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GENETIC DIVERSITY IN F_2 SEGREGATING POPULATION OF SINGLE-CUT AND MULTI-CUT VARIETY OF SORGHUM

ANITA YADAV[1], PARDEEP KUMAR*[,2], K. S. BOORA[1] AND S. K. PAHUJA[3]

[1]Department of Molecular Biology, Biotechnology & Bioinformatics
CCS Haryana Agricultural University,
Hisar-125 004 (Haryana), India
**(e-mail : pardeep1@icar.gov.in)*

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SUMMARY

The F_2 segregating population of sorghum [*Sorghum bicolor* (L.) Moench] of cross HJ 541 (single-cut) × SSG 59-3 (multi-cut) was studied by using simple sequence repeats (SSR) markers to measure genetic divergence within the population. Out of 50 SSR markers, 21 were found to be polymorphic which showed 90.3 per cent polymorphism. Cluster tree analysis grouped the 32 F_2 progeny lines into two major clusters : Cluster I comprised three F_2 progeny lines with parental genotype, HJ 541, whereas cluster II had the remaining 29 F_2 progeny lines with parental genotype, SSG 59-3. Genetic similarity among F_2 progeny lines ranged from 0.45 to 0.81 with average similarity of 0.66. Parental genotypes of sorghum were found to be most diverse and F_2 progeny lines viz., G2, G18, G20, G21 and G29 showed high similarity with multi-cut parental sorghum genotype, SSG 59-3. Hence, these F_2 progeny lines could be selected for early variety development from the F_2 segregating population in order to meet ever increasing demand of green fodder for the livestock.

Key words : Genetic diversity, SSR markers, Single-cut sorghum, multi-cut sorghum, segregating population

It has been also estimated that need for forage crops will increase two to three folds up to 2050 in Asian countries (Devendra and Leng, 2011). To narrow down the gap between demand and supply of fodder for the increasing livestock population, multi-cut varieties of sorghum can provide the solution. Multi-cut sorghum varieties with quick regeneration lead to early vigour of sorghum crop which not only increases the fodder yield per unit area but also escapes the attack of pests at initial stages of plant growth (Grewal *et al*., 2005). Hence, there is an urgent need to re-orient forage sorghum improvement strategies to give more emphasis to develop multi-cut forage sorghum varieties in order to obtain a quantum jump in the production of quality fodder. There is an extremely large amount of variation between the cultivated taxa in the species of *Sorghum bicolor*. Pahuja *et al*. (1999) observed 24 genotypes of multi-cut sorghum from different geographical area for green and dry fodder yield along with the stability parameters. But, SSG 59-3 developed in 1974 in India has been used as a check variety in All India Coordinated Sorghum Improvement Project (AICRP) on forage sorghum, because a multi-cut variety that could yield better than SSG 59-3 was not released at the national level till date. SSG 59-3 was developed by advanced-generation selection of a cross between non-sweet Sudan grass and the sweet sorghum variety (Paroda and Lodhi, 1978). HJ 541 is a promising single-cut variety with 13.08 per cent increase in green fodder per day productivity over the best check of Haryana, HC 308. This variety has been found to be resistant against three major foliar diseases viz., grey leaf spot, zonate leaf spot and sooty stripe and moderately resistant against shoot fly (Pahuja *et al*., 2012). HJ 541 × SSG 59-3 cross was attempted in order to get maximum diversity, and to further advance this cross to get recombinant inbreed lines (RILs) in order to study the basis of regeneration in multi-cut sorghum genotypes. A large degree of genetic diversity among its populations will help an organism to adapt and evolve under unfavourable conditions.

Molecular markers are recognized as significant tools to accurately estimate the genetic structure for

[2]Plant Quarantine Divison, ICAR-National Burearu of Plant Genetic Resources, New Delhi-110 012, India.
[3]Department of Genetics & Plant Breeding , CCS HAU, Hisar (Haryana), India.

species of interest because molecular markers are not affected by the environment and can be detected in all tissues at all stages of development. There are many types of molecular markers like RAPD, RFLP, AFLP, ISSR, SSR, SNP etc. which have been developed and applied to study genetic diversity of sorghum (Morris *et al*., 2013; Motlhaodi *et al*., 2014). But, SSR markers have been found to be more reliable and useful than other markers. In addition, SSR markers are highly polymorphic, co-dominant, abundant and uniformly dispersed in the plant genome. SSR markers have been utilized for assessment of genetic diversity in sorghum Rajarajan and Ganesamurthy, 2011; Motlhaodi *et al*., 2014). The objective of the present study was to estimate the genetic structure using SSR markers and to find out new alleles in F_2 progeny lines of cross HJ 541 × SSG 59-3.

MATERIALS AND METHODS

Development of F_1 and F_2 Segregating Populations

F_2 segregating populations were generated by crossing parental genotypes viz., HJ 541 (single-cut) and SSG 59-3 (multi-cut) at the farm of Forage Section, CCS Haryana Agricultural University, Hisar, India. At panicle emergence and flowering stage, the florets of female parent, HJ 541 were hand emasculated early in the evening time i. e. around 4 p. m. and next day pollination was done using pollen of SSG 59-3 during morning hours i. e. before 8 a. m. when the stigma was receptive. To avoid contaminations from foreign pollens, emasculated panicles were covered with butter paper packet. The seeds set on female plants were harvested as F_1 seeds. F_1 seeds were sown next year (2011) to get F_1 plants and these were selfed to get F_2 seeds. F_2 seeds were sown in next year (2012) to get F_2 plants. Thirty-two high fodder yielding segregating plants were selected for molecular marker analysis.

SSR Genotyping

Genomic DNA was isolated from young leaves of 32 F_2 progeny lines of cross HJ 541 × SSG 59-3 along with their parents following modified CTAB (Cetyltrimethyl ammonium bromide) extraction method as reported by Murray and Thompson (1980) and modified by Saghai-Maroof*et al.* (1984) and Xu *et al.* (1994). PCR was performed in a volume of 20 µl, containing 1X PCR buffer (10 mMTris-HCl pH 8.3, 50 mMKCl, 1.5 mM MgCl2), 100 µM of each dNTPs, 1.0 units Taq DNA polymerase (Geno-Bioscience Pvt. Ltd.), 10 µM of each forward and reverse primer, and 50 ng of template genomic DNA. Amplifications via SSR markers were carried out using PTC-100 Thermal Cycler (MJ Research and Biometra personal, USA), using the following profile : initial denaturation at 94°C for 10 min, followed by 35 cycles at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. PCR products were resolved by electrophoresis on 3.0 per cent agarose gels and visualized under gel documentation system after staining with ethidium bromide.

Statistical Analysis

A binary (0/1) matrix was prepared using the banding pattern obtained from SSR analysis for each primer where 1 and 0 are representing the presence and absence of amplification, respectively. This matrix was used to calculate the genetic similarity among F_2 progeny lines using the Jaccard similarity coefficient. Further, cluster analysis was carried out using the similarity coefficients obtained from the above analysis through a sub program of NTSYS-pc version 2.2 (Rohlf, 1998). The dendrogram was constructed by unweighted pair group method with arithmetic averages (UPGMA) within the Sequential Agglomerative Hierarchical Nested (SAHN) analysis module of NTSYS-pc.

RESULTS AND DISCUSSION

SSR Markers Analysis

To identify the polymorphism, a total of 50 SSR markers, nearly having 50% G+C content were selected from published literature. Out of 50 SSR markers, nine markers (Xtxp 10, Xtxp 149, Xtxp177, Xtxp 197, Xtxp 205, Xtxp 215, Xtxp 225, Xtxp 227 and Xtxp 309) did not exhibit any amplification, 20 were found to be monomorphic and rest showed polymorphism.

A total of 58 sharp and reproducible alleles were obtained from 21 polymorphic SSR markers. Out of which 52 alleles were polymorphic and six alleles were monomorphic which showed 90.38 per cent polymorphism among the F_2 progeny lines similar to that found by Pei *et al.* (2010) and Pecina-Quintero *et al.*

(2012), while Zhan *et al.* (2012) reported polymorphism rate of 78.64 per cent using 31 SSR primers. The number of amplified alleles per primer ranged from 1 to 7. Primer Pep C amplified highest number of alleles that were 7, while primers Xtxp 56, Xtxp 92, Xtxp 105, Xtxp145, Xtxp 159, Xtxp 258, Xtxp 273, Xtxp 339, Xtxp 343, Xtxp 348, Xtxp 358 and Cbaf amplified with lowest number of alleles that was 2 with an average of 1.90 alleles per primer. The number of alleles amplified by all other primers was in between this range (Table 1). The minimum level of polymorphism i. e. 50 per cent was produced by primers Xtxp 295 and Xtxp 339, while 100 per cent polymorphism was obtained by all remaining primers except primers Xtxp 210, Xtxp 228 and Xtxp 335 which gave 66 per cent polymorphism (Table 1). The estimates of genetic parameters such as percentage polymorphism, allelic richness depend on a number factors such as diversity in the genotypes, the sampling schemes, number of SSR used, the size of the SSR repeats and the location of the SSR on the genome, etc. (Pejic *et al*., 1998). The banding profile and polymorphism generated using one of the primers (Xtxp 115) is shown in Fig. 1. The size of the bands obtained

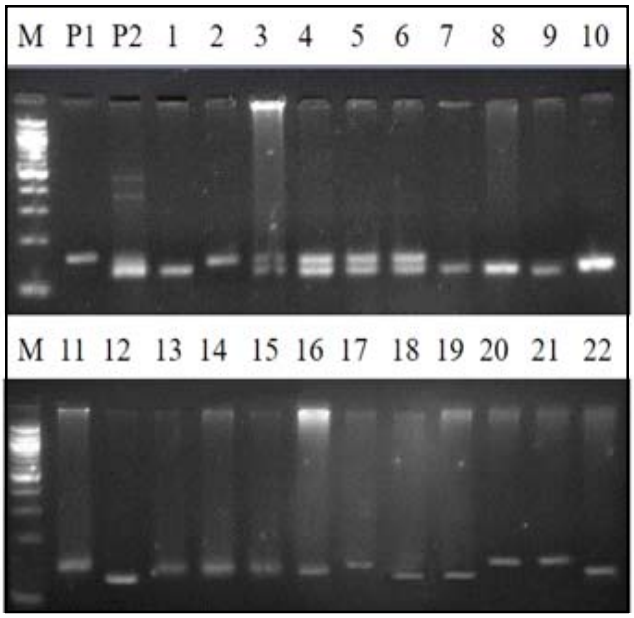

Fig. 1. SSR banding profile of F_2 progeny lines of cross HJ 541 × SSG 59-3 along with parental sorghum genotypes using Xtxp 115 marker.

P1- HJ 541, P2- SSG 59-3, G1-G22 : F_2 progeny lines of cross HJ 541 × SSG 59-3, M : 100bp plus DNA ladder.

TABLE 1

Details of polymorphic SSR markers used to study 32 F2 progeny lines of cross HJ 541 × SSG 59-3 along with parental sorghum genotypes

S. No.	Marker	Total numbers of alleles	Polymorphic alleles	Per cent polymorphism	Range of molecular size (bp)
1.	Pep C	7	7	100	150-1000
2.	Kaf 2	3	3	100	150 -500
3.	Cba f	2	2	100	200-300
4.	Xtxp 47	4	4	100	150-800
5.	Xtxp 56	2	2	100	120-180
6.	Xtxp 65	3	3	100	190-650
7.	Xtxp 92	2	2	100	200-210
8.	Xtxp 105	2	2	100	220-230
9.	Xtxp 115	2	2	100	120-500
10.	Xtxp 145	2	2	100	180-190
11.	Xtxp 159	2	2	100	170-190
12.	Xtxp 210	3	2	66	200-400
13.	Xtxp 228	3	2	66	190-400
14.	Xtxp 258	2	2	100	180-190
15.	Xtxp 273	2	2	100	280-300
16.	Xtxp 295	4	2	50	150-500
17.	Xtxp 335	3	2	66	140-400
18.	Xtxp 339	2	1	50	250-600
19.	Xtxp 343	2	2	100	300-350
20.	Xtxp 348	2	2	100	130-180
21.	Xtxp 358	2	2	100	200-210
	Mean	2.67	2.38	90.38	

as a result of the present study on the sorghum genotypes varied from 120-1000 bp and was comparable with some of the previous studies on sorghum (Singh and Boora, 2006; Ji *et al*., 2011). Primer, Pep C, amplified the maximum size of the allele, i. e. 1000 bp whereas primer Xtxp 56 amplified minimum size of allele i. e. 120 bp . The rest of the primers used in the present study gave bands having size in between this range (Table 1).

Unique Alleles

After analyzing all the bands amplified by the 21 primers across 32 F_2 progeny lines of cross HJ 541 × SSG 59-3, we had been able to identify some new/ unique alleles in F_2 progeny lines under study. Primer Xtxp 210 gave a unique allele of size 400 bp in progeny line, G12. Primer Pep C gave unique alleles of size 400 bp, 500 bp, 700 bp, 900 bp in F_2 progeny lines, G8, G10, G21, G23, respectively. The importance of unique alleles lies in the fact that they are associated with a particular progeny line/genotype (Agrama and Tuinstra, 2003). New alleles may be generated because of recombination (Ghebru *et al*., 2002). Rare alleles were also reported by Somers *et al*. (2007) and Motlhaodi *et al*. (2014) in sorghum genotypes.

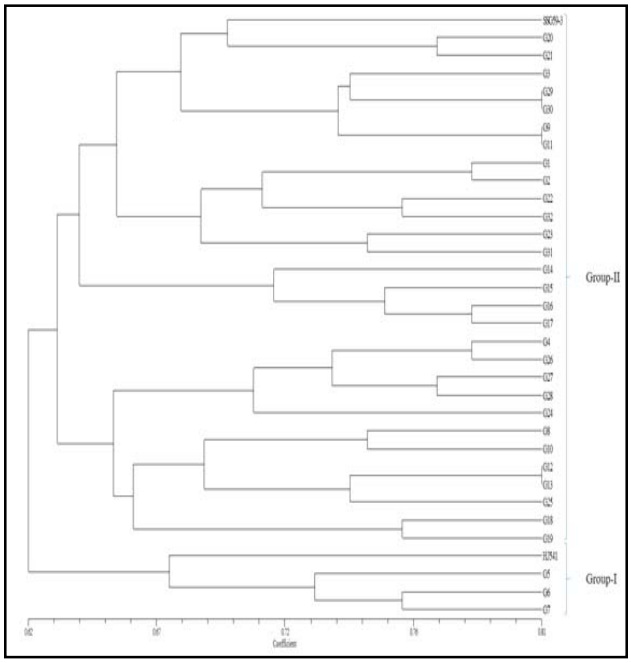

Fig. 2. Dendrogram of F_2 progeny lines of cross HJ 541 × SSG 59-3 along with parental genotypes based on SSR marker analysis.

HJ 541 : Single-cut sorghum genotype, SSG 59-3 : Multi-cut sorghum genotype, G1-G32 : F_2 progeny lines of cross HJ 541 × SSG 59-3.

Cluster Tree Analysis

The average linkage between 32 F_2 progeny lines of cross HJ 541 × SSG 59-3 along with the parental sorghum genotypes was used for constructing a phylogenetic tree depicting the relationship among progeny lines. The association amongst different progeny lines has been presented in the form of a dendrogram (Fig. 2). The cluster tree was broadly grouped into two major groups at a similarity coefficient of 0.62. Group I consisted of three progeny lines *viz.*, G5, G6 and G7 with single-cut parental genotype, HJ 541, while group II consisted of remaining 29 progenies along with multi-cut parental genotype of sorghum, SSG 59-3. Group II again was subdivided into two sub-groups at a similarity coefficient of 0.63. Sub-group I consisted of 17 F_2 progenies along with parental genotype, SSG 59-3. Sub-group II consisted of remaining 12 F_2 progenies of group II. These sub-groups were further divided into sub sub-groups. Progeny lines G12 and G13; G9 and G11; G9 and G30; G29 and G30 showed maximum similarity i. e. 0.81. Cluster tree analysis based on SSR markers data showed that F_2 progeny lines of cross HJ 541 × SSG 59-3 were interspersed between the two parental sorghum genotypes with inclination towards multi-cut parental sorghum genotype, SSG 59-3.

Similarity Matrices Analysis

The similarity matrices revealed the relationships among the 32 F_2 progeny lines of cross HJ 541 × SSG 59-3 along with their parental sorghum genotypes. The similarity matrices among the progeny lines ranged from 0.45 to 0.81. The average similarity across all the progenies was found out to be 0.66, indicating a high level of genetic divergence among the 32 F_2 progeny lines. The average genetic diversity observed in the present study was similar to that found by Agrama and Tuinstra (2003), who obtained values of 0.62 and 0.58, respectively, but was higher than the values reported by Schloss *et al.* (2002) and Ali *et al.* (2008) who obtained values of 0.46 and 0.40, respectively. Progeny line, G29 showed maximum similarity (0.75) with multi-cut parental sorghum genotype, SSG 59-3, while progeny line, G4 showed maximum similarity (0.70) with single-cut parental sorghum genotype, HJ 541. The maximum similarity value of 0.81 obtained between progenies G12 and G13, G9 and G11, G9 and G30, G29 and G30, while minimum was observed between progenies G7 and G18 (0.45).

CONCLUSION

This study provides the quantification of genetic diversity present in F_2 progeny lines of cross HJ 541 × SSG 59-3. The data obtained demonstrate that there was substantial genetic diversity within F_2 progeny lines. The progeny lines showing high genetic similarity with multi-cut sorghum genotype may be directly selected for development of new varieties. The presence of unique/ new alleles in some progeny lines indicates the need for further evaluation in next generations to identify progeny lines with desirable traits.

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