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Evaluation of Shoot Fly Resistance through SSR Markers in Sorghum [Sorghum Bicolor (L.) Moench]

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Keywords : *sorghum, shoot fly, ssr etc.*

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I. Introduction

Sorghum bicolor (L.) Moench is one of the most important crops in the world because of its adaptation to a wide range of ecological conditions, suitability for low input cultivation and diverse uses [6]. It is grown on about 7.93 M ha in India, with an annual grain production of 7.78 Mt (Ministry of Agriculture, 2007-08). During its cultivation, the crop is exposed to several stresses, starting from the seedling stage to harvest, and biotic stresses have the maximum impact on crop growth [4,5]. More than 150 species of insect pests damage the sorghum, of which sorghum shoot fly, Atherigona soccata (Rondani), is the most important pest in Africa, Asia and Mediterranean Europe [18] during early stage of crop growth and establishment. Shoot flies of the genus Atherigona are also known to cause 'dead hearts' in a number of tropical grass species [16] and wheat [17]. In India, the losses due to shoot fly damage have been estimated to reach as high as 90 percent of grain, and 45 percent of fodder yield [12]. That leads annual economic losses US$200 million (ICRISAT, 1992).

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). Molecular markers have been used for rapid, detailed, and directed genetic manipulation of crop plants, and to identify and characterize quantitative trait loci (QTLs) associated with plant height and days to maturity [15], plant domestication [14], resistance to diseases [10], resistance to insects [1], and tolerance to drought [22]. The advent of PCR based molecular marker techniques has facilitated the analysis of sorghum genome [24]. A high level of genetic variation has been detected among the sorghum accessions, which was high for bicolor, and guinea races and low for kafir race [8]. The SSR primers and linkage map locations have been published for sorghum [2, 3, 13, 19, 20 & 21]. Accordingly present study was undertaken to identify the SSR marker associated with QTL responsible for shoot fly resistance.

II. Materials and Methods

In present investigation three shoot fly resistant *viz*, IS 2312, IS 2205 and SUENT 11, one having recovery resistance *viz*, CSV 17 and four susceptible *viz*, 27B, AKMS 14 B, DJ 6514 and CSV 23 genotypes were crossed in diallele fashion during *kharif* 2010 at instructional farm, RCA, Udaipur and during summer 2011 at sorghum research station, Warangal (AP) to obtained the F_1 seed. The parents and F_1 were sown during *kharif* 2011 to score the value of early vigour (1- Plants having minimum vigour, 2- Plants having medium vigour, 3- Plants having maximum vigour), glossiness (1- No glossy (dark green, dull broad and dropping leaves), 2- Medium glossy (medium green and shining Leaves), 3- Highly glossy (light green, shining, narrow and erect leaves) and trichome density (No./mm^2) and to obtain the leaves for DNA extraction. These traits were identified for shoot fly resistance by [9]. The genomic

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DNA was isolated from soft leaf tissue using the CTAB method described by [7] and treated with RNase to eliminate RNA. Concentration and quality of DNA was estimated by measuring the optical density at 260 nm and 280 nm using a nano spectrophotometer and the integrity of the purified DNA was verified by visualization of DNA on 0.8 percent Agarose gel with DNA standard uncut lambda DNA.

The PCR amplification reactions for SSR markers were carried out with a total volume of 25 µl containing 2 µl of 30 ng of genomic DNA, 1.5 µl of each forward and reverse primer, 19 µl of ready to use 1X PCR buffer and 1 µl of 1 unit *Taq* polymerase. Amplification was performed for 45 cycles (1 min at 94°C, 1 min at 57°C and 1 min at 72°C) followed by a final extension of 10 min at 72°C. Amplified products were separated by 1.2 percent agarose gel electrophoresis.

PCR based SSR analysis was carried out for 28 crosses and eight parents using 21 SSR primers to identify the linked polymorphic markers for component traits of shoot fly resistance. To study the relationship between polymorphic bands and traits independent student t test, correlation and stepwise regression analysis was carried out using SAS 9.2. Similarity coefficient was calculated using following formulae based on Jaccards coefficient [11].

$$S_{ij} = \frac{2X_{ij}}{X_{ij} + Y_i + Y_j}$$

$$D_{ij} = 1 - S_{ij}$$

Where, S_{ij} = Similarity coefficient between i^{th} and j^{th} genotype.

D_{ij} = Distance coefficient between i^{th} and j^{th} genotype.

X_{ij} = Number of common bands present in both i^{th} and j^{th} genotypes.

Y_i = Number of bands present in i^{th} genotype but absent in j^{th} genotype.

Y_j = Number of bands present in j^{th} genotype but absent in i^{th} genotype.

III. Results and Discussion

Out of 21 SSR primers 6 were amplified and produced 12 bands and 327 clearly amplified fragments. Five bands were polymorphic lead 41.67 per cent polymorphism. The most informative primers was *Xtxp88* having 64 clear and reproducible amplicons from one monomorphic and one polymorphic bands. The minimum number of amplified amplicons was observed in primer *Xtxp69* and *Xtxp75 i.e.* 36 amplicons from one monomorphic band in each.

All the three shoot fly resistance component traits were positively correlated with each other. The correlation of early vigour with glossiness and trichome density was 0.46 and 0.35 and glossiness with trichome density was 0.71 (table 1). These results confirm the findings of [2]. In the genotypes where *Xtxp88* 250 bp band was present, value of glossiness (p=0.00) and trichome density (p=0.02) was significantly low (table 2). Marker *Xtxp88* (250 bp) showed significant and negative correlation with glossiness (-0.58) and trichome density (-0.38) (table 1) [2,23]. In the stepwise regression analysis only one band *Xtxp88* 250 bp was entered and explained 33.20 and 14.70 percent variability of glossiness and trichome density, respectively, indicating that the band *Xtxp88* 250 bp was associated with susceptibility to shoot fly, whereas [23] reported positive correlation with shoot fly resistance components. But, it explained very low variability.

Table 1 : Correlations between primers and shoot fly resistance component traits

	Xtxp37 300bp	*Xtxp88* 250bp	*Xtxp248* 200bp	*Xtxp248* 250bp	Early Vigour	Glossiness	Trichome Density
Xtxp32 250bp	-0.17	-0.04	0.23	0.07	0.32	0.18	0.25
Xtxp37 300bp		0.06	-0.36*	-0.24	-0.22	0.04	0.19
Xtxp88 250bp			0.16	0.20	-0.21	-0.58**	-0.38**
Xtxp248 200bp				0.44**	0.16	-0.29	-0.28
Xtxp248 250bp					0.17	0.02	-0.13
Early Vigour						0.46**	0.35*
Glossiness							0.71**

**, ** Significant at the 0.05 and 0.01 level, respectively.*

Table 2 : Mean comparison of polymorphic bands for shoot fly resistance component traits

S. No.	Band	Characters	Bands		Probability
			Absent	Present	
1.	*Xtxp32* 250bp	Early vigour	2.33±0.12	2.78±0.15	0.06
		Glossiness	1.85±0.13	2.11±0.20	0.31
		Trichome density	2.11±0.51	3.56±0.58	0.14

2.	*Xtxp37* 300bp	Early vigour	2.48±0.11	2.00±0.00	0.19
		Glossiness	1.91±0.11	2.00±0.58	0.82
		Trichome density	2.33±0.42	4.00±2.08	0.28
3.	*Xtxp88* 250bp	Early vigour	2.67±0.17	2.37±0.12	0.21
		Glossiness	2.56±0.18	1.70±0.10	0.00
		Trichome density	4.11±0.94	1.93±0.42	0.02
4.	*Xtxp248* 200bp	Early vigour	2.33±0.19	2.52±0.11	0.36
		Glossiness	2.13±0.17	1.76±0.14	0.09
		Trichome density	3.13±0.77	2.00±0.44	0.18
5.	*Xtxp248* 250bp	Early vigour	2.36±0.14	2.57±0.14	0.32
		Glossiness	1.91±0.15	1.93±0.17	0.93
		Trichome density	2.73±0.60	2.07±0.52	0.45

The similarity coefficient using all the bands was ranged from 0.58 to 1.00, whereas from polymorphic bands, the value was ranged from 0.00 to 1.00. The trend of the similarity was similar in both the ways. However, similarity was increased after inclusion of monomorphic bands. The correlation between them was 0.80. Looking to this trend the similarity based on all the bands were used.

The maximum similarity coefficient 0.88 was observed between resistant parents IS 2312 & IS 2205 and SUENT 11 followed by 0.78 between IS 2205 & SUENT 11. Between resistant and susceptible parents maximum similarity coefficient 1.00 was observed between IS 2312 & CSV 17 and minimum similarity coefficient 0.70 was observed between IS 2205 & CSV 23 and SUENT 11 & DJ 6514. The maximum similarity coefficient 1.00 was observed between susceptible parents 27 B & AKMS 14B and minimum similarity coefficient 0.78 was observed between CSV 17 & DJ 6514 and CSV 23.This indicated no relationship between these bands and resistance.

The maximum similarity coefficient 0.88 was observed between resistant parents and resistant x resistant F_1's IS 2205 & IS 2205 X IS 2312 and minimum similarity 0.58 was observed between IS 2205 & IS 2205 X SUENT 11. For resistant parent and resistant x susceptible F_1's maximum similarity coefficient 0.89 was observed between IS 2205 & IS2205 X 27 B and SUENT 11 & SUENT 11 X CSV 17 and minimum similarity 0.64 was observed between IS 2205 & IS 2205 X CSV 23 and IS 2205 X CSV 17. The maximum similarity coefficient 0.80 was between resistant parent and susceptible x susceptible crosses SUENT 11 & 27 B X AKMS 14 B, 27 B X CSV 17, 27 B X CSV 23 and AKMS 14 B X DJ 6514 while minimum similarity coefficient 0.58 was between IS 2205 & 27 B X DJ 6514. The maximum similarity coefficient 0.89 was observed between susceptible parents and susceptible x susceptible F_1's AKMS 14 B & AKMS 14 B X CSV 17 and AKMS 14 B X CSV 23 and minimum similarity 0.73 was observed between 27 B & 27 B X DJ 6514. For susceptible parent and susceptible x resistant F_1's maximum similarity coefficient 1.00 was observed between 27 B & 27 B X IS 2312 and minimum similarity 0.64 was observed between DJ 6514 & DJ 6514 X SUENT 11. The maximum similarity coefficient 1.00 was observed between susceptible parent CSV 17 and resistant x resistant cross IS 2205 X IS 2312 while minimum similarity coefficient 0.64 was between DJ 6514, CSV 23 & IS 2312 X SUENT 11.

The average similarity was maximum between susceptible parents and crosses involved at least one susceptible parent followed by resistant parent and crosses involved both resistant parents, susceptible parents and crosses without susceptible parents, resistant parents and crosses without one resistant parent and resistant parents and crosses without resistant parents. The difference between the similarity coefficients was very low as well as there was no specific trend. This indicated that most of the polymorphic bands obtained were not related to resistance and susceptibility except *Xtxp88* 250 bp. This band was present in all the susceptible parents (except CSV 17) *viz;* 27 B, AKMS 14 B, DJ 6514 and CSV 23 and their crosses except IS 2205 (R) X DJ 6514 (S) and DJ 6514 (S) X SUENT 11 (R). This band was absent in CSV 17 though it was not having trichomes but having medium early vigour and glossiness that caused recovery resistance. Its cross with SUENT 11 was also not having this band. These results indicated that this marker was dominant and present in all the crosses where susceptible parents were involved.

In all we can concluded that resistance was dominant over susceptibility and band *Xtxp88* was associated with susceptibility though it can explained only 33.20 and 14.70 percent variability of glossiness and trichome density, respectively. Therefore, to identify QTL responsible for resistance some other markers may be used.

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