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Diagnostic set of microsatellite markers for hybrid purity testing and molecular identification of hybrids and parental lines in sorghum

Research Article

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Abstract

Ten sorghum hybrids (seven kharif, two dual purpose and one rabi types) along with their thirteen parental lines were utilized for molecular identification and hybrid purity testing in the present study. Ten out of 11 microsatellite (or SSRs-simple sequence repeats) markers used were polymorphic (90.9%) with 2 to 6 alleles (average 3.4 alleles) and PIC (Polymorphism Information Content) value ranged from 0.28 to 0.61. All the ten polymorphic markers revealed polymorphism between the male and female parents of one or more hybrids and would be useful for their hybridity testing. Further, Sb5-236 and Sb4-15 have been identified as the two diagnostic markers for the genetic purity testing of all the ten sorghum hybrids included in this study. Sb6-42, Sb4-22 and Sb5-236 individually or a combination of Sb6-42/Sb4-22 and Sb5-236 could distinguish three and five hybrids respectively and can be used as referral set of markers for identification and protection of sorghum hybrids (CSH 13, CSH 15R, CSH 1, CSH 14 and SPH 837). All the kharif/dual purpose and rabi sorghum hybrids were grouped in separate clusters. Diagnostic/referral set of SSR markers will aid in hybrid breeding programme of sorghum.

Keywords: Sorghum; Microsatellite markers; Hybrids; Purity testing

Introduction

Sorghum is a very important high biomass producer C_4 plant and has been considered as a 4F crop for its usage as food, feed, fodder and fuel crop. In India it is grown in many states from Tamil Nadu to Uttarakhand in two seasons, namely rainy (kharif) and post-rainy (rabi) season. Sorghum is the second crop after maize in developing high yielding hybrids using cytoplasmic-genic male sterility system. So far, approximately 30 hybrids and 26 varieties have been released nationally besides many more state releases. Hybrid purity is very much essential for its high commercial value and production of certified hybrid seeds. Compared to conventionally used 'grow-out test (GOT)' for hybrid purity testing, molecular markers based testing is more effective as it is not affected by environmental factors and time constraints. Among the available molecular markers, SSRs offers an important DNA marker system for hybrid purity testing because of their co-dominance, reproducibility, robustness, and multi-allelic nature. SSR markers can be used to generate unique DNA fingerprints useful for the identification/molecular characterization of parental lines and hybrid purity testing in sorghum. The SSR markers have been used for the assessment of purity of hybrids and parental lines in other crops [1-5] and in sorghum RAPD markers have been used before for hybrid purity testing [6,7]. The present study was undertaken with the objective to identify or develop diagnostic set of SSR markers showing polymorphism between parental lines of different hybrids of sorghum.

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Materials and Methods

Plant materials

A total of 23 sorghum genotypes (Table 1) including 10 hybrids and 13 parental lines [A-line (cytoplasmic male sterile line/female parent) and R-line (fertility restorer line/male parent)] were used for SSR profiling.

DNA extraction

Genomic DNA of bulk (20 plants/genotype) leaves was extracted using CTAB method [8]. DNA quantification was done using H33258 dye in a fluorometer (Hoeffer Scientific, San Francisco, USA) with calf thymus DNA as standard.

PCR amplification

For SSR analysis the following reaction components were used: 30 ng of genomic DNA, 2.5 mM $MgCl_2$, 1U Ampli*Taq* Gold DNA polymerase (PE Applied Biosystems), 1x PCR buffer without $MgCl_2$, 0.3 µM forward and reverse SSR primers each and 0.2 mM dNTP mix. A total of 11 labeled (forward primers labeled with FAM/HEX/TET dyes) SSR primers were used for SSR profiling [9,10]. Thermocycling conditions used for PCR amplification were: denaturation at 95°C for 10 min. followed by twenty five cycles of denaturation at 95°C for 1 min, primer annealing at 55°C for 2 min, and primer extension at 72°C for 2 min, and final extension step was done at 72°C for 10 min. All the PCR amplification was carried out in Gene Amp PCR system 9600 (PE Applied Biosystems). PCR products (1.0 µl) were mixed with 0.5 µl of Gene Scan 500 ROX (in case of HEX and FAM labeled primers) or TAMRA 500 (in case of TET labeled primers) internal size standard, 12.0 µl of de-ionized formamide and were denatured at 96°C for 6.0 minutes. The denatured PCR products were run on ABI PRISM 310 Genetic Analyser.

Data analysis

Data was analysed by Gene Scan^R 3.1 analysis software. Electropherograms were generated and the data was converted to binary unit characters (1.0= present, 0= absent) using Genotyper (Version 2.5, Perkin-Elmer/ABI) software. Polymorphism Information content (PIC) value was calculated as described by Botstein [11]. Then NTSYS-pc version 2.1 [12] was used to calculate Jaccard's similarity coefficient [13] and UPGMA based cluster analysis.

Results and Discussion

Genetic purity testing of hybrids is mandatory for the successful seed production. Co-dominant DNA markers like SSRs produce unambiguous banding patterns useful for hybridity and hence purity testing. In the present study eleven SSR markers were used for the identification of ten sorghum hybrids along with their parental lines, indicating the application of this technique to distinguish the hybrids from its parental lines and hybrid purity testing (Figure 1). Of all the 11 primers pairs used in this study, Sb5-256 was monomorphic, showing only one allele of 171 bp in size. Ten markers showed polymorphism (90.9%) with 2 to 6 alleles (average 3.4 alleles) and 0.28 to 0.61 PIC value. All the ten polymorphic markers showed polymorphism between the parents of one or more hybrids (Table 2). And for a particular SSR marker parental lines of one to three hybrids showed unique fingerprints. Sb6-42 produced highly polymorphic patterns with six alleles of size 181, 183, 185, 187, 191, 193 bp among different hybrids and their parents. Banding patterns for Sb5-236, Sb1-1 and Sb4-22 also displayed high polymorphism, with four alleles each. Maximum number of seven sorghum hybrid combinations and their parental lines could be distinguished easily by using only one primer pair Sb5-236 followed by Sb4-15 (six sorghum hybrids and their male and female parents). All the ten sorghum hybrids and their parental lines could be distinguished using two primer pairs Sb5-236 and Sb4-15 and would serve as potential set of markers for genetic purity testing of these ten sorghum hybrids. Out of ten polymorphic SSR primer pairs eight showed unique fingerprints for one to three hybrids viz. Sb1-1 (CSH 1), Sb6-57 (CSH 9), Sb6-84 (CSH 15R), Sb6-42 (CSH 13, CSH 15R and CSH 1), Sb4-72 (CSH 17), Sb4-22 (CSH 13, CSH 15R and CSH 1), Sb5-236 (CSH 14, CSH 15R and SPH 837), Sb4-121 (CSH 6). Sb6-42, Sb4-22 and Sb5-236 individually or a combination of Sb6-42/Sb4-22 and Sb5-236 could distinguish three and five hybrids respectively and can be used as diagnostic set of markers for identification and protection of these sorghum hybrids.

Table 1: Characteristics of hybrids and parental used for the present study.

Hybrids	Parental lines	Characteristics
CSH 1	*IS 84 X CK 60A	Kharif hybrid, early type (100 days maturity)
CSH 5	CS 3541 X 2077A	Kharif hybrid, average yield 34 q/ha, medium type (100-120 days maturity)
CSH 6	CS 3541 X 2219A	Kharif hybrid, average yield 34 q/ha and is suitable for inter-cultivation with pigeon pea, early type 90-95 days to maturity
CSH 9	CS 3541 X 296A	Kharif hybrid, average yield 40 q/ha, medium type 100-120 days to maturity
CSH 13	RS 29 X 296A	Medium type, average yield 39q/ha, dual purpose
CSH 14	*AKR 150 X AKMS 14A	Kharif hybrid, early type, average yield 38q/ha
CSH 15R	RS 585 X 104A	Rabi hybrid
CSH 16	C 43 X 27A	Kharif hybrid, average yield 41q/ha, medium type
CSH 17	RS 673 X AKMS 14A	Kharif hybrid, average yield 40q/ha, early type
SPH 837	SU 556 X AKMS 14A	Early type (85-90 days maturity), average yield 35-40q/ha, dual purpose

*parental lines not included in the present study

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Table 2: Allele sizes (bp) of different polymorphic SSR markers in sorghum hybrids and parental lines.

Hybrids/parents	Sb1-1	Sb6-57	Sb6-84	Sb6-42	Sb4-72	Sb4-22	Sb6-34	Sb4-15	Sb5-236	Sb4-121
RS 29	250	283	196	193	189	317	196	128	172	226
CSH 13	250	9	*182/196	**187/193	189	**299/317	*196/200	128	*172/178	*226/214
296 A	250	305	182	187	189	299	200	128	178	214
CSH 9	250	**283/305	*182/196	*187/191	189	299	200	9	*172/178	*226/214
CS 3541	250	283	196	191	189	299	200	128	172	9
CSH 6	250	283	9	191	189	299	9	*120/128	172	**216/226
2219 A	250	283	194	191	189	299	200	120	172	216
CSH 14	*250/256	303	9	*187/191	189	9	9	*120/128	**174/178	9
AKMS 14A	256	303	196	191	189	299	196	120	174	226
CSH 17	*250/256	*283/303	9	191	**189/181	299	9	*120/128	174	226
RS 673	250	283	194	191	181	9	9	128	174	226
104 A	250	305	194	185	191	299	200	128	172	214
CSH 15R	250	305	**182/194	**181/185	191	**299/301	9	9	**172/170	214
RS 585	9	305	182	181	191	301	200	128	170	214
27 A	250	283	196	191	189	299	200	120	172	214
CSH 16	250	283	196	191	189	299	200	*120/128	172	*226/214
C 43	250	283	196	191	189	299	200	128	172	226
SPH 837	*250/256	*283/303	196	191	189	299	*196/200	*120/128	**172/174	226
SU 556	250	283	196	191	189	299	200	128	172	226
CSH-1	**252/262	*283/303	*182/196	**183/191	189	**299/313	200	120	*172/178	9
CK 60A	252	283	196	191	189	299	200	120	172	216
CSH-5	250	*283/303	196	191	189	299	200	*120/128	*172/178	9
2077A	250	303	196	191	189	299	200	120	178	214
No. of alleles	4	3	3	6	3	4	2	2	4	3
PIC	0.35	0.58	0.54	0.52	0.33	0.29	0.28	0.49	0.61	0.60

* Hybrids showing polymorphic alleles between parental lines; **hybrids with unique fingerprints; 9 missing data; PIC Polymorphism Information Content

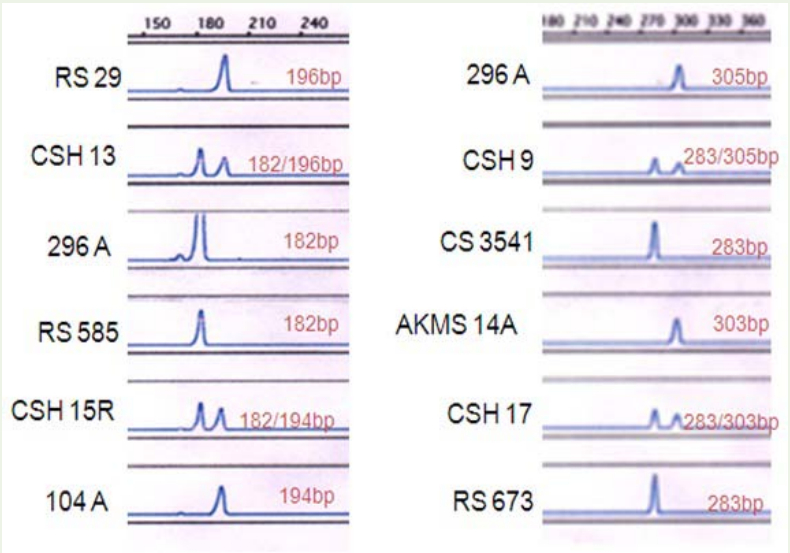

Figure 1: SSR electropherogram of four hybrids and parental lines of sorghum with primers Sb6-84 and Sb6-57 highlighting the importance of SSR markers in hybrid purity testing.

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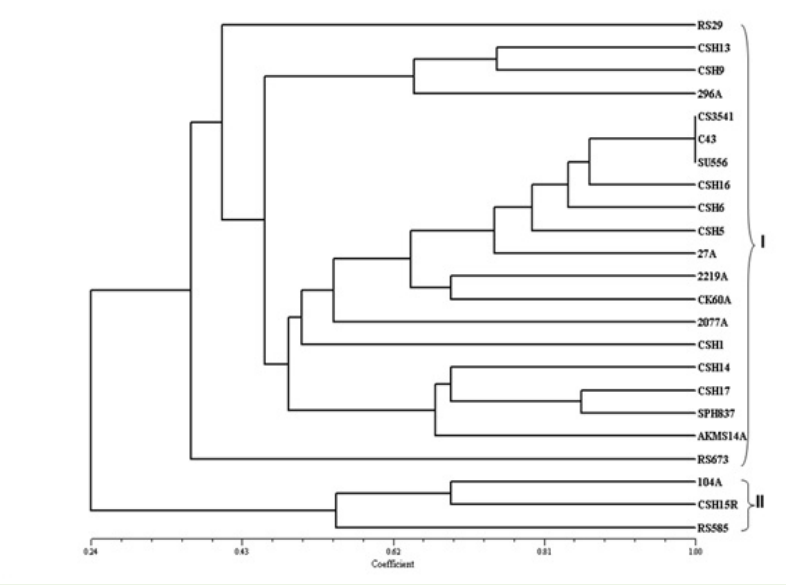

Figure 2: UPGMA clustering of sorghum hybrids and their parental lines.

Genetic relatedness

Allelic data was converted to 0/1 data and Jaccard's similarity coefficient and UPGMA cluster analysis was done using NTSYS-pc version 2.1. Average Jaccard's similarity coefficient observed was 0.46 that revealed average genetic diversity of 54% among the experimental material studied. Two major clusters I and II were observed (Figure 2). Cluster I consisted of kharif and dual purpose hybrids and their parental lines, while cluster II consisted of rabi sorghum hybrid CSH 15R and its parental lines. Hybrids CSH 13 and CSH 9 sharing common female parent 296 A were closely grouped. Similarly CSH 5 and CSH 6 were closely grouped with its common male parent CS 3541. And CSH 16 was clustered with its male parent C 43. Three hybrids viz. CSH 14, CSH 17 and SPH 837 having AKMS 14A as its common female parent were placed in one cluster. All the ten sorghum hybrids, seven female and three male parental lines could be distinguished with the set of SSR markers used in this study.

The study showed the usefulness of SSR markers as authentic technology in demarcation of different hybrids and their parental lines as well as in genetic purity assessment of sorghum hybrids, which otherwise based on conventional methods is labor intensive, environment effected and time consuming. The identified set of SSR markers will be beneficial for the protection of sorghum hybrids and facilitate hybrid breeding programmes in sorghum.

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