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RESEARCH ARTICLE

Screening for sorghum shoot fly (*Atherigonna soccata* Rond.) resistance QTL's in $F_{2:3}$ generation of the cross K8 x IS 18551 in sorghum

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ABSTRACT

The major biotic factor affecting sorghum production during its early growth period is the sorghum shoot fly (*Atherigonna soccata* Rond.). There are numerous ways to minimize yield losses from shoot fly attack but, it is necessary to develop the genetically resistant lines through marker-assisted breeding. The current study was carried out to screen the F_3 generation developed from the cross K8 x IS 18551 for the major four sorghum shoot fly resistance QTLs in linkage groups SBI-05 and SBI-10. IS 18551 is used as a resistance source. The segregating population was subjected to genotyping. Foreground selection was carried out using SSR markers flanking the QTLs. As a result of Marker Assisted Selection (MAS), 5 plants with 4 QTLs and 37 plants with 3 QTLs, 22 plants with 2 QTLs and 32 plants with 1 QTL was identified by genotyping. From the component trait analysis, it has been evident that the plants with resistant QTLs recorded very less shoot fly infestation. The homozygous lines harbouring resistant QTL's can be evaluated in yield trials and may also be useful in further breeding programmes for management of shoot fly in sorghum.

Keywords: *sorghum shoot fly, screening, foreground selection, MAS, SSR markers and resistant QTL's*

INTRODUCTION

Sorghum bicolor L. Moench is the fifth most important cereal crop globally, and third most important crop in India. It is mostly raised in semi-arid tropics of Asia and Africa for food, feed and fuel (Kumar *et al.*, 2013) because of its drought-tolerant nature (Ashok Kumar *et al.*, 2011). It is the staple food for the poorest people because of its micro-nutritional value and energy (Kumar *et al.*, 2009). Recently, the area under sorghum cultivation and its yield are limited due to biotic and abiotic factors (Kiranmayee *et al.*, 2015; Mohammed *et al.*, 2015; Sharma *et al.*, 2015).

Among the biotic factors affecting sorghum production, sorghum shoot fly (*Atherigonna soccata* Rond.) is the most economically important pest in Asia, America, Australia and Africa. Shoot fly infestation results in the typical "Dead Heart" symptom leading to the production of side tillers with reduced yield (Kiranmayee *et al.*, 2015; Sharma *et al.*, 2015). Usually, sorghum is raised after monsoon showers as a post-rainy season crop. It is not possible for the poor farmers to go for chemical sprays for insect control as it involves cost and manpower (Gorthy *et al.*, 2017; Mohammed *et al.*, 2016). Development of host plant resistance is the cost-effective and long term control to reduce pest incidence (Gorthy *et al.*, 2017; Kiranmayee *et al.*, 2015; Mohammed *et al.*, 2018). For selecting the plants with resistance based on phenotypic observations alone is not reliable as shoot fly resistance is a quantitative trait regulated by environmental interactions (Gorthy *et al.*, 2017). Breakthrough in sorghum improvement occurred only after the availability of sorghum sequence which facilitated fine characterization, synteny studies and comparative QTL studies through the integration of genetic and physical maps (Paterson *et al.*, 2009). Shoot fly resistance is a quantitative trait associated with morphological traits such as leaf glossiness, dead heart per cent, seedling vigour and trichome density (Kiranmayee *et al.*, 2015). The QTLs associated with shoot fly resistance were identified by several scientists (Apotikar *et al.*, 2011; Aruna *et al.*, 2011b; Gorthy *et al.*, 2017; Kiranmayee *et al.*, 2016; Satish *et al.*, 2012; Satish *et al.*, 2009). Sorghum shoot fly resistant QTLs reported by Satish *et al.* (2012) were used for marker-assisted screening (MAS). The K8 is an elite sorghum cultivar

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released from Kovilpatti research station from the cross IS 12611 x Sc 108. Duration of the crop is about 85-95 days but is susceptible to shoot fly. The IS 18551 is the internationally used check for shoot fly resistance with poor agronomic traits and yield. The aim of this study is to screen the F_3 generation plants derived from the cross K8 x IS 18551 for shoot fly resistance through molecular markers along with phenotypic confirmations.

MATERIAL AND METHODS

Field screening of sorghum F_3 generation was carried out during Kharif 2018 at New Area field no. 4 C under the Department of Millets, TNAU, Coimbatore at the Latitude 11.0136 °N and longitude 76.9378 °E with an altitude of 436.8 m. The segregating plants from the F_2 generation of K8 x IS 18551 cross harbouring the resistant QTL's (Table 1) for sorghum shoot fly resistance were evaluated in F_3 generation. The selected plants were sown with a spacing of 45 x 15 cm along with the parents. General agronomic practices were followed as per Crop Production Guide (2012) except for insecticide spray.

Simple Sequence Repeat (SSR) markers (Table 2) associated with shoot fly resistance, which is closely linked to the major QTL's were used for screening the F_3 population raised in the field. In addition to genotyping, important component traits of shoot fly resistance such as leaf glossiness, seedling vigour, oviposition percentage, dead heart percentage, number of side tillers were phenotypically observed and recorded.

DNA isolation and genotyping

The F_3 generation plants were tagged individually. Leaf samples were collected from the young leaves for isolation of DNA. DNA was isolated by using a modified DNA isolation protocol given by Doyle and Doyle (1990). The isolated DNA was quantified by Nanodrop spectrophotometer and diluted to the final concentration of 50ng/µl. The diluted DNA was used as a template for the further PCR reaction. The markers linked to four major QTLs were used in the study and the respective characteristics associated with QTLs taken for research were provided in Table 1. The primer details were provided in table 2. The PCR was performed with the profile of about 94°C for 5 min of initial denaturation, 94°C for 1 min for denaturation, 55 °C or 58 °C for 1min for annealing and 72°C for 1 min for extension and the cycle was repeated 35 times and final extension of about 72°C for 7 min with hold temperature of 4°C. The PCR product was resolved using 3.5% agarose gel and the progenies were scored (Fig 1).

Study for shoot fly resistant component traits

Seedlings with high leaf glossiness showed resistance to shoot fly infestation. Leaf glossiness has been observed during the early morning hours at 10 DAE based on the scale from 1 - 5 (score of 1 indicates highly glossy light green leaves which are shining, narrow and erect and score of 5 is given to plants with non –glossy dark green dull broad and drooping leaves) (Aruna *et al.*, 2011a).

Seedling vigour has a direct correlation with shoot fly resistance as the plants with high vigour escapes shoot fly incidence. The seedling vigour was observed at 10 DAE using vigour scale of 1-5, a score of 1 was given to highly vigorous, robust plants having a maximum height with more number of fully expanded leaves with good adaptation and a score of 5 indicates plants with poor seedling vigour, growth and less adaptation (Aruna *et al.*, 2011a).

Seedlings with a lower oviposition frequency show shoot fly resistance (Figure 2 A). Oviposition percentage was calculated on the 21st and 28th day after emergence. It was calculated using the formula

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

Dead heart percentage was calculated after 40 DAE as it was the direct measure of shoot fly resistance (Figure 2B). It was calculated using the formula

$$\text{Dead Heart (\%)} = \frac{\text{Number of seedlings with dead heart}}{\text{Total number of seedlings}} \times 100$$

As a result of dead heart, side tillers were produced. Total number of side tillers were recorded (Fig 2C).

RESULTS AND DISCUSSION

DNA isolation and genotyping

As the classical conventional breeding involves time and labour a new technique of Marker Assisted Breeding (MAB) came into existence.

Table 1. Sorghum SSR markers flanking QTL's and QTL-related biophysical traits

QTLs and SSR markers	Chromosome number	Traits associated with QTL	
QTL 1 Xtxp65- XnhsbmSFC61* *Candidate gene markers	SBI-5	Leaf surface glossiness, Dead heart % and Oviposition	
QTL 2 Xtxp 020 -Xnhsbm1011	SBI-10	Leaf surface glossiness, Seedling vigour, Dead heart %, Oviposition and trichome density	Satish *et al.* (2012)
QTL 3 XnhsbmSFC1034*-Xnhsbm SFCILP30 *Candidate gene markers	SBI-10	Leaf surface glossiness, Oviposition, Dead Heart % and trichome density	
QTL 4 Xtxp129-Xtxp331	SBI-10	Dead Heart % and leaf surface glossiness	

The use of molecular markers in sorghum dates back to the 1990's. The first report on the use of Marker Assisted Backcross Breeding (MABC) for

Table 2. Primer sequence information of Sorghum SSR markers used in genotyping

Primer Name	Primer Sequence (5' to 3')	No. of nucleotides	Melting temp (Tm)	QTL
Xtxp 65 -F	CAC GTC GTC ACC AAC CAA	18	55 C	QTL 5.1
Xtxp 65 -R	GTT AAA CGA AAG GGA AAT GGC	21		
Xnhsbm SFC 61-F	GCA AGA CCC AAA GAG AGA CG	20	58 C	
Xnhsbm SFC 61-R	TTC ACA GCA GCA GCA ACT TC	20		
Xtxp 129-F	TCC TCG ACA TCC TCC A	16	55 C	QTL 10.1
Xtxp 129-R	GAC ACC TCG TAG CAC TCC	18		
Xtxp 331- F	AAC GGT TAT TAG AGA GGG AGA	21	55 C	
Xtxp 331-R	AGT ATA ATA ACA TTT TGA CAC CCA	24		
Xtxp 020-F	TCT CAA GGT TTG ATG GTT GG	20	55 C	QTL 10.2
Xtxp 020-R	ACC CAT TAT TGA CCG TTG AG	20		
Xnhsbm 1011-F	TGG GAT GCC ATA TTC TTT TTG	21	58 C	
Xnhsbm 1011-R	GTT CCT GGT GTT CGT TTG CT	20		
Xnhsbm SFC34-F	GCT CAA CTG TGG GTC GTT CT	20	58 C	QTL 10.3
Xnhsbm SFC34-R	TCG CAG TCA ATG ATC TCC TG	20		
Xnhsbm SFC IL P30- F	GGG AAC TGT GGG AAG AAG GT	20	58 C	
Xnhsbm SFC IL P30-R	AGA GAG CCT GTT TGG CAA GA	20		

shoot fly resistance was given by Gorthy *et al.* (2017). Based on the earlier studies carried out by Satish *et al.* (2012) the four major QTLs associated with major component traits of shoot fly resistance were found to be present in SBI-05 and SBI-10. A total of 96 plants were screened. As a result, five plants with 4 QTLs, thirty-seven plants with 3 QTLs, twenty-two plants with 2 QTLs and thirty-two plants with 1 QTL was observed. The plants with maximum QTLs were forwarded to the next generation for further studies.

Table 3. Correlation of F3 generation plants with resistant QTLs and shoot fly resistance component traits

Genotypes and Generation	Plants With Respective Number OF QTL's	Seedling vigour	Leaf glossiness	Oviposition (%)	Dead heart (%)	No. Of side tillers
F3 Progenies	1	4.5	4.53	48.3	33.3	4.9
	2	3.84	3.95	36.5	19.7	3.86
	3	2.34	2.63	28.6	15.8	1.83
	4	1.68	1.53	22.3	11.2	0.53
Parents	5 Recurrent parent(K8)	5	5	79.4	59.8	0.98
	Donor parent (IS 18551)	1	1	11.3	6.3	0.13
CD (5%)		0.053	0.046	0.65	0.058	0.107

*Mean of five plants with respective number of QTLs

Study for shoot fly resistant component traits

Leaf glossiness is known as the reflectance of light from the leaf surface. A negative correlation was observed between leaf glossiness to oviposition and dead hearts. The intensity of glossiness is directly proportional to the resistance in seedlings (Dhillon

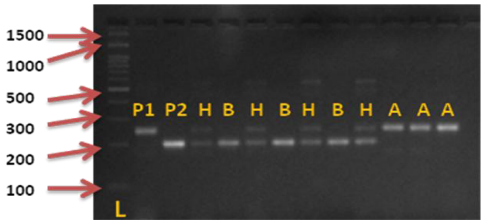

Figure. 1 - Foreground selection for presence of shoot fly resistant QTL and scoring of gel

L- NEX-GEN 100 bp DNA ladder,
P1- Susceptible parent (K8)
P2- Resistant parent (IS 18551),
A- Progeny with homozygous allele of parent A (K8)
B- Progeny with homozygous allele of parent B (IS 18551)
H- Progeny with alleles from both the parents

et al., 2005; Gorthy *et al.*, 2017). The plants with maximum QTLs show high glossiness thereby a low number of eggs and dead heart. Seedling vigour is a negatively associated trait with dead heart and oviposition (Dhillon *et al.*, 2005; Gorthy *et al.*, 2017). Plants with resistant QTLs recorded high seedling vigour than susceptible ones.

The plants with more number of eggs have higher rates of dead heart formation indicating that oviposition has a direct correlation with dead heart incidence (Dhillon *et al.*, 2006). This correlation was found to go in line with this study also. Susceptible plants were found to have more oviposition percentage and dead heart, whereas resistant plants with QTL's recorded significantly high level of shoot fly resistance. All the above component traits were found to be connected with a resistance mechanism known as non-preference for oviposition (Gorthy *et al.*, 2017). The consolidated results of component traits from the mean of 5 plants with 1-4 number of QTLs were given in table 3.

Figure 2. A) Egg of shoot fly (*Atherigonna soccata Rondani*) on the abaxial side of sorghum leaf
B) Typical dead heart symptom caused by maggot feeding on central whorl
C) Side tillers produced by shoot fly infestation

CONCLUSION

The segregating population of 96 plants were screened using the markers associated with the resistant QTLs for the presence of major QTLs. Eight SSR markers flanking the QTLs were used for genotyping and the component characteristics associated with shoot fly resistance were recorded. The lines identified with shoot fly resistant QTLs can be used for further evaluation and in future breeding programmes.

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