep 4
1	J 2668-12	+A	153	209	347	440
2	J 2680-2	+A	112	233	355	456
3	J 2650-3	-A	129	212	354	423
4	J 2651-2	-A	101	257	327	460
5	J 2662-5	-A	123	214	310	454
6	J 2674-5	-A	114	234	309	401
7	J2699-9	+E	164	226	311	422
8	J 2708-3	+E	137	246	346	403
9	J 2714-3	+E	127	228	332	402
10	J 2743-3	+E	104	230	340	435
11	J 2707-4	-E	144	241	339	413
12	J 2712-3	-E	150	237	306	461
13	J 2716-7	-E	156	206	303	414
14	J 2726-4	-E	140	216	338	464
15	J 2614-1	+G	133	261	345	442
16	J 2614-2	+G	159	259	307	426
17	J 2614-3	+G	141	217	357	439
18	J 2614-5	+G	147	252	316	462
19	J 2752-7	+J1	122	240	333	430
20	J 2758-5	+J1	139	232	321	406
21	J 2767-5	+J1	125	221	335	449
22	J 2779-4	+J1	163	215	362	407
23	J 2749-5	-J1	143	211	308	455
24	J 2771-2	-J1	134	224	325	431
25	J 2777-2	-J1	154	231	349	425
26	J 2785-4	-J1	118	250	328	418
27	J 2833-11	+J1+J2	136	223	304	419
28	J 2834-6	J1+J2	158	220	312	444
29	J 2799-1	-J1&2	149	248	314	409
30	J 2814-5	-J1&2	121	264	359	429
31	J 2658-6	+A	162	244	324	457
32	J 2698-7	+A	131	249	353	410
33	RIL153-5		111	210	317	427
34	RIL153-6		120	247	351	448
35	RIL 153-7		102	243	330	452
36	RIL 153-8		145	227	358	451
37	RIL 189-1		110	205	326	438
38	RIL 189-2		126	235	343	417
39	RIL 189-3		119	202	336	405
40	RIL 189-4		116	229	301	441
41	RIL 252-3		157	213	331	411
42	RIL 252-4		138	204	352	433
43	RIL 252-5		151	253	360	443
44	RIL 252-9		152	258	334	424
45	BTx623		105	208	313	432
46	BTx623		107	225	342	415
47	BTx623		124	222	320	412
48	BTx623		115	218	348	463
49	IS 18551		109	254	319	434
50	IS 18551		106	219	356	458

Contd..

(BTx623 background of BC4F3) **RP 1C Kharif 2007 Patancheru**

Alpha Design 64 entries x 4 replications - 8 Blocks/rep x 8 entries/block

2 rows plots of 4 meters

51	IS 18551	161	255	337	447
52	IS 18551	135	238	364	416
53	Swarna	142	260	350	408
54	Swarna	113	242	363	421
55	Swarna	108	239	323	420
56	Swarna	103	256	302	404
57	IS 1051	117	245	305	437
58	IS 1051	148	251	344	428
59	IS 1051	146	236	329	445
60	IS 1051	160	201	341	453
61	IS 2312	130	207	318	446
62	IS 2312	155	263	322	450
63	IS 2312	128	203	361	459
64	IS 2312	132	262	315	436

Randomisation for Shoot fly resistance QTL introgression lines Observation Nursery RP01/K07
Alpha Design randomizations 84 entries x 2 replications - 12 Blocks/rep x 7 entries/block
(296B background of BC4F3) **84ent x 1rows x 2reps in 2meters**

Treat	GH/S06	Target	Rep 1	Rep 2
1	J 3022	+A	160	202
2	J 3042	+A	181	244
3	J 3054	+A	137	237
4	J 3044	-A	179	257
5	J 3059	-A	125	214
6	J 3062	-A	166	275
7	J 3089	+LgE	101	239
8	J 3296	+LgE	106	204
9	J 3307	+LgE	103	248
10	J 3308	+LgE	157	263
11	J 3319	+LgE	154	264
12	J 3323	+LgE	165	245
13	J 3324	+LgE	113	230
14	J 3282	-LgE	128	255
15	J 3283	-LgE	127	219
16	J 3284	-LgE	162	224
17	J 3295	-LgE	121	222
18	J 3315	-LgE	108	252
19	J 3317	-LgE	112	249
20	J 3063	+J1	170	269
21	J 3072	+J1	164	284
22	J 3075	+J1	109	242
23	J 3089	+J1	168	210
24	J 3091	+J1	124	203
25	J 3097	+J1	143	201
26	J 3101	+J1	174	277
27	J 3119	+J1	119	282
28	J 3121	+J1	142	216
29	J 3131	+J1	102	273
30	J 3145	+J1	161	236
31	J 3147	+J1	156	274
32	J 3158	+J1	141	213
33	J 3175	+J1	150	212
34	J 3202	+J1	144	218
35	J 3239	+J1?-J2	147	241
36	J 3275	+J1	148	283
37	J 3275	+J1	114	209
38	J 3276	+J1	133	267
39	J 3279	+J1	163	251
40	J 3065	-J	155	221
41	J 3066	-J	183	254
42	J 3068	-J	151	225
43	J 3071	-J1	135	217
44	J 3074	-J	118	207
45	J 3081	-J	146	256
46	J 3082	-J	104	258
47	J 3085	-J1	158	279
48	J 3088	-J	136	233
49	J 3093	-J	131	235

Contd..

Randomisation for Shoot fly resistance QTL introgression lines Observation Nursery RP01/K07
Alpha Design randomizations 84 entries x 2 replications - 12 Blocks/rep x 7 entries/block

(296B background of BC4F3) **84ent x 1rows x 2reps in 2meters**

50	J 3103	-J	105	229
51	J 3106	-J	180	280
52	J 3124	-J	159	211
53	J 3134	-J	132	261
54	J 3154	-J	140	262
55	J 3168	-J	182	231
56	J 3171	-J	169	250
57	J 3197	-J	107	220
58	J 3122	+J2?	111	266
59	J 3180	-J1+J2?	184	215
60	J 3186	-J1+J2?	172	240
61	J 3200	-J1+J2?	126	234
62	J 3242	-J1+J2?	117	238
63	J 3213	+J1+J2	138	243
64	J 3235	+J1+J2	110	271
65	J 3243	+J1+J2	129	246
66	J 3215	-J1-J2	153	253
67	J 3222	-J1-J2	120	247
68	J 3231	-J1-J2	130	228
69	J 3244	-J1-J2	149	270
70	J 3268	-J1-J2	167	260
71	J 3271	-J1-J2	134	205
72	J 3274	-J1-J2	176	206
73	BTx623		145	226
74	BTx623		115	208
75	BTx623		171	281
76	BTx623		139	278
77	296B		177	265
78	296B		116	276
79	296B		173	227
80	296B		175	232
81	IS 81551		178	223
82	IS 81551		123	272
83	IS 81551		122	268
84	IS 81551		152	259