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Short Communication

Validation of foreground and background SSR markers for introgression of QTL governing leaf glossiness into a sorghum variety - NTJ 2

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Abstract

Leaf glossiness and trichomes on leaf surface, two important component traits governing resistance to shoot fly in sorghum is controlled by Quantitative trait loci (QTLs) located on linkage group SBI-05. Foreground and background markers were validated for transfer of the QTLs to NTJ2, a popular variety of sorghum. For foreground selection, five SSR markers, Xtxp268, Xisep1111, Xtxp065, Xtxp303, and Xiabt440 in the region of the QTL were identified from 17 tested markers. From a total of 167 SSR markers spanning the entire genome, 35 polymorphic SSR markers, distributed on all the 10 chromosomes were identified for background selection. The F_1, BC_1F_1 and F_2 generation plants were screened using foreground markers. Based on the results obtained, the hybridity of the F_1's was confirmed and marker-homozygote individuals in F_2 generation were identified for subsequent backcrossing.

Key words: Sorghum, shoot fly, QTL, leaf glossiness, foreground selection, background selection, introgression

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop in the world, cultivated in 40.5 m ha with a production of 55.6 m t during 2010 [1]. In India it is cultivated in an area of 7.7 m ha. Shoot fly (*Atherigonasoccata*) is one of the most important pests in India causing grain and stover losses. The average grain losses in the country are estimated to be 5% [2]. Host plant resistance can play a major role in minimizingthe extent of losses and is compatible with other tactics ofpest management, including the use of natural enemiesand chemical control [3]. Resistance to shoot fly in sorghum in know and various component traits contributing to resistance have been identified, of which, leaf glossiness, leaf trichomes are found to be very important [4]. Several sources of resistance have been identified and used in breeding programs but the complex nature of resistance has limited the success through conventional breeding approaches [3].

The use of molecular markers in breeding programs can be promising to improve quantitative traits. Once major QTLs are detected they can be transferred into desirable parents through marker assisted backcrossing (MABC) where foreground and background markers are used to select target loci and recipient genome, respectively. We report validation of foreground and background markers for introgression of QTL associated with leaf glossiness and thus confer shoot fly resistance in sorghum into an elite parent, NTJ 2 through MABC. The target QTL is on linkage group SBI-05. The foreground validation is important as all the primer pairs in the region of QTL are not expected to detect polymorphism between two parents, thus polymorphism of markers in the target QTL region is essential to begin MABC. The background markers

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hasten the introgression of QTL for leaf glossiness into NTJ 2; evident from the simulation studies, where it was shown that equivalent levels of the recurrent parent genome may be recovered by BC_4 or BC_3 using a marker assisted backcrossing approach [5], thus saving two or three backcross generations. Leaf glossiness is an important component trait contributing to resistance to shoot fly in sorghum and the QTL's governing the trait were reported [6].

NTJ 2 (NandyalTellaJonna 2), used as recurrent parent is a high yielding postrainy season adapted, sweet stalk variety developed at Acharya N G Ranga Agricultural University and released in 1990. It is a pureline selection from E-1966, a *zerazera* land race obtained from ICRISAT, Patancheru. It is valued for grain and fodder yield and quality and is also promoted as sweet-sorghum variety. The donor parent, J 2779-P4 was obtained from ICRISAT, Patancheru, India. This parent, bred at ICRISAT is a backcross derivative in the background of BTx623 with QTL on SBI 05 governing leaf glossiness introgressed from a shoot fly resistant germplasm accession, IS 18551. Crosses were made in 2010 rainy season to raise F_1 plants in 2010-11 post rainy season. The elite parent, NTJ 2 was used as female parent to tap its maternal effects of the variety, if any. The F_1's were backcrossed and selfed to obtain BC_1F_1 and F_2 seeds, respectively. The BC_1F_1 and F_2 plants were raised in glass house during 2011 summer season.

The DNA from parents (NTJ 2 and J 2779-P4), F_1, F_2 and BC_1F_1 generations plants was extracted and checked for quality. Polymerase chain reactions (PCR) were performed in 5 µl reaction volumes and PCR reactions were carried out in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, USA) with a Touchdown (61-51) program. Capillary electrophoresis of denatured pooled products was performed using ABI3130*xl* DNA genetic analyzer. Allele calling was done by GENOTYPER 3.7 v software (Applied Biosystems). The segregation of SSR markers in F_2 generation was tested by Chi-square test.

A set of 17 SSR markers (Xisep0338, Xisep1029, Xisep1041, Xisep1111, Xisep1133, Xisep1107, Xisep1109, Xisep1208, Xtxp14, Xtxp15, Xtxp065, Xtxp112, Xtxp268, Xtxp303, Xiabt67, Xiabt215, Xiabt440t) were identified for foreground selection based on their location close to desired QTL on SBI 05 governing leaf glossiness from the published data [6]. Five primer pairs were polymorphic between the parents, NTJ 2 and J 2779-P4 that can be used for foreground selection of the target QTL. The differences in allele size among the parents varied between 6 and 11 bp. The five SSR markers, Xtxp268, Xisep1111, Xtxp065, Xtxp303, and Xiabt440 can be used for foreground selection of the target QTL. The F_1 plants were confirmed by the presence of marker-heterozygotes that represent the alleles from both the parents (Fig 1). The F_1 plants confirming to hybridity were backcrossed to NTJ 2 and also selfed.

For background screening, a total of 167 markers spanning the entire genome (24 on SBI-01, 23 on SBI-02, 23 on SBI-03, 15 on SBI-04, 25 on SBI-0525, 12 on SBI-06, 11 on SBI-07, 9 on SBI-08, 12 on SBI-09 and 13 on SBI-10) were identified for the studyfrom published data[6].Of which, 35 primers (Xisep0114, Xisep0132, Xisep0209, Xisep0327, Xisep0422, Xisep0504, Xisep0506, Xisep0522, Xisep0608, Xisep0612, Xisep0716, Xisep0809, Xisep0949, Xisep1008, Xisep1031, Xisep1032, Xxisep1042, Xisep1150, Xisep1231, Xisep1241, Xxtxp001, Xxtxp009, Xxtxp025, Xxtxp027, Xtxp149, Xisep1046, Xtxp278, Xtxp088, Xtxp141, Xmsbcir300, Xxmsbcir238, Xmsbcir 248, Xisep 938, Xxmsbcir223, and Xgap342) were found to be polymorphic between parents, which can distinguish them. The 35 primers are distributed on all the 10 linkage groups and can be used for background selection to recover the maximum genomic content of recurrent parent in early backcross generations. The linkage group, SBI-01 has highest number of polymorphic markers.

Fifteen BC_1F_1 seed were harvested, from which none confirmed for the target QTL as the markers amplified only A type (recurrent parent type) allele. The 88 F_2 seedlings that were genotyped with 35 validated background markers and scoring is given as A/B/H/–/O, where A represents the homozygote for the recurrent parent allele; B represents the homozygote for the donor parent allele; H represents the heterozygote for both of the parent alleles; '–' represents the missing data point (due to PCR failure or DNA isolation failure); and 'O' represents the presence of an unexpected, off-type allele. Since SSRs are co-dominant markers, they are expected to segregate in 1:2:1 ratio in F_2 generation. The segregation of A type (homozygote for allele from P_1 parent, NTJ 2), H type (Heterozygote) and B type (homozygote for allele from P_2 parent, J 2779-P4) was tested for goodness of fit for 1:2:1 ratio. Three makers

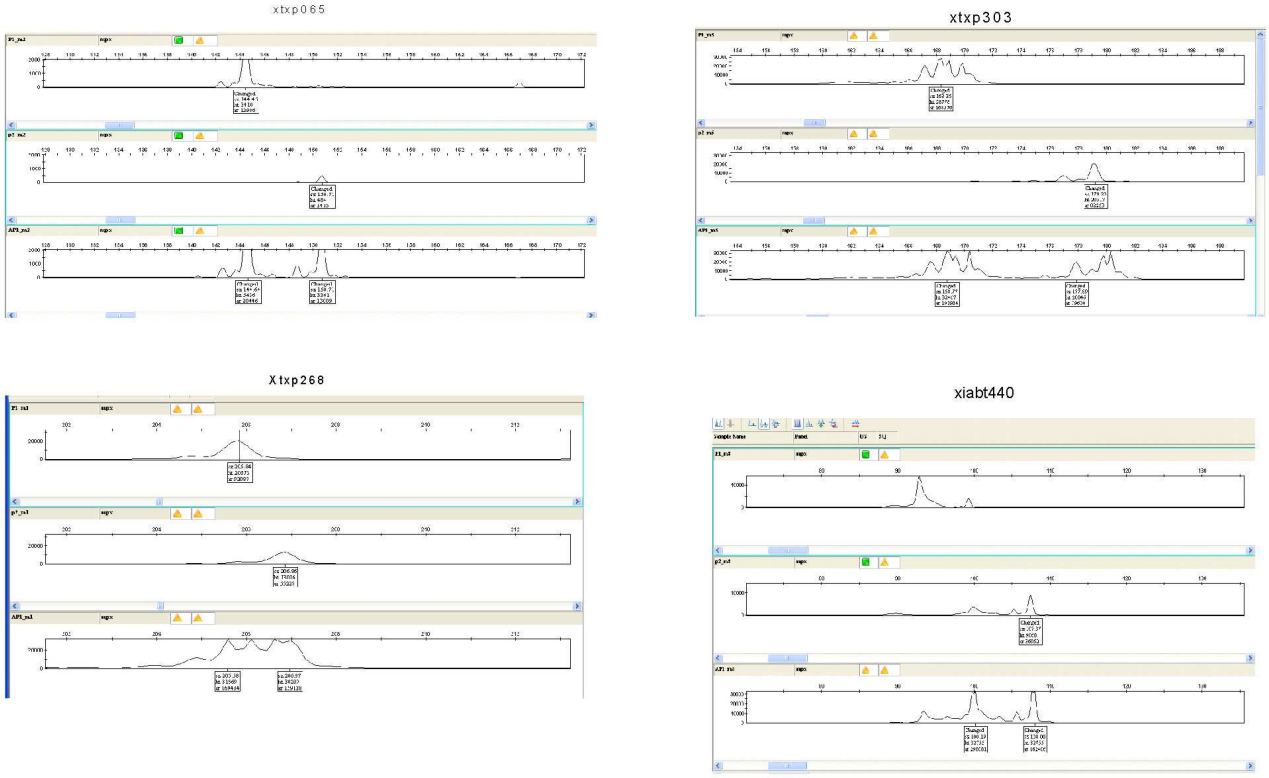

Fig. 1. Graphical representation (chromotogram) of the SSR markers, Xtxp065, Xtxp0303, Xtxp0268 and Xiabt0440in F_1 and parents analyzed through ABI Prism 3700

showed p levels > 0.15 indicating high probability of goodness of fit to the ratio, 1:2:1, while one primer showed a p level <0.005. Thus all the four markers showed goodness to fit to the expected ratio of 1:2:1 ratio. Among them, the markers, Xtxp268, Xtxp303, Xiabt 440 have high probability for goodness to fit (Table 1).

Of the 88 F_2 plants, 18 plants were positive *i.e.*, B/H type all the markers.Among them, five are 'B type' at all the four marker loci, 3 are 'H type' at all marker loci and 9 are a combination of B and H type and five plants were identified for morphological similarity with NTJ 2. Among the five individual plants identified for NTJ 2 phenotype, one plant has 'B type' at all four marker loci and that wasbackcrossed to NTJ 2 to obtain BC_1F_1 seed. Background selection with validated markers will be done after deriving BC_1, BC_2 and advanced generations, i.e., after backcrossing the selected F_2 plants to NTJ 2. The selected NTJ 2 type plants in BC_2F_2 are selfed to obtain BC_2F_3 progenies which will be phenotyped for yield performance and shoot fly resistance, and stable lines will be advanced to multilocation testing.

Table 1. Segregation of SSR markers in F_2 generation and goodness of fit to 1:2:1 ratio

Marker/allele	No. of F_2 plants			
	Observed	Expected	Chi-square value	Proba-bility
Xtxp0268				
A	17	15		
H	25	28		
B	16	15		
Total	58	58	0.65	0.1-0.9
Xtp065				
A	33	21		
H	28	41		
B	22	21		
Total	83	83	11.02	<0.005
Xtxp303				
A	24	16		
H	29	33		
B	12	16		
Total	65	65	5.48	0.05-0.1
Xiabt440				
A	12	13		
H	26	27		
B	15	13		
Total	53	53	0.42	0.1-0.9

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