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COMPARISON OF SHOOT FLY RESISTANCE QTLS IN SORGHUM INTROGRESSION LINES USING SNP GENOTYPING

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ABSTRACT

Shoot fly is a major pest in sorghum that causes significant annual yield loss. Use of pesticide to control this pest is an expensive and environmentally unsafe approach. Present study investigated the host plant resistance mechanism to develop shoot fly resistance (SFR) lines through transfer of shoot fly resistance QTLs (glossiness, trichome density, ovipositional non-preference) using SSR marker assisted backcrossing. Genomic regions for SFR showed four QTLs on SBI 01, SBI 07, SBI 05 and SBI 10 contributing up to 11.5%, 18.3%, 14% and 14.7% phenotypic variation. But QTLs on SBI 05 and SBI 10 for glossiness and trichome density are the major QTLs for which 10 SNPs have been designed. In earlier studies, three QTL regions associated with shoot fly resistance were successfully introgressed into elite cultivar SPV 1411 (Parbhani Moti) and a B line ICSB29004 using donors viz. J2658 (SBI01), J2614 (SBI10), and J2714 (SBI07) (which are derivatives of BTx 623). Phenotyping of introgression lines (ILs) led to the identification of resistant lines for each QTL region present on chromosome SBI-01, SBI-07 and SBI-10. Hence, in this study we have analysed the above developed ILs using single-nucleotide polymorphism (SNP) markers tightly linked to shoot fly resistant QTLs. The results showed that introgression lines with QTL present on chromosome SBI-10 were segregating for favorable alleles for leaf glossiness and for trichome density in homozygous condition. Other introgression lines with QTLs on chromosome SBI-01 and SBI-07 for component traits - oviposition non-preference, seedling vigor are segregated for glossiness trait also thus showing the transfer of non-targeted region, which in this case proved beneficial for SFR. This study showed that these SNPs can be used to analyze introgression lines and can be used as genomic markers for early generation selection of shoot fly resistance lines.

Key words: Sorghum, shoot fly, QTLs, *Sorghum bicolor* (L.) Moench**,** *Atherigona soccata* Rondani, host plant resistance, resistant cultivars, SSR marker, marker assisted selection, shootfly management, IPM

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most important cereal crops grown on 42 million ha in >100 countries in the semi-arid tropics of the world. Shoot fly (*Atherigona soccata* Rondani) is a major grain yield limiting factor that causes damage when sowings are delayed in the rainy season (Sharma et al. 2015). It is a serious pest in sorghum growing areas of Asia, Africa, and the Mediterranean Europe. Crop losses due to insects have been estimated to be over US$1000 million annually in the semi-arid tropics. In India, the losses due to shoot fly damage have been estimated to reach as high as 90% of grain, and 45% of fodder yield (Riyazaddin et al., 2015). Over the years various chemical, genetic and cultural management practices have been developed to manage sorghum shoot fly. The chemical options available for shoot fly management are expensive and environmentally unsafe. Therefore, developing host plant resistance is better suited for managing shoot fly. Shoot fly resistance is a complex trait and various component traits contribute to imparting shoot fly resistance in sorghum. Studies on SFR mechanisms suggested that dead hearts, oviposition, leaf glossiness, trichomes on the abaxial surface of the leaf can be used as marker traits to select for resistance to shoot fly in sorghum (Gorthy et al., 2017; Riyazaddin et al., 2015).

Quantitative trait loci (QTL) for these marker traits have already been identified and will enable to analyze the association between the traits. Satish et al. (2009) identified major QTLs on chromosome SBI 01, SBI 05, SBI 07 and SBI 10 associated with different shoot fly resistance component traits such as leaf trichome density, reduced oviposition and deadhearts incidence and leaf glossiness for shoot fly resistance in a recombinant inbred line (RIL) population. These QTLs were introduced into elite sorghum maintainer lines 296B and BTx623 using marker assisted backcrossing. Using these elite lines carrying shoot fly resistance QTLs as donors, six shoot fly resistant introgression lines were developed from three crosses involving two elite sorghum lines ICSB 29004, SPV1411 and QTL donors J2658, J2714, J2614 (derivatives of BTx623 carrying shoot fly QTLs) and the subsequent backcrosses and selfing using the foreground and background selection. The specific objective of this study was to analyze the introgression lines (ILs) using recently developed10 SNPs and assess the presence of resistance alleles.

MATERIALS AND METHODS

Two recurrent parents were used in the study. SPV1411 has bold and pearl-like grains with excellent grain and fodder quality and is drought tolerant, released by Marathwada Krishi Vidyapeeth, Parbhani. ICSB 29004 is a shoot fly resistant B line.

Donor parents: BTx 623 is a shoot fly susceptible, elite B-line and a standard genotype for sorghum genomics work. The below mentioned J Lines are donor parents (derived from BTx623) carrying QTLs from different chromosomes.

QTLs from Chromosome SBI-01: J2658-6, J2698-7

QTLs from Chromosome SBI-05: J2758-5, J2833-11, J2799, J 2834-6

QTLs from Chromosome SBI-07: J2714, J2743-3

QTLs from Chromosome SBI-10: J2614

Backcrossing was done with recurrent parent and donor parent (Plant × Plant cross) in the field and the hybridity was identified for targeted QTL using SSR markers. Two successive backcrosses were performed. Heterozygous plants were marked for each targeted QTL and crossed with recurrent parent (Plant × Plant cross) to produce $BC_1F_1$and BC_2F_2population. The introgressed lines were then selfed to obtain BC_2F_4. Three crosses ICSB 29004×J2658-6, ICSB29004×J2714 and SPV1411×J2614. Hybridity of the introgression lines were confirmed in each generation. The plants with desired QTL and phenotype were selected. Phenotyping for agronomic performance was done in BC_2F_4 generation in both yield plot as well as in the entomology field. In the entomology field, high shootfly infestation using interlard fishmeal technique was subjected to the introgression lines so as to select the resistant lines.

Genotyping was carried with molecular markers such as Simple Sequence Repeat (SSR) used for foreground selection (FGS) as well as for background selection. The ILs were analysed using newly identified SNPs. Recently, ten SNPs were identified on two chromosomes SBI- 05 with QTL J1, J2 and SBI-10with QTL G. The two chromosomes are associated with two major component traits viz. leaf glossiness and trichome density respectively, however the 6 SNPs on SBI-05 are associated with both the traits and 4 SNPs on SBI-10 are associated with leaf glossiness only. SNP genotyping was performed on all introgressed lines irrespective of the QTL present in it. Leaf discs from introgressed lines along with recurrent, donor and checks were genotyped using ‘Intratek’ platform (SNP based commercial platform) for analyzing the efficacy of SNPs in the introgression lines we developed.

Phenotyping studied in this study with two elite sorghum genotypes viz. SPV1411 (Parbhani Moti) and ICSB 29004 were used as recurrent parents. J2658-6, J2714 and J2614 [introgression lines (ILs) of BTx623, an elite sorghum line] were used as donor parents. Using these parents three crosses were made: ICSB 29004×J 2658, ICSB29004×J2714 and SPV 1411×J2614. The four validated QTLs imparting shoot fly resistance on chromosome SBI-01, SBI-07 and SBI-10 used in this study govern different component traits, such as chromosome SBI-01 for ovipositional non-preference and seedling vigor; SBI-07 for glossiness and ovipositional non-preference, and SBI-10 glossiness, oviposition non-preference, dead hearts, and leaf trichomes. Field evaluation was done by collecting the data on five component traits contributing to shoot fly resistance were recorded in 2019 rainy season, at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (17.53° N, 78.27° E) located at an altitude of 545 m above mean sea level along with checks (shoot fly resistant check IS18551 and susceptible checks Swarna and 296B).

RESULTS AND DISCUSSION

The genotyping of shoot fly introgression lines with newly identified shoot fly SNPs were done in the 2019 rainy season at ICRISAT, Patancheru India. The introgression lines contain QTLs from chromosome SBI-01, SBI-07 and SBI-10 linked to different traits such as oviposition non- preference, trichome density on lower leaf surface and leaf glossiness and their combinations. Variable number of lines were planted for the three introgressions (Table 1). The results indicated the presence of random genomic regions in the introgression lines from the donor parent genome, because of which all the introgression lines were exhibiting the resistance alleles for all three traits. The results showed that out of 8 introgression lines with QTL G with leaf glossiness, all introgression lines were segregating for both the traits in homozygous condition i.e., leaf glossiness as well as leaf trichome density (Fig. 1). Similarly, out of forty introgression lines with QTLA with oviposition non- preference, trichome density on lower leaf surface as main trait also showed the presence on favourable alleles for glossiness trait and out of 29 introgression lines with QTL E with glossiness, oviposition non-preference as main trait, showed the presence of favourable alleles for trichome density on lower surface also. The resistant source parent (IS 18551) is having homozygous favourable alleles for all the SNPs from QTLs for leaf glossiness and trichome density. Recurrent parents (ICSB 29004) also had positive homozygous SNP and one heterozygous and one unfavourable allele for 10 SNPs. Another recurrent parent (SPV1411/ Parbhani Moti) also showed similar results. Phenotyping results showed the introgression lines have lesser dead-hearts compared to their recurrent parents. The donor parent showed highest dead hearts because of shoot fly susceptible background (BTx 623) (Table 2).

To address complex issues like shoot fly resistance, molecular breeding techniques have been deployed in many crop species. Studies on shoot fly resistance mechanisms suggested that dead hearts, oviposition, leaf glossiness, trichomes on the abaxial surface of the leaf can be used as marker traits to select for resistance to shoot fly in sorghum (Dhillon et al. 2005; Anandan et al. 2009). QTL for these marker traits have already been identified and will enable to analyze the association between the traits. Satish et al. (2009) identified major QTLs on chromosome SBI 01, SBI 05, SBI 07 and SBI 10 associated with different shoot fly resistance component traits such as leaf trichome density, reduced oviposition and dead hearts incidence and leaf glossiness

Table 1. Number of plants from three introgression lines and their respective donor parent (DP), recurrent parent (RP), along with resistant check and susceptible check showing resistance alleles from 10 shoot fly SNPs

Genotypes	No. of plants	J1 (Leaf glossiness)	G (Leaf glossiness)	J2 (Trichome density lower)
ICSB 29004 x J2658(IL 1)	40	6	31	0
ICSB 29004 (RP)	4	4	4	4
J 2658 (DP)	5	5	0	5
ICSB 29004 x J2714 (IL 2)	29	23	23	23
ICSB 29004 (RP)	4	4	4	4
J 2714 (DP)	5	4	1	4
SPV 1411 x J2614 (IL 3)	8	8	8	8
SPV 1411 (RP)	3	3	3	3
J 2614 (DP)	5	5		5
IS 18551 (Resistant check)	5	5	5	5
Swarna (Susceptible check)	5	0	0	0

Table 2. Overall shoot fly infestation through dead heart (%) in the introgression lines and their corresponding parents under field conditions

Genotype	Plants with shoot fly eggs (%)	Shoot fly dead hearts (%) 28 DAE
ICSB 29004 x J2658(IL 1)	75	73
ICSB 29004 x J2714 (IL 2)	75	75
SPV 1411 x J2614 (IL 3)	73	57
ICSB 29004	89	81
SPV 1411	73	69
IS 18551	95	93

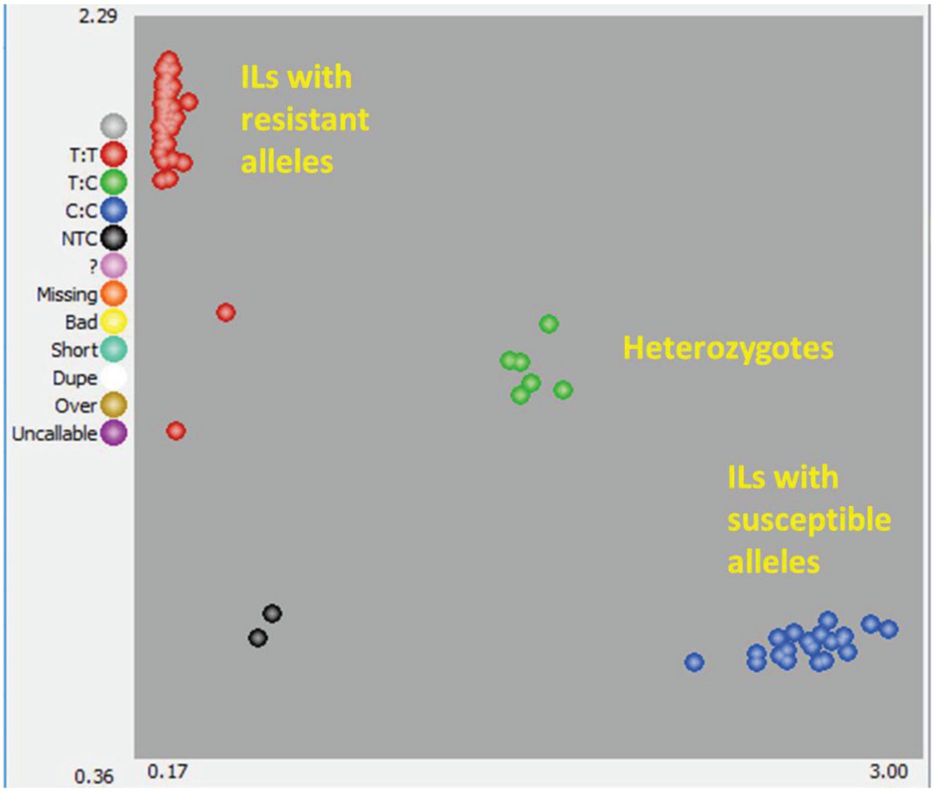

Fig. 1. SNP Genotyping of introgression lines (ILs) using 10 shoot fly SNPs along with resistant and susceptible checks. The SNP view diagram shows the presence of favourable alleles from non-targeted QTLs along with the QTL of interest, resulting in increased shoot fly resistance

for shoot fly resistance in a recombinant inbred line (RIL) population. These QTLs were introduced into elite sorghum maintainer lines 296B and BTx623 using marker assisted backcrossing. Using the elite lines as QTL donors, for the first time we developed six shoot fly resistance introgression lines from crosses involving two elite parental lines ICSB 29004 and SPV1411, and three QTL donors J2658, J2714, J2614 (derivatives of BTx 623 carrying SFR QTLs) and the subsequent backcrosses and selfing using the foreground and background selection. These introgression lines showed better shoot fly resistance and grain yield under field conditions. This study showed that these SNPs can be used to study the introgression lines and can be used as genomic markers for early generation selection of shoot fly resistance lines. The presence of different alleles from different QTLs in introgression lines shows the presence of some nonspecific regions has also been transferred at random and at these regions there are SNPs which have relevant role in shoot fly resistance mechanism (Ferreira et al., 2017). During phenotyping the shoot fly infestation was very high. The introgression lines were more resistant towards shoot fly compared to their recurrent parents. This resistance could be the effect of the presence of genomic regions from other chromosomes shows that this proportion could be higher than expected, which may be due to an overestimation of the introgressed fragment sizes. Thus, from this study we found that this back crossing method has introgressed few non targeted regions into other chromosomes which in this case has positive effect as it contains more shoot fly resistance loci and may help in the future for stacking of shoot fly resistance traits. The development of new SNP markers linked to shoot fly resistance has helped in the identification of genomic regions present in the introgressed lines. The presence of SNPs from SBI-10 in the QTL region has showed that, the elite sorghum cultivar SPV 1411 (Parbhani Moti) has been introgressed with QTL for shoot fly resistance. The occurrence of non-targeted genomic regions from other chromosomes has been an added benefit for increasing shoot fly resistance. These markers can further be used for genomic selection programs and for early generation selection of shoot fly resistance lines.

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