Agricultural Communication

Biosci. Biotech. Res. Comm. 9(2): 179-188 (2016)

BBRC Bioscience Biotechnology Research Communications

Marker assisted selection for shoot fly tolerance in sorghum, *Sorghum bicolor*

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ABSTRACT

The marker assisted selection for shootfly tolerance in sorghum (*Sorghum bicolor* (L.) Moench) was aimed at to introgress the glossiness and trichome density resistant QTLs using specific SSR markers. A total 136 BC_3 and 30 BC_4 plant progenies were obtained by crossing BC_2 X AKSV 13 R and BC3 X AKSV 13 R respectively which were then evaluated for phenotyping and genotyping using specific foreground primers. Phenotypic correlations between the component traits of shoot fly resistance were estimated based on individual progeny from each of BC_3 and BC_4 populations under study. Yield showed highly significant and positive correlation with trichome density, glossiness and pigmentation in all BC_3 and BC_4 populations under study. Foreground selection of BC_3 progenies using markers associated with QTL co-localized with genomic region for glossiness and trichome density revealed heterozygote alleles in plant no. BC_3-7, BC_3-16, BC_3-49 and BC_3-113 compared to their donor parent. Foreground selection of BC_4 revealed heterozygote alleles in plant no. BC_4-14 and BC_4-24. Amplification with foreground markers revealed QTL specific allele recovery from donor parent (IS 18551) in BC_3 and BC_4 population. Plant no. BC_3-50, BC_3-75 and BC_3-133 among BC_3 population and plant no. BC_4-6, BC_4-7 and BC_4-17 of BC_4 population showed maximum alleles with expected size.

KEY WORDS: QTL, SSR, FOREGROUND SELECTION, HETEROZYGOTE, TRICHOME, GLOSSINESS.

INTRODUCTION

Sorghum bicolor, commonly called sorghum and also known as durra or jowari, is a grass species cultivated for its edible grain. *S. bicolor* is typically an annual, but some cultivars are perennial. It grows in clumps that may reach over 4 meters high. The grain is small, ranging from 3 to 4 mm in diameter. Sorghum was domesticated in North East Africa near the equator (De wet, 1978). It belongs to family Poaceae, subfamily Panicoidae, tribe Andropogonae and the subtribe Sorghastrae (Price *et al.*, 2005). It is predominantly a self pollinated crop. Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop globally after wheat, maize,

ARTICLE INFORMATION:

*Corresponding Author: sgangurde40@gmail.com
Received 18th March, 2016
Accepted after revision 14th June, 2016
BBRC Print ISSN: 0974-6455
Online ISSN: 2321-4007

Thomson Reuters ISI SCI Indexed Journal
NAAS Journal Score : 3.48
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Online Contents Available at: http//www.bbrc.in/

rice and barley (FAO, 2006). Sorghum grain is one of the main staples for the world's poorest and most food-insecure people. Sorghum is now grown in more than 80 countries, mostly in tropical and sub-tropical regions.

Shoot fly (*Atherigona soccata*) is a major grain yield limiting factor that causes damage when sowings are delayed in rainy season (Ashok Kumar, 2008).The early sown crop escapes from shoot fly damage but the late sown crop in most cases is affected. Shoot fly infestation is high when sorghum sowings are staggered due to erratic rainfall distribution which is common in the semi arid tropics (Kumar *et al*., 2008). 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 (Sukhani and Jotwani,1980; Jotwani, 1982). Shootflies of the genus *Atherigona* are known to cause 'deadhearts' in a number of tropical grass species (Deeming 1971).

To understand the nature of gene action for inheritance of shoot fly resistance, 10 parents was evaluated, 45 F1's and their reciprocals in replicated trials during the rainy and postrainy seasons. The genotypes ICSV 700, Phule Anuradha, ICSV 25019, PS 35805, IS 2123, IS 2146, and IS 18551 exhibited resistance to shoot fly damage across seasons. Crosses between susceptible parents were preferred for egg laying by the shoot fly females, resulting in a susceptible reaction. ICSV 700, ICSV 25019, PS 35805, IS 2123, IS 2146, and IS 18551 exhibited significant and negative general combining ability (gca) effects for oviposition, deadheart incidence, and overall resistance score. (Mohammad *et al.* 2016).

Before the development of molecular markers, morphological markers were found to be useful in varietal identification and assessing genetic diversity, but they had certain limitations. Later markers based on protein variants were used with limited success. Isoelectric variants of proteins, referred to as isozymes or allozymes, were found to be useful and there were many studies made to assess genetic diversity of different crops using such protein markers (Morden *et al*., 1989). Marker-assisted selection (MAS) is a new paradigm in plant breeding. It involves the selection of genotypes carrying a desirable gene, or gene combination, via linked marker(s). Breeders practice marker-assisted selection when an agronomically important trait that is difficult to assess. Through MAS, the transfer of traits from exotic donor parents to more elite locally adapted crop cultivars is possible. Backcrossing has been a widely used technique in plant breeding for almost a century.

The shootfly dead heart percentage has been estimated to be 43.5% during the Rabi 2012-13 in Maharashtra (Anonymous 2013). Given the economic impact of shoot fly, the improvement of genetic resistance to this pest is one of the major goals in sorghum breeding programs in India. In this context, a better understanding of the inheritance of resistance and identification of genomic regions/QTL that influence resistance can help the breeders to develop more efficient and effective breeding and selection schemes through marker-assisted selection (MAS).

MATERIAL AND METHODS

The experimental sorghum seed material comprised of backcross populations developed from the cross AKSV-13R X IS-18551. Experimental seed material was developed by crossing BC_2 with recurrent parent (recipient parent) to obtain BC_3 seed in kharif 2013 and BC_4 seed was obtained by crossing BC_3 with recurrent parent (recipient parent) in rabi 2013-14. The experiment was carried out at Sorghum Research Unit, Dr. PDKV, Akola. Plant protection measures are avoided for recording morphological observations. Developed population was grown at a spacing of 45 cm X 15 cm with simple lattice design.

Following observations were recorded on each of the parent and the individual plants in each population. Pink pigment on plumule and leaf sheath was assessed at 5 DAE at 1 to 5 rating scale as per Sharma *et al.* (1997) as (1-Plumule and leaf sheath with dark pigment, 2- Plumule and leaf sheath with fair pink pigment, 3- Plumule and leaf sheath with light pink pigment, 4-Plumule and leaf sheath with very light pink pigment, 5-Plumule and leaf sheath with green colour).

Leaf glossiness was evaluated on a 1 to 5 rating as given below at 10 DAE in the early morning hours. When there was maximum reflection of light from the leaf surfaces as 1- Highly glossy (Light green, shinning, narrow and erect leaves), 2 - Glossy (Light green, less shinning and erect leaves), 3 - Moderate glossy (Fair green, light shinning, medium leaf width and less dropping leaves), 4 - Moderate non glossy (Green, broad, dropping leaves), 5 - Non glossy (Dark green, dull, head and dropping leaves).

The observations of dead heart count were taken on 14th and 21st DAE for each entry. Dead Heart percentage was calculated as the number of dead hearts with respect to total number of plants in the entry multiplied by 100. The observations were taken on 14th and 21st DAE for each entry. Seedling with eggs laid by shoot fly larvae was expressed in terms of percentage of the plants. The seedlings with eggs percentage was calculated by number of seedlings with eggs with respect to total number of plants in the entry multiplied by 100.

Observations Trichome density (mm^2) were taken on 14th DAE, on fifth leaf from apical point as per the procedure outlined by Sharma *et. al.* (1997). leaf samples were

kept in 20 ml of acetic acid: alcohol (2:1) solution for overnight then transferred in 90 percent lactic acid in small vials and stored for further observations. The leaf samples were mounted on a slide in a drop of water and observed under a binocular microscope at magnification of 10x. The number of trichomes per mm^2 was counted on abaxial (lower side) leaf surface.

For Genotypic observations, genomic DNA was isolated from each of the genotype using a modified cetyl tri methyl ammonium bromide (CTAB) method (Sharma *et al*., 2002). For DNA extraction two gram leaf samples of three to four weeks old seedlings of each BC_3 and BC_4 population were used. DNA quantified on 0.8% agarose gel followed by Ribonuclease A treatment.

PCR AMPLIFICATION

SSR primer pairs were used for the present investigation. The list of forward and reverse primers used for amplification of the genomic DNA with their sequences, is given in table 1. The synthesis of following primers was done from Genaxy Scientific Pvt. Ltd., India.

Table1: The list of SSR (primer) markers used for amplification of the genomic DNA.

SN	Primer Code	Forward and Reverse	Annealing Temp. (°C)	Product Size (bp)	Linkage group
1.	*Xtrp* 37	F-AACCTAAGAGGCCTATTTAACC	57	165	A
		R-ACGGCGACTCTGTAACTCATAG			
2.	*Xtrp* 75	F-CGATGCCTCGAAAAAAAAACG	57	149	A
		F-CCGATCAGAGCGTGGCAGG			
3.	*Xtrp* 141	F-TGTATGGCCTAGCTTATCT	55	200-205	G
		R-CAACAAGCCAACCCAAA			
4.	*Xgap* 1	F-TCCTGTTTGACAAGCGCTTATA	56	180-254	G
		R-AAACATCATACGAGCTCATCAATG			
5.	*Xtrp* 15	F-CACAAACACTAGTGCCTTATC	55	215	J
		R-CATAGACACCTAGGCCATC			
6	*Xtrp* 65	F-CACGTCGTCACCAACCAA	55	310	J
		R-GTTAAACGAAAGGGAAATGGC			
7	*Xtrp* 94	F-TTTCACAGTCTGCTCTCTG	50	232	J
		R-AGGAGAGTTGTTCGTTA			
8	*Xisp*10263	F-TATCTTCTCCGCCCTTTC	52	330-360	G
		R-TAAGNGCCAAGGGAATG			
9	*Xtrp*10258	F-GCAGGACCGGATAGAGAT	53	190-270	J
		R-ATCCCGGAATGATGAAGT			
10	*Xtrp* 1	F-TTGGCTTTTGTGTGGAGCTG	49	192	B
		R-ACCCAGCAGCACTACACTAC			
11	*Xtrp* 4	F-AATACTAGGTGTCAGGGCTGTG	61	150	B
		R-ATGTAACCGCAACAACCAAG			
12	*Xtrp* 67	F-CCTGACGCTCGTGGCTACC	59	178	F
		R-TCCACACAAGATTCAGGCTCC			
13	*Xgap* 88	F-CGTGAATCAGCGAGTGTTGG	57	118	A
		R-TGCGTAATGTTCCTGCTC			
14	*Xtrp* 298	F-GCATGTGTCAGATGATCTGGTGA	63	187	B
		R-GCTGTTAGCTTCTTCTTCTAATCGTCGGT			
15	*Xtrp* 319	F-TAGACATCTGAATTAAGGAGC	53	170	A
		R-CATGCCCCTGAAAGAGA			
16	*Xtrp* 331	F-AACGGTTATTAGAGAGGGAGA	55	220	G
		R-AGTATAATAACATTTTGACACCCA			
17	*Xtrp*159	F-ACCCAAAGCCCAAATCAG	49	180	E
		R-GGGGGAGAAACGGTGAG			

The PCR was carried out in small reaction tubes, containing a reaction volume typically of 16 µl that was inserted into a thermal cycler (Applied Biosystems Gene Amp PCR System 2700) that heated and cooled the reaction tubes within it to the precise temperature required for each step of the reaction. The working concentration of master mix for 16 µl (final volume) was prepared as 0.9mM 10 X Taq polymerase assay buffer with $MgCl_2$, 2.4mM dNTPs and 0.5 U Taq polymerase. 2 µl of DNA sample was used with 1.5 µl of each primer. The amplified products were resolved on 10% PAGE on a Genei's Vertical Gel Electrophoresis System (glass plate size 24 cm x 19 cm).To resolve the SSR products the silver staining was carried out following Tagelstrom (1992).

DATA ANALYSIS

The banding patterns obtained from PCR amplification of the various SSR primer in each of the BC_3 and BC_4 population was visualized with silver staining were scored as 'A' [Homozygote for the allele for parental strain P1 (IS18551)], 'B' [Homozygote for the allele for recurrent parental strain P2 (AKSV 13 R)], 'H' (Heterozygote carrying the alleles from both P1 and P2 parental strains), '-' (Missing data for the individual).

Phenotypic correlation

Phenotypic correlation coefficients were estimated using the formula of Singh and Choudhary (1996).

$$r_p = \frac{Cov_p (X, Y)}{Var_p (X)\ Var_p (Y)}$$

Where,

r_p = Phenotypic correlation coefficient
Cov_p (X,Y) = Phenotypic covariance between x and y
Var_p (X) = Phenotypic variance in X
Var_p(Y) = Phenotypic variance in Y

The observed values of correlation coefficients were compared with the tabulated values for (n-2) degrees of freedom to test their significance.

RESULTS AND DISCUSSION

The BC_3 population was derived from crossing BC_2 with recipient parent and BC_4 population derived from BC_3 population crossed with recipient parent. To identify the reaction linked to the shoot fly tolerance components traits SSR markers were used and morphological observations were recorded. The experiment was conducted in late kharif and late rabi 2013 at Sorghum Research Unit, Dr. Panjabrao Deshmukh Krish Vidyapeeth Akola. The results obtained from morphological and molecular analysis using specific SSR primers for shoot fly resistance are discussed in detail in this chapter.

Phenotypic performance of parents AKSV- 13R and IS 18551 revealed that both the parents differ for the shootfly resistance contributing traits like leaf glossiness (1-5 scale), pigmentation (1-5 scale), percent seedling with eggs at 14th and 21st days after emergence, dead heart percentage at 14th and 21st days after emergence and trichome density measured on abaxial surface per mm^2 of leaf. The mean performance of parents for these traits are summarized below:

Table 2: Mean phenotypic performance for shoot fly resistance component traits in the parents.

Character	IS18551 (resistant)	AKSV-13R (susceptible)
Leaf glossiness (1-5 scale)	1	4
Pigmentation (1-5 scale)	1.5	4
Seedling with egg per cent 14th and 21th DAE	10,12	45,50
Deadhearts per cent at 14th and 21th DAE	9, 11	47, 52
Trichome density (per mm^2)	28	3

The resistant donor parent IS18551 recorded rating of one on scale five leaf glossiness as compared to rating four leaf glossiness in parent AKSV-13R on 1-5 scale. Similarly differences for mean performances for pigmentation was recorded in two parents as IS18551 (1.5) and AKSV-13R (4) on 1-5 scale. The mean percent seedlings with eggs recorded after 14th and 21st days after emergence were 10 and 12 percent respectively in IS 18551. Which were numerically lower than AKSV- 13R as 45 and 50 percent after 14th and 21st days after emergence respectively. Similarly dead heart percentage at 14th and 21st days after emergence varied in two parents, AKSV- 13R (47% and 52% on 14th and 21st DAE) and IS18551 (9% and 11% on 14th and 21st DAE). Trichome density was higher in IS 18551 (28 per mm^2) as compared to AKSV -13R (3 per mm^2).

Total 136 plants of BC_3 and 30 plants of BC_4 population were studied. BC_3 population was studied for phenotypic traits related to shoot fly resistance such as glossiness, pigmentation, trichome density, egg laying percent, dead heart percent and yield per plant. Mean phenotypic performance for shoot fly resistance component traits in the BC_3 of cross AKSV-13R X IS18551.

In BC_3 population of AKSV -13 R x IS18551 cross, pigmentation ranged from 1.5 to 4.5. Highest pigmentation was recorded in plant no. BC_3-7 which was 1.5 and lowest pigmentation observed in plant no BC_3-111 which was 4.5. Whereas pigmentation in remaining plants

ranged from 2.5 to 4. In BC_3 population of AKSV-13 R x IS18551 cross, glossiness was ranged from 1 to 4. Out of the 136 plants, plant no. BC_3- 111, BC_3-127, BC_3-128, BC_3-129, BC_3-131, BC_3-132, BC_3-133, BC_3-136 showed lowest glossiness rating 4 and plant no. BC_3-7 and BC_3-113 showed highest glossiness rating 1. Whereas glossiness in remaining plants ranged from 1 to 4.

In BC_3 population of AKSV -13 R x IS18551 cross, trichome density ranged from 1 to 56.33 trichomes/mm^2. Highest trichome density of 56.33 trichomes/mm^2 was found in plant no. BC_3-7 and lowest trichome density of 1 trichome/mm^2 was found in plant no. BC_3-111. Seedlings with eggs on abaxial surface were recorded on 14th and 21st day after emergence (DAE). The presence of eggs on 14th and 21st DAE was found in plants BC_3- 38, BC_3-84, BC_3-111, BC_3-127, BC_3-132, BC_3- 136. Whereas the presence of eggs on 21st DAE was found in BC_3-4, BC_3-5, BC_3-18, BC_3-19, BC_3-31, BC_3-32, BC_3-128.

Total dead heart damage observed at 14th DAE and 21st DAE. The presence of dead heart damage on 14th and 21st DAE was found in plants BC_3- 38, BC_3-84, BC_3-111, BC_3-127, BC_3-132, BC_3- 136. Whereas the presence of dead heart damage on 21st DAE was found in BC_3-4, BC_3-5, BC_3-18, BC_3-19, BC_3-31, BC_3-32, BC_3-128. Yield per plant in BC_3 population ranged from 22 gm to 86 gm. Plant no. BC_3-7 and BC_3-113 showed highest yield 86 gm whereas plant no. BC_3-136 showed lowest yield 22 gm. Total 69 plants showed more yield than mean yield that is 53.47.

Mean phenotypic performances for shoot fly resistance component traits in the BC_4 of cross AKSV-13R X IS18551. In BC_4 population of AKSV -13 R x IS18551 cross, pigmentation ranged from 1.5 to 4. Highest pigmentation was recorded in plant no. BC_4-14 and BC_4-20 which was 1.5 however lowest pigmentation observed in plant no. BC_4-18 which was 3.5. In BC_4 population of AKSV -13 R x IS18551 cross, glossiness was ranged from 1 to 3.5. Out of the thirty plants, plant no. BC_4-18 showed lowest glossiness of 3.5 and plant no. BC_4-14 and BC_4-20 showed highest glossiness of 1 where as glossiness in remaining plants ranged from 1 or 3.

In BC_4 population of AKSV -13 R x IS18551 cross, trichome density ranged from 4 to 59.33 trichome/mm^2. Highest trichome density was found in plant no. BC_4-14 as 59.33 trichomes/mm^2 and lowest trichome density was found in plant no. BC_4-18 as 4 trichomes/mm^2. Seedlings with eggs on abaxial surface were recorded on 14th and 21st day after emergence (DAE). The presence of eggs on 14th and 21st DAE was found in plant BC_4-18. Whereas presence of eggs on 21st DAE was found in plants BC_4-22 and BC_4-27. Total dead heart damage observed at 14th DAE and 21st DAE. The presence of dead heart damage on 14th and 21st DAE was found in plant BC_4-18. Whereas presence of dead heart damage on 21st DAE was found in plants BC_4-22 and BC_4-27.

Yield per plant in BC_4 population ranged from 12 gm to 83 gm. Plant no. BC_4-14 showed highest yield 83 gm and plant no BC_4-18 showed lowest yield 12 gm. Total 13 plants BC_4-1, BC_4-2, BC_4-4, BC_4-11, BC_4-12, BC_4-14, BC_4-15, BC_4-16, BC_4-17, BC_4- 20, BC_4-21, BC_4-24, BC_4-28 showed more yield than mean yield that is 48.83.

Simple linear correlation coefficient between shoot fly resistance components were estimated based on each plant of BC_3 and BC_4. This helps to understand the

Table 3: Phenotypic correlation coefficient among the four morphological traits in BC_3 derived from cross AKSV-13R X IS18551

Trait	Pigmentation	Leaf glossiness	Trichome density	Yield
Pigmentation	1	0.84**	0.57*	0.66**
Leaf glossiness		1	0.68**	0.74**
Trichome density			1	0.93**
Yield				1

**Significant at P= 0.01= highly significant *Significant at P = 0.05 = significant, N. S. = Non Significant

Table 4: Phenotypic correlation coefficient among the four morphological traits in BC4 derived from cross AKSV-13R X IS18551

Trait	Pigmentation	Leaf Glossiness	Trichome Density	Yield
Pigmentation	1	0.86**	0.76**	0.76**
Leaf Glossiness		1	0.77**	0.72**
Trichome Density			1	0.97**
Yield				1

** Significant at P= 0.01= highly significant *Significant at P = 0.05 = significant, N. S. = Non Significant

Table 5: The score sheet indicating the genotypic status for trichome density and glossiness QTLs among BC3 progenies derived from AKSV 13R X IS 18551 (Foreground Selection)

Plant no.	Xtxp-15	Xtxp-75	Xtxp-94	Xtxp-37	Xisp-10258	Xtxp-141
BC_3-3	A	A	H	H	A	A
BC_3-7	H	H	H	H	H	H
BC_3-16	H	H	H	H	H	H
BC_3-17	A	H	A	A	A	A
BC_3-19	H	A	A	H	A	A
BC_3-21	A	H	A	A	A	A
BC_3-25	A	H	A	H	A	A
BC_3-27	A	A	H	A	A	A
BC_3-28	H	A	A	A	A	A
BC_3-29	A	H	A	A	A	A
BC_3-35	A	H	H	H	H	A
BC_3-39	A	H	A	H	A	A
BC_3-42	A	H	A	A	A	A
BC_3-48	A	H	A	A	A	A
BC_3-49	H	H	H	H	H	A
BC_3-55	A	H	A	A	A	H
BC_3-61	A	H	A	A	H	A
BC_3-66	A	H	A	H	A	A
BC_3-69	A	A	H	A	A	H
BC_3-71	H	A	A	A	A	A
BC_3-74	H	A	A	A	A	A
BC_3-75	A	A	H	A	H	A
BC_3-76	A	H	A	A	A	A
BC_3-78	H	A	A	H	A	A
BC_3-82	A	H	A	A	H	A
BC_3-84	A	A	H	A	A	H
BC_3-87	A	H	A	A	H	A
BC_3-89	H	A	A	A	A	H
BC_3-96	A	H	A	H	H	A
BC_3-98	A	H	A	A	A	H
BC_3-110	A	H	A	H	H	A
BC_3-113	H	H	H	H	H	H
BC_3-117	A	H	A	H	A	H
BC_3-118	A	A	H	A	A	H
BC_3-126	A	A	H	H	A	A
BC_3-132	A	H	A	A	H	A
BC_3-135	A	H	A	H	H	A

Where, 'A'- Homozygote at marker locus as that of donor parent (IS 18551)
'B'- Homozygote at marker locus as that of recurrent parent (AKSV 13R)
'H' – Heterozygote at marker locus as that of donor parent

varying degree of association and contribution of each character in building up total genetic architecture of resistance considering the varying degree of phenotypic values.

Marker-assisted selection appeared as a promising tool for breeding quantitative resistance. Two problems are inherent in backcross breeding, one being the large number of generations required to recover the genome of the inbred parent and the other being linkage drag, i.e. the presence of the inbred portions of the donor's genome (which can be linked to non-favourable alleles) surrounding the introgressed allele. As previously described by Tanksley et al. (1996), the main advantage of using DNA markers as opposed to conventional selection is to accelerate the fixation of recipient alleles in non target regions and to identify the genotypes containing crossovers close to target genes.

In present investigation a set of markers are used to screen the parents of the backcross populations AKSV-13R and IS18551 (resistance) to identify polymorphic one for further genotyping the BC_3 and BC_4 population. Screening was done on 10% PAGE gel where silver staining recipe was adopted. Marker showed polymorphism between parent were selected for screening of the BC_3 and BC_4 population. Total 17 markers were used for parental screening out of these 8 showed polymorphism. Recurrent parent, AKSV 13R, susceptible for shoot fly while being agronomically superior were backcrossed with donor parent IS 18551. All the backcross progenies obtained for AKSV 13R were screened with foreground markers to identify the progenies with target QTLs.

The target QTL from donor parents is insured in recurrent parent background by selecting for heterozygous for the flanking markers. A total 136 plants of the BC_3 population screened with six polymorphic foreground markers (Xtxp-37, Xtxp-75, Xtxp-141, Xtxp-94, Xtxp-15, Xisp-10258). The band in the gels was scored as A, B, H and "_" based on the pattern compared with those of the parents. Only the plants with marker status 'H' for the all target marker were scored and selected. Out of the 136 plants, only plant no. BC_3-7, BC_3-16, BC_3-113, were found to be heterozygous (H) for six primers for target QTL region and plant no. BC_3-49 showed heterozygous banding pattern in five primers highlighted in table 5.

Foreground selection for QTL introgression in AKSV 13R X IS 18551 BC_4 populations:

A total 30 plants of the BC_4 populations screened with nine polymorphic foreground markers (Xtxp-37, Xtxp-75, Xtxp-141, Xtxp-94, Xtxp-15, Xisp-10258, Xisp 10263, Xgap 1, Xtxp 65). Only the plants with marker status 'H' for the all target marker were scored and selected. Out of the 30 plants, plant no. BC_4-14 showed heterozygous banding pattern in seven primers while plant no. BC_4-24 showed heterozygous banding pattern in six primers highlighted in table 6.

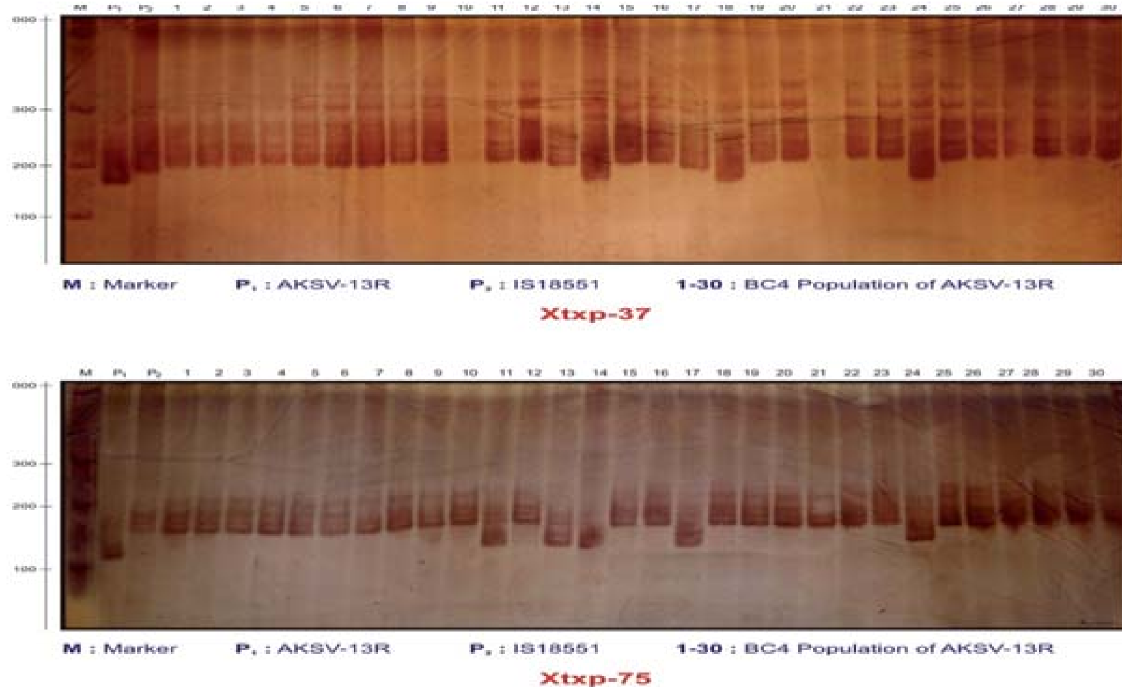

Table 6: Summary statistics of foreground selection for AKSV 13R X IS 18551 derived BC3 progenies		
Sr. No.	Particulars	AKSV-13R X IS18551
1	Number of plants screened	136
2	Number of polymorphic marker used	6
3	Scorable marker data points generated	816
4	Number of progeny satisfying the foreground selection for all the targeted QTL regions	4
5	Marker status of selected plants at target QTL regions	Heterozygous

Table 7: The score sheet indicating the genotypic status for trichome density and glossinessQTLs among BC4 progenies derived from AKSV 13R X IS 18551 (Foreground Selection)

Plant no.	Xtxp-37	Xisp-10263	Xtxp-141	Xtxp-75	Xtxp-15	Xisp- 10258	Xtxp-94	Xgap-1	Xtxp-65
BC_4-2	A	A	A	A	A	A	A	H	A
BC_4-3	A	H	H	A	A	A	A	A	A
BC_4-4	A	A	A	A	A	A	H	A	A
BC_4-6	A	A	A	A	A	A	A	H	A
BC_4-7	A	A	A	A	A	A	A	A	H
BC_4-8	A	H	A	A	A	A	A	A	A
BC_4-11	A	A	A	H	A	A	A	A	A
BC_4-12	A	A	A	A	A	H	A	H	A
BC_4-13	A	A	A	H	H	A	A	A	H
BC_4-14	H	H	H	H	H	H	H	A	A
BC_4-15	A	A	A	A	A	A	A	A	H
BC_4-17	A	A	A	H	A	A	A	A	A
BC_4-18	H	A	A	A	A	A	A	A	A
BC_4-24	H	H	H	H	H	H	A	A	A
BC_4-25	A	A	A	A	A	A	A	A	H
BC_4-26	A	A	H	A	A	A	A	A	A

Where, 'A'- Homozygote at marker locus as that of donor parent (IS 18551)
'B'- Homozygote at marker locus as that of recurrent parent (AKSV 13R)
'H' – Heterozygote at marker locus as that of donor parent

Table 8: Summary statistics of foreground selection for AKSV 13R X IS 18551 derived BC_4 progenies		
Sr. No.	Particulars	AKSV-13R X IS18551
1	Number of plants screened	30
2	Number of polymorphic marker used	9
3	Scorable marker data points generated	270
4	Number of progeny satisfying the foreground selection for all the targeted QTL regions	2
5	Marker status of selected plants at target QTL regions	Heterozygous

QTL specific allele recovery from donor parent in BC_3 and BC_4 population of various crosses

All the BC_3 and BC_4 progenies were tested for the presence of trichome density and glossiness specific donor allele from donor parent using polymorphic foreground markers. The data is scored for presence and absence for specific allele.

QTL specific allele recovery from donor parent IS18551 in BC_3 population of AKSV-13R x IS 18551:

Allele specific for trichome density and glossiness were recovered using six polymorphic foreground markers Xtxp-37, Xtxp-75, Xtxp-141, Xtxp 15, Xtxp 94, Xisp 10258. Plant no. BC_3-50, BC_3-75 and BC_3-133 showed presence of specific allele using all the primers with expected size.

QTL specific allele recovery from donor parent IS18551 in BC_4 population of AKSV-13R x IS 18551:

Allele specific for trichome density and glossiness were recovered using nine polymorphic foreground markers Xtxp-37, Xtxp-75, Xtxp-141, Xtxp-94, Xtxp-15, Xisp-10258, Xisp 10263, Xgap 1, Xtxp 65. Plant no. BC_4-6, BC_4-7 and BC_4-17 showed maximum alleles using all the primers with expected size.

CONCLUSION

The results obtained from morphological observations revealed that the two parents differed considerably for all the observed shoot fly resistance component traits and the wide variation was observed among the BC_3 and BC_4 population for shoot fly resistance component traits. Phenotypic correlations between the component traits of shoot fly resistance were estimated based on individual progeny from BC_3 and BC_4 means. The results so obtained can be briefly summarized as yield showed highly significant and positive correlation with trichome density, leaf glossiness and pigmentation in BC_3 and BC_4 population of AKSV-13R X IS 18551. For molecular marker studies, genomic DNA was isolated from the parent and all BC_3 and BC_4 population using CTAB method. DNA quality was confirmed by running them on 0.8 per cent agarose gel stained with ethidium bromide. DNA samples were treated with RNase A (10 mg/ml) @100 µl / ml of sample. Quantification of DNA was done and accordingly, the samples were diluted to a final concentration of 15 ng/µl. 30 ng of DNA was used for each PCR reaction.

Foreground selection using specific markers for glossiness and trichome density QTLs of BC_3 and BC_4 plants were carried out. Out of 136 BC_3 populations, Plant no. BC_3-7, BC_3-16, BC_3-113 were found to be heterozygous for six primers and plant no. BC_3-49 showed heterozygous banding pattern in five primers. Whereas in BC_4 population plant no. BC_4-14 showed heterozygous banding pattern in seven primers and plant no. BC_4-24 showed heterozygous banding pattern in six primers. QTL specific allele recovery from donor parent IS 18551 in BC_3 and BC_4 population was carried out. Out of 136 BC_3 population plant no. BC_3-50, BC_3-75 and BC_3-133 showed presence of specific alleles using six polymorphic foreground markers. Whereas plant no. BC_4-6, BC_4-7 and BC_4-17 showed maximum alleles using all the primers with expected size in BC_4 population.

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