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Bulk Segregant Analysis for the Identification of Shoot Fly Resistance Linked Molecular Marker in Sorghum [*Sorghum bicolor* (L.) Moench]

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ABSTRACT

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most important crops in the semi-arid regions of the world. One of the important biotic constraints to sorghum production in India is the shoot fly which attacks sorghum at the seedling stage. The study was undertaken during 2021–22 at the Centre for Millets Research, Sardarkrushinagar Dantiwada Agricultural University, Deesa, Gujarat, India to assess linked molecular markers for sorghum shoot fly resistance using the bulk segregant analysis (BSA) method with simple sequence repeat (SSR) marker from developed F_2 mapping population with two genetically diverse parental lines, SWARNA (susceptible to shoot fly) and IS 18551 (resistant to shoot fly). Sixty-five SSRs primers pair were used for the parental polymorphism survey using two contrasting parents to detect the primers exhibiting polymorphism. Eight out of sixty-five primers showed polymorphism (12.30%) between two contrasting parental lines in sorghum. Two out of eight polymorphic SSRs primer pairs *i.e.,* Xtxp 67 and Xgap 88 were found polymorphic between resistance and susceptibility in parents and bulks and thus reported to be putatively linked with shoot fly. Bulk segregant analysis (BSA) was extended to identify the traits controlled by minor genes with additive effects, which increased the power and efficiency of this molecular technique to construct genetic map in the sorghum crop improvement program. The identified SSRs markers *i.e.,* Xtxp 67 and Xgap 88 might be useful to screen resistance for shoot fly infestation in future sorghum improvement program.

KEYWORDS: Bulk segregant analysis, linked molecular marker, polymorphism, shoot fly

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1. INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is a C4 photosynthetic cereal crop commonly known as sorghum, great millet, or jowar. It belongs to grass species cultivated for its edible grain, fodder and biofuel purposes (Patel et al., 2018, Rathod et al., 2020). *Sorghum bicolor* (L.) is typically an annual, but some cultivars are also found perennial. It grows in clumps that may reach over 4 metres height. 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,* sub-family *Panicoi-dae*, tribe *Andropogonae* and the sub-tribe *Sorghastrae* (Price et al., 2005). Sorghum is truly diploid species having 2n=2x=20 chromosome number with a whole draft genome sequence of 730 Mb size. Sorghum has an excellent photosynthetic efficiency and biomass production capacity (Peterson et al., 2002). Sorghum is the world's fifth most important cereal crop and ranks fourth in India (Goswami et al., 2020, Gami et al., 2021). It is potentially the number one cereal for sub-Saharan Africa's semi-arid environments (Taylor, 2003). The pollination behaviour is often cross pollinated.

Many of sorghum varieties were highly susceptible to shoot fly [*Atherigona soccata* (L.) Rondani] which often resulted in severe damage to the crop (Jotwani et al., 1970, Sharma et al., 2015). Shoot fly of the genus *Atherigona* are known to cause 'deadhearts' in a number of tropical grass species (Deeming, 1971). Adoption of chemical control is not economically feasible for resource-poor farmers of the semi-arid tropics (SAT), as the low crop value per acre precludes the use of insecticides. 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, Mohammed et al., 2016). In India, the losses due to shoot fly damage have been estimated to reach as high as 90.00% of grain and 45.00% of fodder yield (Sukhani and Jotwani, 1980). Glossy leaves (Agrawal and House, 1982), seedling vigour (Jadhav et al., 1986) and trichomes on the abaxial leaf blade (Maiti and Bidinger, 1979) are reported to be the main factors responsible to shoot fly resistance. To identify sources of resistance to shoot fly, a large number of sorghum germplasm accessions were screened and resistance sources were identified (Kumar et al., 2014).

The biotechnological approaches to address the traits/ mechanisms for improving shoot fly resistance were more promising (Kiranmayee et al., 2015). A compelling understanding of oligogenic and quantitative traits is offered to plant breeders by recent advances in genetic marker technology (Young, 1999). A major advantage of using molecular markers for the introgression of useful genes from the wild and related species into cultivars is a gain in time by guiding and expediting conventional plant breeding program by reducing number of breeding cycles (Melchinger, 1990). Molecular technology must always be integrated with conventional crop improvement methods to better understand the genetics of important attributes linked to insect pest resistance. The inheritance of features from one generation to the next is governed by the normal principles of heredity, and molecular markers are identifiable DNA sequences located at certain sites in the genome.

Molecular marker associated with the characters can be used to screen genotypes in the laboratory condition (Gupta et al., 2010). In vitro screening using molecular marker provide more precision compare to field screening (Bohra et al., 2017). In the field condition, many factors affect plant growth and development that may reduce phenotyping accuracy. In the many crop characters linked marker, have been utilized for crop improvement programmes (Salgotra and Stewart, 2020). Among the various DNA-based markers, SSR is a class of genetic markers that have proven to be abundant and well-distributed throughout the genome of plants (Wu and Tanksley, 1993 and Chin et al., 1996). Simple sequence repeats markers require only small amounts of DNA sample without involving radioactive labels and are simpler as well as faster. Also, SSR markers can discriminate genotypes into homozygotes and heterozygotes due to co-dominant nature and high level of polymorphism compared to any other molecular marker assay is achieved (Powell et al., 1996, Lekgari and Dweikat, 2014).

Bulk segregant analysis (Michelmore et al., 1991) was carried out for two bulks along with the parental genotypes using a set of SSR primers to identify the linked polymorphic markers for resistance to shoot fly component traits in sorghum. Considering the pest's economic importance, improving the plant's genetic makeup is an important objective in the sorghum breeding programmes. Therefore, in order to better understand the inheritance of resistance, identification of the linked marker is important. In the present study, shoot fly resistant linked marker were identified using bulk segregant analysis in F_2 population.

2. MATERIALS AND METHODS

The study was undertaken during 2021–22 at the Centre for Millets Research, Sardarkrushinagar Dantiwada Agricultural University, Deesa, Gujarat.

2.1. Development of F_2 mapping population using parental lines

For the molecular analysis, female parent SWARNA was taken as susceptible for shoot fly, while male parent IS 18551 was taken as a resistance for shoot fly. The parent SWARNA has short plant height and longer panicle length and shows poor growth, low leaf expansion and poor adaptation. Parent IS 18551 having longer plant height,

more leaf expansion and robustness seedling vigour. The genotype, SWARNA was flowered an average five days, earlier than the genotype IS 18551. However, both parents matured in same time.

2.1.1. Development of mapping population

The experimental material comprised a single sorghum cross involving two genetically diverse parents, SWARNA and IS 18551, for shoot fly resistance.

2.1.2. Crossing programme

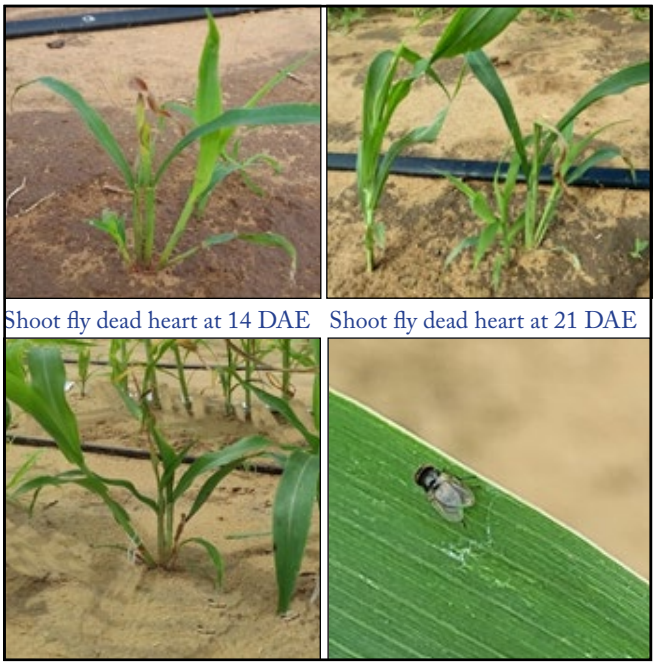
The genotypes SWARNA and IS 18551 were sown at Centre for Millets Research, S. D. A. U., Deesa during summer (February, 2021) and the crosses were made. Confirmation of F_1's hybrid carried out through molecular evaluation in *kharif* (August, 2021).

2.1.3. Development of F_2 population and leaf sample collection

The F_1's were sown at Centre for Millets Research, S. D. A. U., Deesa during *kharif* (July, 2021). Whole panicles of F_1 plants were covered with white parchment paper bags to prevent cross-pollination. Selfed seeds of F_1 plants were sown along with their parental lines to develop F_2 population in the summer (February, 2022). Leaf samples were collected individually from 15 days old tagged plants of F_2 along with parents and stored at -20°C in the deep freezer.

2.2. Phenotypic scoring (screening) of the F_2 population

For phenotypic scoring the observations recorded for individual plant basis damage appearing of shoot fly (seedling vigour, seedling glossiness, shoot fly dead heart at 14, 21 and 28 days after emergence). Seedling vigour directly correlates with shoot fly resistance as the plants with high vigour escape shoot-fly incidence (Aruna et al., 2011). Seedling vigour was recorded at 16 DAE (Days after emergence) on a 1 to 5 rating scale. Scale 1 was given for poor seedling vigour (plants showing poor growth and weak seedlings), 2 for less vigorous (less plant height with poor leaf expansion and poor adaptation), 3 for moderately vigorous (moderate plant height with moderate number of fully expanded leaves and fairly good seedling growth), 4 for vigorous (good plant height, good number of fully expanded leaves, and good adaptation and seedling growth), 5 for highly vigorous (plants showing maximum height, more number of fully expanded leaves, good adaptation and robust seedlings). Seedling glossiness was evaluated on a 1 to 5 rating at 21 days after emergence in the early morning hours when there was maximum light reflection from the leaf surfaces. Scale 1 fornon-glossy (dark green, dull, broad and drooping leaves), 2 for moderate non-glossy (green, pseudo-shine, broad and drooping leaves), 3 for moderate glossy (fair green, light shining, medium leaf width and less drooping leaves), 4 for glossy (light green, less shining, narrow and erect leaves), 5 for highly glossy (light green, shining, narrow and erect leaves). Shoot fly dead heart recorded on a 1 to 5 rating at 14, 21 and 28 days after emergence (Figure 1). Scale 1 stand for higher dead heart (unhealthy, poor growth) observed on plant at 14, 21, and 28 days after emergence, 2 for moderate with higher dead heart observed, 3 for moderate dead heart observed (fairly good seedling growth), 4 for moderate with low dead heart observed (plant having good adaptation and healthy seedling growth), 5 for less dead heart (more number of fully expanded leaves with good adaptation and plant possessing good health) on seedling observed at 14, 21, and 28 days after emergence. Chi-square (χ^2) analysis was used to calculate the goodness of fit to 1:1 segregation ratio of F_2 generations by using this formula, $\chi^2=(O-E)^2/E$, where O is the observed value and E is the expected value.



Shoot fly dead heart at 14 DAE Shoot fly dead heart at 21 DAE

Shoot fly dead heart at 28 DAE Sorghum shoot fly adult

Figure 1: Dead heart caused by sorghum shoot fly at seedling stages

2.3. Molecular characterization

The lab experiment was conducted at the Biotechnology Laboratory, Department of Genetics and Plant Breeding, C. P. College of Agriculture, S. D. A. U., Sardarkrushinagar, District: Banaskantha, Gujarat, India. Genomic DNA was isolated from resistant and susceptible genotypes of F_2 and parental lines using the modified CTAB method of Doyle and Doyle (1987). The genotypes used for the bulking of DNA in F_2 were carried out.

2.3.1. Quantification of extracted genomic DNA by spectrophotometer

Spectrophotometric analysis was performed on the quantity (in terms of protein and RNA contamination) of isolated

genomic DNA. The concentration of DNA and absorbance were measured at 260 nm and 280 nm. Pure DNA was considered as the one having A_{260}/A_{280} ratio of 1.8 to 2.0.

2.3.2. Parental polymorphism survey

Two diverse parental lines were initially screened with all the SSRs primer to find polymorphic markers before taking actual BSA in segregating F_2 mapping population. For this, DNA from SWARNA and IS 18551 were subjected to PCR amplification with each available SSR primer pair. A total of 65 SSRs primers pairs were used to screen the parents. The sequence information of sorghum SSR primers used for polymorphism survey between parents is given in Table 1. From this screening, SSR primer pairs detecting scorable polymorphism between the parents were noted and used for further analysis in BSA.

2.4. Bulk segregant analysis (BSA)

For BSA, an equal quantity of DNA from thirty resistance lines and thirty susceptible lines of F_2 population was bulked separately to constitute resistance and susceptible bulks, respectively (Michelmore et al., 1991). The parents and bulked DNA were screened with polymorphic simple sequence repeats (SSRs) markers. The primers distinguishing two near isogenie bulks as well as parents were identified as tagged markers.

2.5. PCR-based amplification using SSR primers

The PCR for SSR primers were carried out in small reaction tubes, containing a reaction volume typically of 15 µl that was inserted into a thermal cycler (Applied Biosystems Gene Amp PCR System) 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 15 µl (final volume) was prepared as 1.5 µl Taq buffer B (10 X) with 0.4 µl dNTP mix (10 mM), 1.5 µl each primer (5 pmol μl^{-1}), 0.1 µl Taq DNA polymerase (3 $U\mu l^{-1}$), 1.0 µl template DNA (50 ng μl^{-1}) and 10.5 µl nuclease-free water. The isolated and purified genomic DNA was utilized in Polymerase Chain Reaction (PCR) based amplification by using SSR, dNTPs, Taq polymerase, buffers etc.Reaction conditions were as follows: initial denaturation for 5 min at 94°C, subsequently 35 cycles of denaturation for 1 min at 94°C, annealing at 48–63°C for 1 min and extension at 72°C for 1 minas well as final extension7 min at 72°C and product store at 4°C. The amplified products were analyzed by horizontal electrophoresis on 3.0% agarose gel at 80 V for 2 h 30 minutes and stained with ethidium bromide (0.5 µg ml^{-1}). The gel was visualized under UV in gel documentation system.

2.5.1. Resolution of amplified products

All the PCR amplified products were resolved on 3.0% agarose gel for SSR containing ethidium bromide.

Table 1: Sequence information of SSR primers

Sl. No.	Primer		Primers sequence 5' -3'	No. of nucleotides
1.	Xnh 1008	F	TGAATGGCAATGTGTTTGGT	20
		R	ACGTGTTCCCGTAGGTTGTC	20
2.	Xnh 1013	F	GCAACTCGTGACACCAGAGA	20
		R	TGCCGATTCATCTTCCAAAT	20
3.	Xnh 1033	F	GGCCTTTTGGTTATGATTGC	20
		R	GGGTCTATTGTGCCTTGACG	20
4.	Xnh 1043	F	TTTCTCATCGCGACTCACAC	20
		R	TGGATGAGACATCGACCTTG	20
5.	Xnh 1044	F	GCGCACCAGAGTCATATTGTT	21
		R	GCCCTTTTGCAACGTCTAAA	20
6.	Xnh 1048	F	CGAACCCCCTACTCCACTCT	20
		R	CGCGATTTTCTTTCACACAA	20
7.	Xtxp 1	F	TTGGCTTTTGTGTGGAGCTG	20
		R	ACCCAGCAGCACTACACTAC	20
8.	Xtxp 4	F	AATACTAGGTGTCAGGGCTGTG	22
		R	ATGTAACCGCAACAACCAAG	20
9.	Xtxp 12	F	AGATCTGGCGGCAACG	16
		R	AGTCACCCATCGATCATC	18
10.	Xtxp 15	F	CACAAACACTAGTGCCTTATC	21
		R	CATAGACACCTAGGCCATC	19
11.	Xtxp 20	F	TCTCAAGGTTTGATGGTTGG	20
		R	ACCCATTATTGACCGTTGAG	20

Table 1: Continue...

Sl. No.	Primer		Primers sequence 5' -3'	No. of nucleotides
12.	Xtxp 37	F	AACCTAAGAG-GCCTATTTAACC	22
		R	ACGGCGACTCTGTA-ACTCATAG	22
13.	Xtxp 57	F	GGAACTTTTGAC-GGGTAGTGC	21
		R	CGATCGTGAT-GTCCCAATC	19
14.	Xtxp 65	F	CACGTCGTCAC-CAACCAA	18
		R	GTTAAAC-GAAAGGGAAATGGC	21
15.	Xtxp 67	F	CCTGACGCTCGTG-GCTACC	19
		R	TCCACACAA-GATTCAGGCTCC	21
16.	Xtxp 75	F	CGATGCCTC-GAAAAAAAAACG	21
		R	CCGATCAGAGCGTG-GCAGG	19
17.	Xtxp 94	F	TTTCACAGTCT-GCTCTCTG	19
		R	AGGAGAGTTGTTC-GTTA	17
18.	Xtxp 129	F	TCCTCGACATCCTC-CA	16
		R	GACACCTCGTAG-CACTCC	18
19.	Xtxp 141	F	TGTATGGCCTAGCT-TATCT	19
		R	CAACAAGC-CAACCTAAA	17
20.	Xtxp 149	F	AGCCTTGCATGAT-GTTCC	18
		R	GCTATGCTTGGTG TGGG	17
21.	Xtxp 159	F	ACCCAAA GCCCAAATCAG	18
		R	GGGGGAGAAACG GTGAG	17
22.	Xtxp 278	F	CACGACGTTG-TAAAACGACGGG TTTCAACTC-TAGCCTACCGAACT	45
		R	ATGCCTCATCATGG TTCGTTTTGCTT	26
23.	Xtxp 289	F	AAGTGGGGT-GAAGAGATA	18
		R	CTGCCTTTCC-GACTC	15
24.	Xtxp 298	F	GCATGTGTCAGAT-GATCTGGTGA	23
		R	GCTGT-TAGCTTCTTCTT CTAATCGTCGGT	29
25.	Xtxp 317	F	CCTCCTTTTCCTCCT-CCTCCC	21
		R	TCAGAATCCTAGC-CACCGTTG	21
26.	Xtxp 319	F	TAGACATCTGAAT-TAAGGAGC	21
		R	CATGCCCCT-GAAAGAGA	17
27.	Xtxp 320	F	GGAGGA CCTAGCA AGCAAG A	20
		R	TAAACTAGAC-CATATACT GCCATGATAA	28
28.	Xtxp 329	F	CACGACGTTGTA-AAACGACACTAC-GAAGGTGTTTAGTT-TAAGGG	44
		R	CATTCATAAAACTA-AACGAAAAACG	25
29.	Xtxp 331	F	AACGGT-TATTAGAGAGGGA-GA	21
		R	AGTATAATAA-CATTTTGACACCCA	24
30.	Xtxp 10258	F	GCAGGACCG-GATAGAGAT	18
		R	ATCCCGGAATGAT-GAAGT	18
31.	msbCIR 238	F	AGAAGAAAAGGGG-TAAGAGC	20
		R	CGAGAAACAATTA-CATGAACC	21
32.	msbCIR 240	F	GTTCTTG-GCCCTACTGAAT	19
		R	TCACCTGTAACCCT-GTCTTC	20

Table 1: Continue...

S l . No.	Primer		Primers sequence 5' -3'	No. of nucleotides
33.	SFC 34	F	GCTCAACT-GTGGGTCGTTCT	20
		R	TCGCAGTCAAT-GATCTCCTG	20
34.	SFC 61	F	GCAAGACCCAA-AGAGAGACG	20
		R	TTCACAGCAGCAG-CAACTTC	20
35.	SFC 112	F	TATTGCTG CT-GTCCT GTTGG	20
		R	CATCCAA AGGGGCCTTTATT	20
36.	SFCILP 94	F	GAGCCTC AGTTC-GATTCTGG	20
		R	CCGGAA GAGGCG ATAAAGA	19
37.	Xcup 53	F	GCAGGAGTATAG-GCAGAGGC	20
		R	CGACAT-GACAAGCT-CAAACG	20
38.	mSFC 107	F	CCTCCTG ATC-CATTTTGCTG	20
		R	CATGCTT CAT-GCTTTGACCA	20
39.	mSFC 106	F	GAGGTGT CGTG-GATTTGACC	20
		R	CCCGTAA GCAG-GCCATAGTA	20
40.	Xgap 1	F	TCCTGTTT-GACAAGCGCTTATA	22
		R	AAACATCATACG-AGCTCATCAATG	24
41.	Xgap 001	F	AATCATGCTTGCA-CACTTGC	20
		R	TCCTGTTT GA-CAAGC GCTTATA	22
42.	Xgap 88	F	CGTGAATCAGC-GAGTGTTGG	20
		R	TGCGTAAT-GTTCCTGCTC	18
43.	Xnhsbm 1011	F	TGGGATGC-CATATTCTTTTTG	21
		R	GTTCCTGGTGTTC-GTTTGCT	20
44.	Xisep 0101	F	CAGATCTCCGGTT-GAAGAGC	20
		R	TGAGCCGAGCTCAA-CATACA	20
45.	Xisep 0131	F	TCAGTCTTGACA-CAAGCAAGC	21
		R	CGCTTCTTCCT-GAGCTTGAG	20
46.	Xisep 0327	F	CTGTTTGTGCTTG-CAACTCC	20
		R	TCATCGATG-CAGAACTCACC	20
47.	Xisep 0444	F	ATGATCCGTCG-GAGTTAGCA	20
		R	GGATGCAGGACAG-CATCTCT	20
48.	Xisep 0449	F	CCGCTCATCAGTCAT-CACAT	20
		R	ACAAAATCCATCCCA-CAACG	20
49.	Xisep 0523	F	ACGACATGGACGA-CATCAGA	20
		R	AACAAAAACACAC-GGGAAGG	20
50.	Xisep 0634	F	CACGACGTTGTAA-AACGA CGCATAGCCACCA-GATCTTCC	39
		R	TATTGCTG CTGTC-CTGTTGG	20
51.	Xisep 0639	F	GTGCAAATAAGGGC-TAGAGTGTT	23
		R	CACGACGTTGTA-AAACGACTCG GAC-GGAGT-CATCAGATA	39
52.	Xisep 0643	F	AAACATC ATA C G A GCTCATC	20
		R	CACGACGTTGTAAAA CGACCTCACCTTGG	29
53.	Xisep 0747	F	AGGCAGCCTGCTTAT-CACAA	20
		R	ACAAGCTCAG-GTGGGTGGT	19

Table 1: Continue...

Sl. No.	Primer		Primers sequence 5' -3'	No. of nucleotides
54.	Xisep 0805	F	CTCCCCCGTGATTTGATCT	19
		R	TAAGCAAAAGCACCATCAGC	20
55.	Xisep 0829	F	CGCTGCCAAAATCTAAGCTC	20
		R	CACGGTGGTCACATCAGAAG	20
56.	Xisep 0809	F	GGAAACTCTTGTGGGTTGGA	20
		R	TTGACCTCTCTACAAATGATCCAC	24
57.	Xisep 0841	F	TAGGAATGACGACACCACCA	20
		R	CAAAGGCAAGGGTTTTGCTA	20
58.	Xisep 0938	F	TGCTGTTCTTGAACGTGTTTG	21
		R	TTTTGCACAAAGTTGCGTGT	20
59.	Xisep 1012	F	TAGCAAGCAGAAATCGACCA	20
		R	ACCATTGTCCCTCACTCCTG	20
60.	Xisep 1014	F	ACCGCCGACGTCATAGTAAG	20
61.	Xisep 1028	F	CACGACGTTGTAAAACGACCAGCGACCAT	29
		R	TGGCATGCATCAAACAAGAT	20
62.	Xisep 1035	F	CACGACGTTGTAAAACGACCA CTTTCTACCGCTCCTTCG	39
		R	AGTGATGATGATGACCGAACC	21
63.	Xisep 1140	F	TGGGAGTACTACCCGGAGGT	20
		R	CGCACGTACACCCTTAATCTT	21
64.	Xisep 1202	F	CTACCTCGTGCACCAAATGA	20
		R	CGCAAACAGATCCTTGCTTT	20
65.	Xisep 10263	F	TATCTTCTCCCGCCCTTTC	18
		R	TAAGNGCCAAGGGAATG	17

Amplified DNA product (20 µl) was mixed with 3 ul of 6X gel loading dye and loaded into the well of 3.0% agarose gel. The standard DNA marker (100 bp or 50 bp) was also run along with the samples. The electrophoresis was carried out at 80 V (constant) for about 3 hours using Genei sub-merge gel electrophoresis system. After electrophoresis, the gel was carefully removed from the casting tray and photographed using Fluor Chem FC 2 gel documentation system (Alpha Innotech Corporation, U.S.A.).

2.5.2. Data scoring

Scoring of the bands on the agarose gel was photographed using gel documentation system. The distance run by amplified fragments from the well was translated to molecular size with reference to DNA ladder using Fluor Chem FC 2 software.

3. RESULTS AND DISCUSSION

3.1. Development and screening of F_2 mapping population against shoot fly resistance

3.1.1 Development of mapping population

Parents were screened and selected based on shoot fly infestation at initial stage. The F_1 was (SWARNA×IS 18551) also confirmed by SSR marker prior to development of F_2 population. The SSR marker Xtxp 67and Xnh 1048 were found polymorphic across the parental line. The marker Xtxp 67 was produced ~150 bp band in the susceptible genotype (SWARNA) and ~176 bp band in the resistant genotype (IS18551), while the marker Xnh 1048 was produced ~168 bp band in the susceptible genotype (SWARNA) and ~136 bp band in the resistant genotype (Figure 2). In the F_1 line, both parental alleles appeared, which indicated the F_1 was true to type. The same F_1 was further utilized for development of F_2 mapping population.

3.1.2. Screening of F_2 mapping population against shoot fly resistance

For bulk segregant analysis, phenotyping was done in F_2 population from the initial seedling stage when shoot fly infestation (damage) was appeared. Total 192 F_2 plants were sown in field along with the parental lines for screening of mapping population. Out of 192 plants 45, 100 and 47

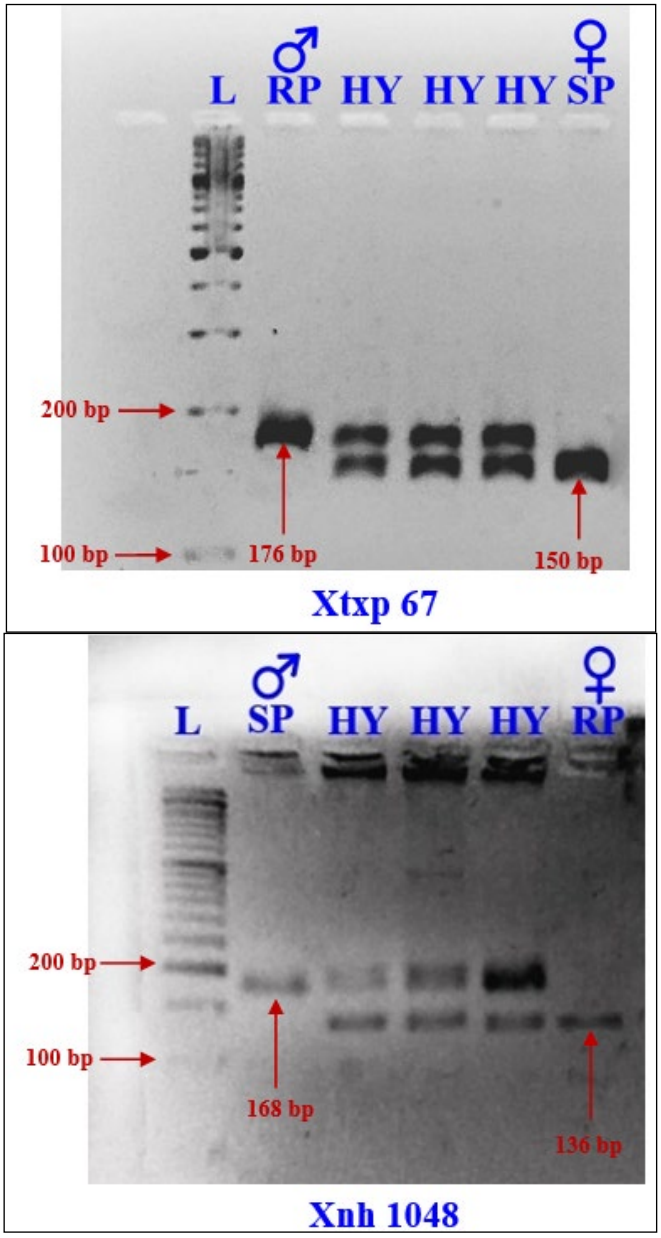


Figure 2: Confirmation of F1's hybrid after crossing between two divers' parents using Xtxp 67 and Xnh 1048 marker; Where, SP: Susceptible parent (SWARNA); HY(F_1): First filial generation of cross (F_1 hybrid); RP: Resistant parent (IS 18551); L: Ladder (100 bp)

plants found resistant, moderately resistant and susceptible, respectively. The resistant and susceptible lines were further used further for the identification of linked markers for shoot-fly resistance.

The computed chi-square (χ^2) value was 0.375, whereas the table chi-square ($\chi 2$) value was at P = 0.05 level and 2 d.f. (3-1=2) is 5.99 (Table 2). Therefore, the result was non-significant, indicating that the difference between observed and expected data only due to chance or either error. Hence, null hypothesis was accepted and concluded that observed data in F_2 generation were in agreement (goodness of fit) with an expected ratio of 1:2:1. The chi-square result indicated a co-dominant (additive) effect for shoot fly resistance.

3.2. Bulk segregant analysis for identification of shoot fly resistance marker

3.2.1. Genomic DNA isolation

DNA was isolated from tender fresh leaves of 15 days old plants of each selected individual from F_2 population along with parents using the modified CTAB method of Doyle and Doyle (1987). The 30 susceptible and 30 resistance lines were randomly selected from F_2 population for genomic DNA isolation.

3.2.2. Quality assessment of genomic DNA

Electrophoresis of genomic DNA was carried out in agarose gel as described by Primrose and Twyman (2013). The 0.8% agarose gel was prepared for qualitative analysis of genomic DNA showing the samples' intactness or compactness of bands. The separation of DNA on 0.8% agarose gel produced intact/compact band in the gel documentation system. Intact band of genomic DNA proves quality of DNA is appropriate. A single sharp band was observed in the isolated DNA of susceptible and resistant parent, their F_1 and F_2 selected lines.

3.2.3. DNA quantification through UV spectrophotometer

The quality and quantity of extracted leaves sample were done through a nano-drop spectrophotometric analysis, an essential step before PCR amplification. The concentration of genomic DNA was ranged from 313.60 ng μl^{-1} to 1710.31 ng μl^{-1}. The ratio of genomic DNA was ranged from1.61

Table 2: Calculation of chi-square (χ^2) value from F_2 mapping population of crosses between SWARNA×IS 18551

Character	Observed frequency (O)	Expected frequency (E)	Calculation (d.f=3-1=2)		
			Deviation (O-E)	Squares of deviation $(O-E)^2$	$\chi^2=\frac{(O-E)^2}{E}$
Resistance	45	48	-3	9	0.188
Moderately resistance	100	96	4	16	0.167
Susceptible	47	48	-1	1	0.020
Total	192	192	-	-	0.375

to 1.94 with an average value of 1.78. The average O.D. ratio (A260/A280) indicated the DNA's appropriateness for further use.

3.2.4. Parent polymorphism survey

In the parent-polymorphism survey total 65 SSRs markers pair were used two contrasting parents (SWARNA×IS 18551) to detect the primers exhibiting polymorphism. The amplified products were separated using 3% agarose gel stained with ethidium bromide. Eight markers viz., Xnh 1008, Xnh 1048, Xtxp 4, Xtxp 15, Xtxp 67, Xtxp 141, MsbCIR 240 and Xgap 88 revealed polymorphism (12.30%) between two contrasting parental lines in sorghum (Table 3).

Table 3: Marker identified polymorphic across the parental line

Sl. No.	Marker	Allele size in ~bp	
		P_1 (Susceptible)	P_2 (Resistant)
1.	Xnh 1008	184 bp	201 bp
2.	Xnh 1048	168 bp	136 bp
3.	MsbCIR 240	189 bp	175 bp
4.	Xtxp 4	210 bp	167 bp
5.	Xtxp 141	190 bp	213 bp
6.	Xtxp 15	140 bp	147 bp
7.	Xtxp 67	150 bp	176 bp
8.	Xgap 88	130 bp	161 bp

3.2.5. PCR amplification of polymorphic marker with susceptible and resistant bulk

The bulk segregant analysis was performed in sorghum to identify SSRs markers linked with the gene/s that affect shoot fly resistance. The genomic DNA was extracted from 60 individuals of F_2 population (30 resistance and 30 susceptible).

The equal concentration of DNA from 10 susceptible and 10 resistant individuals was taken from the F_2 population to prepare susceptible and resistant bulks, respectively. Total three resistance bulk and three susceptible bulk prepared.

3.3. Summary of individual marker used in Bulk Segregant Analysis (BSA)

3.3.1. Marker Xnh 1008

The marker was found polymorphic between susceptible and resistant genotype. ~184 bp and ~201 bp bands were observed in susceptible and resistant genotype, respectively. However, no positive correlation was found between susceptible and resistant parent to susceptible and resistant bulk, respectively. Indicated marker was not linked with shoot fly resistance (Figure 3).

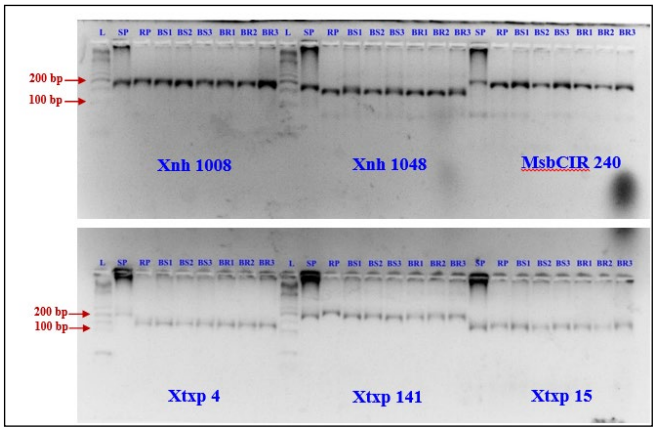


Figure 3: Amplification profiles of SSRs primers for Bulk Segregant Analysis (BSA) using Xnh 1008, Xnh 1048, MsbCIR 240, Xtxp 4, Xtxp 141 and Xtxp 15; Where, SP: Susceptible parent (SWARNA); BS 3: Suceptible bulk 3; RP: Resistant parent (IS 18551); BR 1: Resistant bulk 1; BS 1: Suceptible bulk 1; BR 2: Resistant bulk 2; BS 2 : Suceptible bulk 2; BR 3: Resistant bulk 3; L: Ladder (50 bp)

3.3.2. Marker Xnh 1048

The marker was found polymorphic between susceptible and resistant genotype. In the susceptible genotype ~168 bp band was observed and in the resistant genotype ~136 bp band was observed. However, no positive correlation was found between susceptible and resistant parent to susceptible and resistant bulk, respectively. Indicated marker was not linked with shoot fly resistance (Figure 3).

3.3.3. Marker MsbCIR 240

The marker was found polymorphic between susceptible and resistant genotype. In the susceptible genotype ~189 bp band was observed and in the resistant genotype ~175 bp band was observed. However, no positive correlation was observed between susceptible and resistant parent to susceptible and resistant bulk, respectively. It's indicating marker was not linked with shoot fly resistance (Figure3).

3.3.4. Marker Xtxp 4

The marker was found polymorphic between susceptible and resistant genotype. ~210 bp and ~167 bp bands were observed in susceptible and resistant genotype, respectively. However, no positive correlation was found between susceptible and resistant parent to susceptible and resistant bulk, respectively. Indicated marker was not linked with shoot fly resistance (Figure 3).

3.3.5. Marker Xtxp 141

The marker was found polymorphic between susceptible and resistant genotype. The susceptible genotype ~190 bp band was observed and the resistant genotype ~213 bp band was observed. However, no positive correlation was observed between susceptible and resistant parent to susceptible and

resistant bulk, respectively. It's indicating marker was not linked with shoot fly resistance (Figure 3).

3.3.6. Marker Xtxp 15

The marker was found polymorphic between susceptible and resistant genotype. In the susceptible genotype ~140 bp and in case of the resistant genotype ~147 bp band was observed. However, no positive correlation was observed between susceptible and resistant parent to susceptible and resistant bulk, respectively. It's indicating marker was not linked with shoot fly resistance (Figure 3).

The marker was found polymorphic between susceptible and resistant genotype. In the susceptible genotype ~150 bp band was observed and in the resistant genotype ~176 bp band was observed. The positive correlation found between susceptible and resistant parent to susceptible and resistant bulk, respectively. The result indicated that the marker was linked with shoot fly resistance (Figure 4).

In the case of all three-resistance bulk samples, a clear sharp ~176 bp band was observed, while in all susceptible three, a ~150 bp sharp band was observed. This indicated the marker was closely located with shoot fly resistance gene in the chromosome.

3.3.8. Marker Xgap 88

The marker was found polymorphic between susceptible and resistant genotype. ~130 bp and ~161 bp bands were observed in susceptible and resistant genotype, respectively. Positive correlation was observed between susceptible and resistant parent to susceptible and resistant bulk, respectively. It's indicating marker was linked with shoot fly resistance (Figure 4).

In all the resistance three bulk a clear sharp ~161 bp band was observed, however in all susceptible three bulk ~130 bp sharp band was observed. Its indicating marker was closely located with shoot fly resistance gene in the chromosome.

The positive association between susceptible and resistant parent and bulks was detected by the two SSRs primers Xtxp 67 and Xgap 88. The marker Xtxp 67 had shown the specific band of ~176 bp in the resistance parent and resistant bulk, which was found to be absent in susceptible parent and bulk. In contrast, ~150 bp band appeared in the susceptible genotype and bulks, which was absent in resistance parent and bulk (Figure 4). Similarly, the other polymorphic SSRs primer *i.e.,* Xgap 88 was shown the specific band of ~161 bp in the resistance parent and bulk, which was found to be absent in and susceptible parent and bulk however ~130 bp band was appeared in the susceptible genotype and the susceptible bulks which was absent in resistance parent and bulk (Figure 4).

Similar banding pattern between susceptible parent to susceptible bulk and resistant parent to resistant bulk was

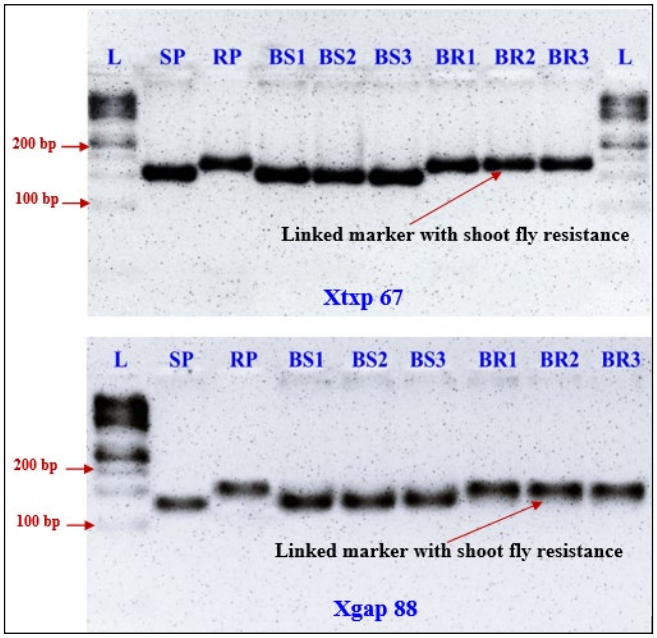


Figure 4: Amplification profiles of SSRs primers for Bulk Segregant Analysis (BSA) using Xtxp 67 and Xgap 88; Where, SP: Susceptible parent (SWARNA); BS 3: Suceptible bulk 3; RP: Resistant parent (IS 18551); BR 1: Resistant bulk 1; BS 1: Suceptible bulk 1; BR 2: Resistant bulk 2; BS 2 : Suceptible bulk 2; BR 3: Resistant bulk 3; L: Ladder (50 bp)

observed with the marker Xtxp 67 and Xgap 88 indicated chromosome location of marker and gene of interest is very closed and further this marker can be utilized for marker assisted programme as well as fine mapping of gene. Apotikar et al. (2011) was also reported the marker (*i.e.,* Xtxp 88) linked with shoot fly tolerance loci in sorghum.

4. CONCLUSION

Bulk segregant analysis (BSA) was extended to identify the traits controlled by minor genes with additive effects, increasing the power and efficiency of this molecular technique for crop improvement program in sorghum. The identified SSRs markers *i.e.,* Xtxp 67 and Xgap 88 might be useful to screen resistance with shoot fly infestation in future sorghum improvement program.

5. REFERENCES

Agrawal, B.L., House, L.R., 1982. Breeding for pest resistance in sorghum. In: International Symposium on Sorghum, Andhra Pradesh, India, 435–446.

Apotikar, D.B., Venkateswarlu, D., Ghorade, R.B., Wadaskar, R.M., Patil, J.V., Kulwal, P.L., 2011. Mapping of shoot fly tolerance loci in sorghum using SSR markers. Genetics 90, 59–66.

Aruna, C., Bhagwat, V.R., Madhusudhana, R., Sharma, V., Hussain, T., Ghorade, R.B., Khandalkar, H.G.,

Audilakshmi, S., Seetharama, N., 2011. Identification and validation of genomic regions that affect shoot fly resistance in sorghum, [*Sorghum bicolor* (L.) Moench]. Theoretical and Applied Genetics 122(8), 1617–1630.

Bohra, A., Jha, R., Pandey, G., Patil, P.G., Saxena, R.K., Singh, I.P., 2017. New hypervariable SSR markers for diversity analysis, hybrid purity testing and trait mapping in pigeonpea (*Cajanus cajan* L.). Frontiers in Plant Science 8, 377.

Chin, E.C., Senior, M.L., Smith, J.S., 1996. Maize simple repetitive DNA sequence: abundance and allelic variation. Genome 39(5), 866–873.

De Wet, J.M., 1978. Systematics and evolution of sorghum. American Journal of Botany 65(4), 477–484.

Deeming, J.C., 1971. Some species of *Atherigona rondani* (L.) from northern Nigeria, with special reference to those injurious to cereal crops. Bulletin of Entomological Research 61(1), 133–190.

Doyle, J.L., Doyle, J.L., 1987. Genomic plant DNA preparation from fresh tissue CTAB method. Phytochemical Bulletin 19(11), 11–15.

Gami, R.A., Patel, R.N., Patel, P.R, Jain, S.K., 2021. A novel high yielding dual-purpose sorghum variety GDJ 1 (Banas Surya) for semi-arid region of Gujarat. Electronic Journal of Plant Breeding 12(1), 109–121.

Goswami, S.J., Patel, P.T., Gami, R.A., Patel, R.N., Khatri A.B., 2020. Correlation and path analysis study of different characters for grain yield and fodder purpose in sorghum [*Sorghum bicolor* (L.) Moench]. Electronic Journal of Plant Breeding 11(4), 1053–1061.

Gupta, P.K., Kumar, J., Mir, R.R., Kumar, A., 2010. Marker-assisted selection as a component of conventional plant breeding. Plant Breeding Reviews 33(4), 145–217.

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

Jotwani, M.G., Marwaha, K.K., Srivastava, K.M., Young, W.R., 1970. Seasonal incidence of shoot fly (*Atherigona soccata* (L.) Rond.) in jowar hybrids at Delhi. Indian Journal of Entomology 32(1), 7–15.

Kiranmayee, K.N., Hash, C.T., Deshpande, S.P., Kavikishor, P.B., 2015. Biotechnological approaches to evolve sorghum (*Sorghum bicolor* (L.) Moench) for drought stress tolerance and shoot fly resistance. Current Trends in Biotechnology and Pharmacy 9(3), 281–292.

Kumar, A.A., Gorthy, S., Sharma, H.C., Huang, Y., Sharma, R., Reddy, B.V.S., 2014. Understanding genetic control of biotic stress resistance in sorghum for applied breeding, in Genetics, Genomics and Breeding of Sorghum. ISBN 978-1-4822-1009-5 (e book). 198–225.

Kumar, A.A., Reddy, B.V.S., Sharma, H.C., Ramaiah, B., 2008. Shoot fly (*Atherigona soccata* L.) resistance in improved grain sorghum hybrids. The Journal of Semi-Arid Tropical Agricultural Research 6, 1–4.

Lekgari, A., Dweikat, I., 2014. Assessment of genetic variability of 142 sweet sorghum germplasm of diverse origin with molecular and morphological markers. Open Journal of Ecology 4(7), 371.

Maiti, R.K., Bidinger, F.R., 1979. A simple approach to identification of shoot fly resistance in sorghum. Indian Journal of Plant Protection 7(2), 135–140.

Melchinger, A.E., 1990. Use of molecular markers in breeding for oligogenic disease resistance. Plant Breeding 104(1), 1–19.

Michelmore, W.R., Paran, I., Kesseli, R.V., 1991. Identification of marker linked to diseases resistance genes by bulked segregant analysis, a rapid method to detect the markers in specific genetic region by using the segregating population. Proceedings of the National Academy of Sciences USA 88(21), 9828–9832.

Mohammed, R., Rajendra, S.M., Kumar, A.A., Kavi, B.P., Reddy, B.V.S., Sharma, H.C., 2016. Components of resistance to sorghum shoot fly (*Atherigona soccata* L.). Euphytica 207, 419–438.

Patel, Y.D., Patel, R.N., Gami, R.A., Patel, P.R., Parmar, N.R., 2018. Study of *per se* performance and heterosis for yield and its attributing traits in forage sorghum [*Sorghum bicolor* (L.) Moench]. Forage Research 44(2), 81–85.

Peterson, D.G., Schulze, S.R., Sciara, E.B., Lee, S.A., 2002. Integration of cot analysis, DNA cloning, and high-throughput sequencing facilitates genome characterization and gene discovery. Genome Research 12(5), 795–807.

Powell, W., Margenta, M., Andre, C., Tingey, S., Rafalsky, A., 1996. The utility of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding 2, 225–238.

Price, J.H., Dillon, S.D., Hodnett, G., Rooney, W.L., 2005. Genome evolution in the genus sorghum (Poaceae). Annals of Botany 95(1), 219–227.

Primrose, S.B., Twymen, R., 2013. Principles of gene manipulation and genomics. ISBN 1-4051-3544-1 (book). Wiley, New York.

Rathod, D.P., Gami, R.A., Chauhan, R.M., Kugashiya, K.G., Patel, R.N., 2020. Study of per se performance and heterosis for forage yield and its attributing traits in sorghum [*Sorghum bicolor* (L.) Moench]. Forage Research 45(4), 258–263.

Salgotra, R.K., Stewart, C., 2020. Functional markers for precision plant breeding. International Journal of

Molecular Sciences 21(13), 4792.
Sharma, H.C., Bhagwat, V.R., Munghate, R.S., Sharma, S.P., Daware, D.G., Pawar, D.B., Kumar, A.A., Reddy, B.V., Prabhakar, K.B., Mehtre, S.P., Kalpande, H.V., 2015. Stability of resistance to sorghum shoot fly. Field Crops Research 178, 34–41.
Sukhani, T.R., Jotwani, M.G., 1980. Ovipositional preference and damage of sorghum shoot fly on different stages of tillers of ratoon crop. Indian Journal of Entomology 42(3), 488–493.
Taylor, J.R., 2003. Overview: Importance of sorghum in Africa. Workshop on the proteins of sorghum and millets: enhancing nutritional and functional properties for Africa, Pretoria 2(4), 1–21.
Wu, K.S., Tanksley, S.D., 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. Molecular and General Genetics 241, 225–235.
Young, N.D., 1999. A cautiously optimistic vision for marker-assisted breeding. Molecular Breeding 5, 505–510.