Volume : 5 | Issue : 12 | December-2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 78.46

Validation of foreground microsatellite markers for introgression of shootfly *(Atherigona soccata)* resistant QTLs into elite sorghum varieties

Agricultural Science

KEYWORDS: Polymorphism, CTAB, Shootfly Sorghum, shoot fly, QTL, leaf glossiness, foreground selection

Sunita Gorthy	International Crops Research Institute for Semi-Arid Tropics, Patancheru, Hyderabad, Telangana, India Jawaharlal Nehru Technological University, Hyderabad, Telangana, India
Lakshmi Narasu	Jawaharlal Nehru Technological University, Hyderabad, Telangana, India
A Ashok Kumar	International Crops Research Institute for Semi-Arid Tropics, Patancheru, Hyderabad, Telangana, India

ABSTRACT

Sorghum is an important cereal and fodder crop which plays pivotal role in human nutrition. Important challenge that threatens the long term production of sorghum is shootfly. Shootfly is the major limiting factor in sorghum as it effects both production and productivity. Leaf glossiness, trichomes on the leaf surface, Ovipositional non-preference and seedling vigor are the major component traits governing shootfly resistance in sorghum. The quantitative trait loci's (QTLs) responsible for these traits are present on chromosome number SBI 01, SBI 05, SBI 07 and SBI 10 respectively. So, to provide ready to use markers for foreground selection in marker assisted breeding for sorghum we have used 20 recurrent parents and 9 donor parents for shootfly resistance. These simple sequence repeat (SSR) markers are validated for introgressing the QTL's into elite postrainy sorghum cultivars. The results reveal, out of 58 SSR markers, 33 markers showed distinct polymorphism among the donor and recurrent parents. For each QTL minimum of five markers from the QTL flanking region were polymorphic for all the parents and are used for foreground selection for the presence of QTLs.

Introduction

Sorghum is the staple crop for more than 500 million people in over 30 countries across Africa and Asia. In addition to grain, its stover is prized for feed value. Sorghum production is greatly influenced by various biotic and abiotic factors. Shootfly is one of the most destructive pest causing around 80% grain losses and 68% fodder losses. Detailed knowledge of the relationship between sorghum breeding lines is important not only for parental selection but also for genetic analysis and breeding system design (Hallaur et al, 1981). Unless there is sufficient genetic diversity in the germplasm it is practically not possible to increase the yield and other desirable characters of a crop, because the selection of improved genotypes depends on the availability of genetic variability within breeding material. Management of sorghum shootfly by the application of insecticides is not very feasible for the resource poor farmers because of its high cost. Therefore management of shootfly with host plant resistance is the most effective means to reduce shootfly damage. Quantitative trait mapping coupled with marker assisted breeding (MAB) provide a means to better handle traits with polygenic inheritance, which was earlier not feasible or was met with limited success using conventional procedures. The DNA markers have been used to identify QTLs and MAS has been applied for integrating different resistant QTLs into sorghum cultivars lacking the desired trait. Among different types of available molecular markers, Simple sequence repeats (SSRs) are useful for a variety of applications in plant genetics and breeding because of their reproducibility, multiallelic nature, co-dominant inheritance, relative abundance and good genome coverage. SSR markers have been more useful for parental polymorphism study.

Studies on shoot fly resistance mechanisms suggested that deadhearts, oviposition, leaf glossiness, trichomes on the abaxial surface of the leaf can be used as marker traits to select for resistance to shoot fly in sorghum (Dhillon et al. 2005; Anandan et al. 2009). Satish et al. (2009) identified major QTLs on chromosome SBI 01, SBI 05, SBI 07 and SBI 10. Present study was undertaken to validate the SSR markers present in the QTL region for shoot fly resistance which can further be used to introgress these QTLs into desired cultivars.

Material and Methods

Plant material

Twenty sorghum genotypes were used as recurrent parents collected from ICRISAT. Two genotypes IS18551 and 296B were used as resistant and susceptible check. BTx 623 is used as standard as it is the background of donor parents. For each shootfly resistant QTL two versions of validated introgression lines were used as donors, developed by Marker Assisted Backcrossing (MABC) of these target QTLs from germplasm line IS 18551 into BTx623 (a shoot fly susceptible, elite B-line and a standard genotype for sorghum genomics work). The donor parents J2658-6, J2698-7 corresponds to QTL on SBI 01, J2758-5, J2833-11, J2799, J 2834-6 on on SBI 05, J2714, J2743-3 on SBI 07 and J2614 on SBI 10. The recurrent parents and donor parents are listed below.

Recurrent Parents	BTx623, IS18551, 296B, ICSB75, ICSB11001, ICSB11005, ICSB11029, P.MOTI, P.VASUDHA, M35, NTJ2, ICSB29001, ICSB29004, ICSB29005, ICSB29007, ICSB29014, ICSB29016, ICSB48, ICSB101, PHULE CHITRA, PHULE YASHODHA,PHULE ANURADHA, PHULE JYOTI
Donor Parents	J2658-6,J2698-7 J2714,J2743-3 J2614 J2758-5,J2833-11,J2799,J 2834-6

Table 1. Donor and recurrent parents used for polymorphism study

The four validated QTLs on SBI 01, SBI 05, SBI 07 and SBI 10 govern different shootfly resistant component traits such as leaf trichome density, reduced oviposition, deadhearts incidence, leaf glossiness and seedling vigor.

Development of Backcross population

The F_1's were generated by emasculating florets of recurrent parents and further crossing those with QTL donor lines in BTx623 background during *rabi* 2011. The crosses were executed in a plant × plant crossing manner for complete MABC program across generations. The F_1's were further backcrossed with the recurrent parents to produce BC_1F_1. Parents (8 seeds) were staggered with one week difference in two sowing dates. F1's were sown in the second sowing date. This staggered sowing ensured nicking of F_1s and corresponding recurrent parent lines.

DNA extraction

Around 16 seeds of recurrent parent and 2 seeds of donor parents were sown in cement blocks in the glasshouse. Bulk DNA was obtained from approximately 30mg representative leaf tissue that was collected from at least 20 one-week old plants per parental line by using a modified CTAB method (Mace et al., 2003). DNA was

further purified by RNase digestion followed by extraction with phenol/chloroform/iso-amylalcohol (25:24:1) and ethanol precipitation as described by Mace et al. (2003)

Polymerase chain reaction (PCR)

Polymerase chain reactions (PCRs) were performed in 5µl reaction volumes and PCR reactions were carried out in a PCR system PE9700 thermal cycler (Applied Biosystems, USA) with touchdown program. Reaction conditions were as follows: initial denaturation for 15 min at 94°C, subsequently 10 cycles of denaturation for 10 sec at 94°C, annealing at 61-52°C for 20 sec, the annealing temperature for each cycle is reduced with 1°C and extension at 72°C for 30 sec and 35 cycles of denaturation for 10 sec at 94°C, annealing at 54°C for 20 sec and extension at 72°C for 30 sec. The last PCR cycle was followed by a 20 minutes extension at 72°C to ensure amplification to equal length of both DNA strands.

SSR Data Analysis

PCR products of two to four primer pairs amplifying SSRs were pooled on basis of product size and dye/color for each QTL across donor-recurrent parent combination. These PCR products were pooled with 0.5µl of VIC, and 1.0µl each of FAM, NED and PET dyes with 7.0µl Hi-Di formamide to a final volume 12.0µl per reaction. These pooled products were denatured at 94°C for 5 min. Capillary electrophoresis of these denatured products was performed using ABI3700*xl* DNA Analyzer. The raw output was sized using Gene-mapper v4.0 software (Applied Biosystems) with help of a standard dye GS500LIZ. Each allele was scored as present (1) or absent (0) at each SSR locus to calculate Jaccard's similarity index.

Results

The present study focussed to validate previously identified markers and to identify the polymorphic SSR markers present in the shootfly resistant QTL region for different crosses which can be used as foreground selection markers.

SSR Polymorphism

A set of 58 SSRs distributed across genomic region of our target QTLs on linkage groups SBI-01 (LG-A), SBI-07 (LG-E), SBI-10 (LG-G) and SBI-05 (LG-J) were tested for polymorphism between the donor lines (introgression lines with QTLs from IS 18551 in BTx623-background), and recurrent parents. PCR components and PCR profiles for all the 58 primer pairs were optimized on one genotypes (IS18551). Among 58 primer pairs, 42 (72.4%) primer pairs provided scorable amplification with a touchdown PCR profile. Subsequently these 33 primer pairs were used for genotyping a set of 32 sorghum genotypes representing parents of which 9 present donor parent and 20 represent recurrent parent for backcrossing (Table 1). As a result, a total of 33 (65.5%)) markers showed polymorphism across 20 donor recurrent combination. The differences in allele size among parents varied between 2 base pairs (bp) and 100bp

QTL containing Chromosome number/Linkage Group	Marker names	Forward primer sequence (5'–3')	Reverse primer sequence (5'–3')	Motif	Reference†
SBI-01/LG A	Xtxp329	CACGACGTTGTAAAACGACACTACGAAGGTGTTTAGTTTAAGGG	CATTCATAAAAACTAAACGAAAACG	(ATC)8 +(CTT)22	Bhattramakki et al. (2000)
	Xtxp149	AGCCTTGCATGATGTTCC	GCTATGCTTTGGTGTGGG	(CT)10	Bhattramakki et al. (2000)
	Xisep1035	CACGACGTTGTAAAACGACCACTTTCTACCGCTCCTTCG	AGTGATGATGACCGAACC	TGAT(5)	ICRISAT_Ramu
	Xisep1028	CACGACGTTGTAAAACGACCAGCGACCAT	TGGCATGCATCAAACAAGAT	GCA(4)	ICRISAT_Ramu
	Xtxp075	CGATGCCTCGAAAAAAAACG	CCGATCAGAGCGTGGCAGG	(TG)10	Bhattramakki et al. (2000)
SBI-07/LG E	mSFC107	CCTCCTGATCCATTTTGCTG	CATGCTTCATGCTTTGACCA	(TA)6	Satish et al 2012
	mSFC106	GAGGTGTCGTGGATTTGACC	CCCGTAAGCAGGCCATAGTA	(GA)7	Satish et al 2012
	XnhsbmSFCILP94	GAGCCTCAGTTCGATTCTGG	CCGGAAGAGGCGATAAAGA	IN2	Satish et al 2012
	XnhsbmSFC112	TATTGCTGCTGTCCTGTTGG	CATCCAAAGGGGCCTTTATT	(AT)7	Satish et al 2012
	Xtxp159	ACCCAAAGCCCAAATCAG	GGGGGAGAAACGGTGAG	(CT)21	Bhattramakki et al. (2000)
	Xtxp278	CACGACGTTGTAAAACGACGGGTTTCAACTCTAGCCTACCGAACT	ATGCCTCATCATGGTTCGTTTTGCTT	(TTG)12	Bhattramakki et al. (2000)
SBI-10/LG G	Xisep0634	CACGACGTTGTAAAACGACGCATAGCCACCAGATCTTCC	AATCATGCTTGCACACTTGC	CAG(5)	ICRISAT_Ramu
	Xgap001	TCCTGTTTGACAAGCGCTTATA	AAACATCATACGAGCTCATC	(AG)16	Brown et al. (1996)
	Xnhsbm1008	TGAATGGCAATGTGTTTGGT	CGTGTTCCCGTAGGTTGTC	(TCTA)18	Satish et al 2009
	Xnhsbm1011	TGGGATGCCATATTCTTTTTG	GTTCCTGGTGTTCGTTTG	(TTC)17	Satish et al 2009
	Xisep0643	CACGACGTTGTAAAACGACCTCACCTTGGA	GGAGGACCTAGCAAGCAAG	TC(7)	ICRISAT_Ramu
	Xtxp320	TAAACTAGACCATATACTGCCATGATAA	GTGCAAATAAGGGCTAGAGTGTT	(AAG)20	Bhattramakki et al. (2000)
	Xisep0639	CACGACGTTGTAAAACGACTCGGACGGAGTCATCAGATA	GCCTTCGTGTCTTCTGTCCT	TCT(6)	ICRISAT_Ramu

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	msbCIR227	CACGACGTTGTAAAACGACTTCACTGTCAAACTTGGAA	TGAATAATATCTTGCATATACGTG	-	Mace et al 2009
	Xcup16	TGCAGTGCTAGCTCATGGTC	CTTTCCAGCCTCCCATATCC	(CTTTT)4	Schloss et al. (2002)
	Xtxp141	TGTATGGCCTAGCTTATCT	CAACAAGCCAACCTAAA	(GA)23	Bhattramakki et al. (2000)
	Xcup07	CTAGAGGATTGCTGGAAGCG	CTGCTCTGCTTGTCGTTGAG	(CAA)8	Schloss et al. (2002)
SBI-05/LGJ	XnhsbmSFC44	GAACAAGTCCGATGCCTCTC	CCACCCTAAGCAGTGGTGTAA	(AT)6+(AT)9	Satish et al 2012
	Drenhsbm057	CACGACGTTGTAAAACGACCAAGCCTTTCCTTGCAGTTC	CACACCTGGCTCTGTTTTCA	-	Satish et al 2012
	XnhsbmSFCILP43	ACGGCAAGACGTTTTTCAAG	TCCAGAGTCCTTCTCGGGTA	IN1	Satish et al 2012
	Xiabtp420	CACGACGTTGTAAAACGACACATGCATGCTTGGAAGTTG	CTCTAGCATGGACCTGCACA	NN	Fakrudin et al.
	Xiabtp454	CACGACGTTGTAAAACGACCCTTCCAGTGAGGATCAGGA	TGAAGCTTTTGATTCAACTTGC	NN	Fakrudin et al.
	XnhsbmSFC61	GCAAGACCCAAAGAGAGACG	TTCACAGCAGCAGCAACTTC	(CA)44	Satish et al 2012
	XnhsbmSFC62	ATGGAAAGCCATGGTGGTAG	CTCTGACGGGTGGTAGCTGT	(GGC)10	Satish et al 2012
	XnhsbmSFC67	CAGCTCACCATTCCAAGCTC	AGGGTGAGGAGGATGAGGAG	(AC)7	Satish et al 2012
	XnhsbmSFC71	CAAGGTTCCCCTGTCATTGT	GCCAGCCTATGACTTTGCTC	(TA)5	Satish et al 2012
	Xisep1029	CACGACGTTGTAAAACGACGACCCTCCTCCTCAACCACT	CATGCATGCACAAGCAGATT	GCAT(3)	Ramu et al 2009
	Xisp10258	GCAGGACCGGATAGAGAT	ATCCCGGAATGATGAAGT	CAA/CCG	Haussmann et al. (2004)

Table 2. List of polymorphic SSR Markers used for introgression study for different shootfly resistant QTLs

These polymorphic markers were used for selecting true hybrids in F1 and BC1F1 generations. The chromatogram some of the SSR markers (Figure 1), Xisep1035, XnhsbmSFC107, Xnhsbm1008 and Dsenhsbm057 all located on different chromosomes, reveals the peak patterns for two parents (recurrent and donor) that are analyzed through ABI Prism 3700xl.

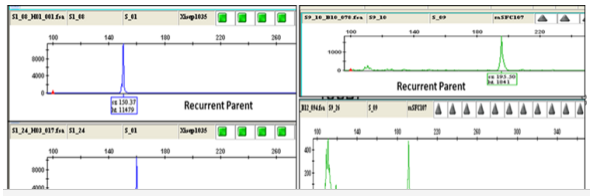

Figure1. Graphical representation (chromatogram) of parental lines screening with SSR markers, analysed through ABI prism 3700xl. The peaks show polymorphism at that locus on different chromosomes for the lines which are to be used as recurrent and donor lines.

SSR Polymorphism in different crosses

Since the SSR markers were screened for all genotypes that can be used as parents in backcrossing, an effort was made to identify the polymorphic markers for twenty backcrosses. In terms of polymorphic markers, the number of polymorphic markers for each QTL on SBI 01, SBI 07, SBI 10 and SBI 05 are 5, 6, 11 and 11 respectively. The percent polymorphism among the different donor recurrent combinations varied from 0 to 100 %. Comparative marker polymorphism among donor recurrent combination has been represented in table 3.

Genotypes	J2658	J2698	J2714	J2743	J 2614	J2758-5	J2833-11	J2799	J2834-6
ICSB75	60	40	16.6	16.6	60	16.6	58.3	25	33.3
ICSB11001	60	40	16.6	16.6	60	66.6	66.6	75	75
ICSB11005	80	40	25	25	70	58.3	66.6	66.6	58.3
ICSB11029	80	80	25	25	50	33.3	66.6	41.6	33.3
ICSB29001	40	80	25	25	50	41.6	16.6	58.3	50
ICSB29004	20	80	50	8.3	50	33.3	66.6	41.6	50
ICSB29005	80	80	25	16.6	80	16.6	66.6	25	33.3
ICSB29007	20	80	16.6	16.6	100	33.3	58.6	41.6	41.6
ICSB29014	80	60	16.6	8.3	90	25	50	33.3	33.3
ICSB29016	60	40	16.6	8.3	60	8.3	58.3	16.6	25
ICSB48	80	60	16.6	16.6	40	0	50	16.6	16.6
ICSB101	60	20	16.6	16.6	30	66.6	50	50	58.3
P.MOTI	80	80	25	25	50	33.3	66.6	41.6	33.3
P.VASUDHA	100	100	25	25	40	33.3	66.6	41.6	33.3
M35	20	80	25	25	60	50	66.6	58.3	50
NTJ2	40	80	16.6	16.6	90	33.3	41.6	50	50
P.CHITRA	80	60	25	33.3	30	41.6	58.3	50	50
P.YASHODHA	80	60	25	33.3	30	25	58.3	33.3	41.6
P.ANURADHA	80	60	16.6	25	60	41.6	50	50	58.3
P.JYOTI	80	60	33.3	25	50	25	58.3	33.3	41.6

Table 3. Comparitive marker polymorphism for different donor recurrent combinations

Genetic Relationships among 20 Sorghum Genotypes

Based on the scoring data (0 or 1) obtained for 33 SSR loci, a similarity matrix was generated manually. Similarity index of the 33 polymorphic maker loci ranged from 0.1 to 1. Lowest similarity index indicates two genotypes are diverse and higher similarity index indicates highest similarity between two genotypes. It was found that IS18551 and BTx623 which are donors of these shootfly resistant QTLs has the highest similarity index (1.0). Apart from these two, highest similarity index was observed for all genotypes for QTL on chromosome SBI 07 (Table 4). On the other hand the 6 most distantly related genotypes were identified with donor J 2614.

	J2658-6	J 2698	J 2714	J2743-3	J2614	J2758-5	J2833-11	J2799	J2834-6
ICSB75	0.40	0.60	0.75	0.83	0.40	0.75	0.33	0.67	0.58
ICSB11001	0.40	0.60	0.75	0.75	0.40	0.33	0.33	0.25	0.25
ICSB11005	0.20	0.40	0.75	0.75	0.20	0.42	0.33	0.33	0.42
NTJ2	0.20	0.20	0.75	0.75	0.40	0.67	0.33	0.58	0.67
ICSB29001	0.20	0.20	0.75	0.75	0.60	0.67	0.33	0.58	0.67
ICSB29004	0.20	0.20	0.75	0.75	0.50	0.67	0.33	0.58	0.67
ICSB29005	0.80	0.20	0.75	0.75	0.30	0.50	0.33	0.42	0.50
ICSB29007	0.60	0.20	0.83	0.83	0.10	0.58	0.50	0.42	0.42
ICSB29	0.60	0.20	0.75	0.75	0.50	0.50	0.67	0.33	0.42
ICSB29016	0.80	0.20	0.75	0.75	0.40	0.58	0.25	0.50	0.42
ICSB11029	0.20	0.20	0.75	0.83	0.20	0.83	0.33	0.75	0.67
ICSB48	0.80	0.20	0.83	0.83	0.10	0.67	0.42	0.58	0.58
P.MOTI	0.20	0.40	0.83	0.91	0.10	0.75	0.50	0.67	0.67
P.VASUDHA	0.40	0.60	0.83	0.91	0.10	0.83	0.33	0.75	0.67
M35	0.20	0.40	0.83	0.83	0.10	1.00	0.50	0.83	0.83
ICSB101	0.40	0.80	0.83	0.83	0.30	0.33	0.50	0.50	0.42
P.CHITRA	0.20	0.40	0.75	0.66	0.60	0.58	0.42	0.50	0.50
P.YASHODHA	0.20	0.40	0.75	0.66	0.60	0.75	0.42	0.67	0.58
P.ANURADHA	0.00	0.20	0.83	0.75	0.40	0.58	0.50	0.50	0.42
P.JYOTI	0.00	0.20	0.66	0.75	0.40	0.75	0.42	0.67	0.58
296B	0.00	0.20	0.75	0.75	0.40	0.58	0.50	0.67	0.58
BTx623	0.80	0.20	0.83	0.83	0.10	0.50	0.50	0.33	0.42
IS18551	0.80	0.60	0.91	1.00	0.80	0.75	0.50	0.83	0.67

Table 4. Jaccards Similarity Index for different sorghum genotypes compared to donor J lines

Genetic similarity (between donor line and recurrent lines) was used to prepare a unweighted pair group method (UPGMA) dendrogram, using the software NTSYS version 2.1. The UPGMA dendrogram for four QTLs are showed in figure 2.

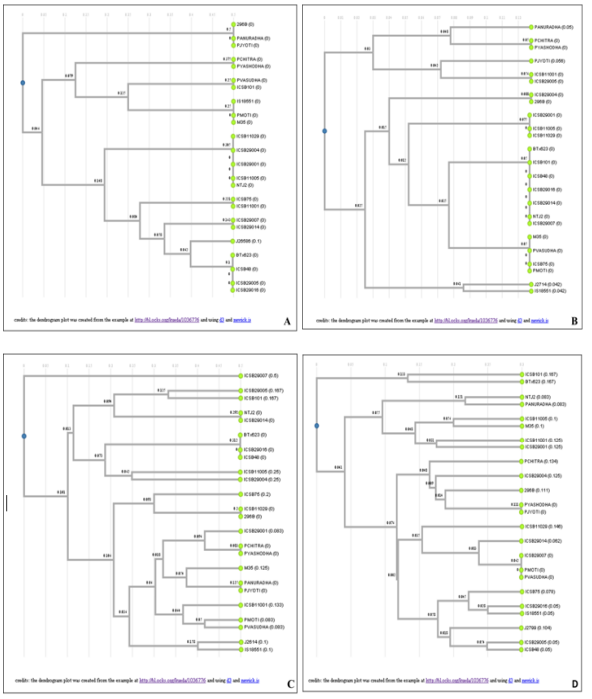

Figure 2. Dendrogram of the 23 genotypes of sorghum (Sorghum bicolor) for four QTLs obtained from simple sequence repeat (SSR) molecular markers, using the similarity index of Nei & Li and the unweighted pair group method with arithmetic mean (UPGMA).

For each QTL different genotypes are showing similarity with the donor. For example figure 2 (A) shows J2658 is clustered with BTx623, ICSB 48, ICSB 29005 and ICSB 29016 exhibiting higher similarity with these recurrent parents and lowest with 296B, P.Anuradha and P. Jyoti. Similarly, J2714 is clustered with IS18551 and distantly related to P. Anuradha, P.Chitra and P. Yashodha. Apart from J2714, IS18551 also clustered with J2614 along with P. Moti and P. Vasudha showing highest similarity in the QTL region, whereas ICSB29007 was found to be diverse with J2614. Lastly, J2799 clustered with ICSB29005 and ICSB 48, but is distantly related to ICSB101 and BTx623.

Discussion

Sorghum is an important cereal crop worldwide, distributed across semi-arid tropics of Asia, Africa and Latin America. It is widely cultivated for food, feed, fodder and bioenergy crop. Even though sorghum is an economically and nutritionally important crop unavailability of appropriate genomic resource hindered molecular breeding applications. The availability of the genome sequence along with the advent of next generation sequencing and genotyping technologies microsatellite or SSR markers have been proven as useful and advantageous molecular markers for genome mapping, genetic diversity studies, QTL mapping and marker assisted selection as they are codominant and multiallelic in nature (Gupta and Varshney, 2000). As a result several laboratories across the world developed a reasonable number and quality SSR markers for sorghum. These in turn have led to the generation of a large number of DNA-based markers and resulted in the identification and fine mapping of QTL associated with grain yield, its component traits, biotic and abiotic stress tolerance as well as grain quality traits in sorghum (Madhusudana et al book chapter, Sorghum Molecular Breeding)

The present study aimed at validation and polymorphism assessment of the previously developed 58 genomic SSR markers present on the four QTL regions on a set of 32 sorghum genotypes of which 20 genotypes can be used as recurrent parents, 9 genotypes (J lines) are donor parents and 3 genotypes are standards (IS18551, 296B and BTx623). Genetic relationships among these all genotypes

Volume : 5 | Issue : 12 | December-2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 78.46

was also studied among the donor lines and recurrent lines. This study reports successful amplification of 42 out of 58 SSR markers with the same PCR components and the same touchdown PCR cycle. SSR Polymorphism was confirmed by genotyping the four QTL regions with the linked markers. The graphical representation of four linked markers belonging to four QTLs shows polymorphism at that particular locus. These markers can further be used as foreground markers in backcross program involving the above mentioned recurrent and donor parents to identify the presence of QTLs.

In the present study, the percent polymorphism between parents ranged from 0 to 100 %. Least or no polymorphism (0 %) was identified between J2758 and ICSB48, followed by J2743 and ICSB29004, ICSB29014, ICSB29016 with 8.3% polymophism. Highest polymorphism was found between three combinations J2658 - P.Vasudha, J2698 - P.Vasudha and J2614 - ICSB29007 with 100% polymorphism. Knowledge of genetic diversity in a crop species is fundamental to its improvement. Substantial diversity exists among J lines and other high yielding sorghum genotypes. In this study, 33 SSR markers showed sufficiently high sensitivity to detect DNA polymorphism among the 20 cultivated genotypes. The dendrogram constructed, not only shows the extent of genetic relationship but also shows the level of similarity between the parents of included in this study (Fig. 2).

All 23 genotypes used in study have revealed the genetic relationship with donor parents giving possibilities to explore their utility for further studies. The highest genetic diversity was detected between J2614 and ICSB 29007, ICSB48, P. Moti, P.Vasudha, M-35, BTx623 with similarity coefficient of 0.1.

Conclusion

The polymorphic markers reported in the present study are optimally placed across the QTL region. We have identified minimum five polymorphic markers on each QTL. In general, three markers optimally placed are sufficient to ensure a target control rate above 99% (Frederic Hospital , book chapter, Marker assisted breeding). On genotyping both recurrent and donor parent with 58 SSR marker flanking four shoot fly resistance QTLs revealed 33 SSR markers that are polymorphic and can further be used to detect hybrids with same donor recurrent combination. The polymorphism was measured in terms of proportion of SSR markers showing atleast 2bp size difference in the alleles amplified. The polymorphism among the parents was calculated to be as high as 100%.

Acknowledgements

The authors sincerely acknowledge the International Crops Research Institute for Semi Arid Tropics (ICRISAT) Patancheru, India for providing work space and Department of Science and Technology, New Delhi for providing funding for this research.

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