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MICROSATELLITE BASED DNA FINGERPRINTING OF SORGHUM [*SORGHUM BICOLOR* (L.) MOENCH] HYBRID CSH-35 WITH ITS PARENTS

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ABSTRACT
The elite sorghum hybrid CSH-35 with its restorer AKR-504-A, Male sterile line 30-A and maintainer AKMS-30-B were fingerprinted using 8 simple sequence repeat (SSR) primers spread throughout the genome. Out of these, 6 primers produced polymorphic profiles, while 2 were monomorphic. In a screen of 4 cultivars, 16 SSR loci produced 17 unique alleles, which provided an opportunity for their unambiguous identification. The DNA profile generated could be used for appropriate identification of hybrid CSH-35, control of violation and to determine seed mixtures. Depending upon the variation in genetic relationship the information can be used to select parental lines for crossing programme for development of hybrid sorghum varieties.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is an important crop in many parts of the world and used for food, fodder and fuel (ethanol) production. It is a major crop in many parts of Africa and some Asian countries. Sorghum ranked fifth among the cereals produced worldwide after rice, wheat, maize and barley (FAOSTAT, 2011). It is estimated that more than 300 million people especially from developing countries rely on sorghum as source of energy (Godwin and Gray, 2000). Some of the industrial uses of sorghum include preparation of beer, adhesives, dye, resins, ethanol and fuel (House, 1985; NAS., 1996). Protection of Plant Varieties and Farmer's Rights Act (2001) requires the registration and protection of new and notified plant varieties based on the criteria of distinctness, uniformity and stability (DUS) of morphological characteristics. The molecular markers can very well support the DUS testing in such cases. However, these morphological traits have not been able to resolve closely related genotypes. In hybrid breeding program, utilization of parental lines with considerable variability is of primary concern for exploitation of maximum level of heterosis or hybrid vigor in the F1 seed production. DNA based molecular markers such as restriction fragment length polymorphism (RFLP) (Botstein *et al*., 1980), randomly amplified polymorphic DNA (RAPD) (Ayesha *et al*., 2011; Williams *et al*., 1990), simple sequence repeats (SSR) (He *et al*., 2012; Kaladhar *et al*., 2004; Tautz, 1989) and amplified fragment length polymorphism (AFLP) (Vos *et al*., 1995) *etc*. have been used effectively to assess the genetic variability in several crop species. Komal *et al*. (2015) used RAPD primers for varietal identification of grapes. The RAPD patterns were served as an ideal tool for identification and characterization of the cultivars as well as evaluation of genetic diversity among various genotypes of the crop. Vikram *et al*. (2015) evaluated twenty rice cultivars (ten mega rice varieties and ten landraces) of India for genetic diversity using 50 microsatellite markers. A total of 98 alleles were detected by 34 polymorphic markers with an average of 2.88 alleles per locus. A maximum of five alleles were observed with the primer pair OSR13 and RM474 and the minimum of two alleles were observed in as many fifteen primer pairs.

It also facilitates identifying of parental line specific molecular markers (Nandakumar *et al*., 2004; Sarao *et al*., 2010), that are useful in detecting hybrids derived from them as well as genome mapping of economically important traits (Chen *et al*., 2011; Kumar *et al*., 2012). The knowledge about the genetic relationship among the most popular cultivars will provide useful information to plant breeders, as it contributes to selection, germplasm resource management and prediction of potential genetic gain (Chakravarthi and Naravaneni 2006, Hai *et al*., 2007). The present study was undertaken to evaluate

the genetic variability among an elite sorghum hybrid and its parental lines with the help of SSR markers based DNA fingerprinting**.** DNA based markers were used to develop "fingerprint" patterns of their varieties so as to identify the varieties thereby to protect their breeder's rights and to avoid disputes arising from variety ownership argues. However, fingerprinting and genetic relationships among sorghum varieties and lines released for production in Ethiopian has not been done to date. Therefore, this study was initiated to develop a DNA fingerprint pattern of released CSH-35 in and to assess their genetic relationship thereby the information generated could be used by breeders and seed companies to distinguish the released varieties and choose potential parental lines for crossing to develop hybrid varieties.

MATERIALS AND METHODS

Plant material

The four genotypes including male sterile line AKR -504-A and 30-A, the maintainer line AKMS-30B and the elite hybrid CSH-35 grown in control condition in the glass house of Biotechnology Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The tender leaves were used for DNA extraction.

SSR markers

SSR markers covering maximum genome of sorghum are selected for fingerprinting. Respectively from each chromosome 8 loci were selected for the profiling (Table 2). Primers were designed on the Primer3 online tool for primer designing by keeping GC content near to 55 percent for proper annealing of the primers.

DNA Extraction

DNA extraction was done using CTAB method. (Murray and Thompson 1980). A set of 8 primers spread across the genome were selected for screening the polymorphism among the hybrid and parental lines using PCR. The PCR was carried out using protocol suggested by Jain and Yadav (Jain and Yadav 2009). Seedlings were grown in the greenhouse during November, 2010. Fresh leaves of 10 individual plants were harvested in bulk from 14 days old seedlings and dried with silica gel in zip locked plastic bags and used for DNA extraction. The DNA was extracted following a modified CTAB (Cetyl Trimethyl Ammonium Bromide) extraction protocol (Mace *et al.*, 2003) at BecA Laboratory, Nairobi, Kenya. A labeled 96 well tube box that contained one stainless steel grinding ball in each tube were put in ice bucket containing liquid nitrogen to chill the tubes. Approximately 1.2 cm2 sorghum leaves were placed into 96 well strip tubes and slid with forceps to about 5 mm above the steel ball strip, caps were put tightly on the tubes, a third-folded paper towel on top of them and covered with a lid and stored at -20EC until ready to grind. Caps were removed carefully and about 450 μL of preheated (65EC) extraction buffer (100 mM Tris-HCl [pH 8], 1.4 M NaCl, 20 mM EDTA, 3% CTAB and 0.17% β-mercaptoethanol) was added to each sample and closed with caps. Samples were then macerated using a Geno/grinder (Geno 2000, Sigma) at 500 strokes/min for 2 min. The macerated samples were incubated for 40 min at 65EC in water bath with occasional mixing. Solvent extraction was done by adding 450 μL chloroform: isoamylalcohol (24:1) to each tube and inverted twice to mix. The tubes were centrifuged at 3500 rpm for 15 min and the entire upper aqueous layer was transferred to fresh strip tubes. About 500 μL of pre-cooled isopropanol (stored at -20EC) was added and inverted three times to mix and kept in -20EC freezer for 2 h. Then the tubes were centrifuged at 3500 rpm for 30-35 min. The supernatant was then decanted, the pellet was air dried for 30 min and dissolved in 200 μL low salt Tris-EDTA (TE) buffer (10 mM Tris, 0.1 mM EDTA, pH 8). To remove RNA from the DNA solution, 3 μL RNase A (10 mg mLG1) was added to each sample and incubated for 30 min at 37EC. A second phase solvent extraction was done by adding 200 μL chloroform: isoamyl alcohol (24:1) to each sample and inverted twice to mix and centrifuged at 3500 rpm for 15 min. The upper layer was transferred to new strip tubes and 500 μL ethanol: sodium acetate solution was added to each sample, then inverted twice to mix and placed in -20EC for 2 h and then centrifuged at 3,500 rpm for 30 min. The supernatant was decanted and the pellets washed with 200 μL 70% precooled ethanol. The tubes were centrifuged at 3,500 rpm for 5 min, the supernatant decanted and the pellet air-dried for about an hour. Finally, pellets from each sample were dissolved in 100 μL low-salt TE buffer and stored at -20EC.

Polymerase chain reaction (PCR)

PCR amplification was performed in 2.0ul of 10X PCR buffer, 3.0ul of dNTPs (1mM), 1.0ul of each of the forward and reverse primers (5M), 0.2ul of Taq polymerase (5Units/ul), 3.0ul of DNA (20ng) and distilled deionised water using a thermal cycler. The PCR profile consisted of initial denaturation at 94ºC for 4 min. and subsequent 35 cycles each with denaturation at 94ºC for 1min, primer annealing at 55ºC for 1 min. and primer extension at 72ºC for 1 min. final extension step was performed at 72ºC for 7 min.

Data analysis

Marker index for SSR markers was calculated in order to characterize the capacity of each primer to detect polymorphic loci among the genotypes. It is the sum total of the polymorphism information content (PIC) values of all the markers produced by a particular primer. PIC value was calculated using the formula PIC = $1\text{-}\Sigma pi\ 2$, where *pi* is the frequency of the i^{th} allele (Smith *et al.*, 1997).

RESULTS AND DISCUSSION

DNA markers are more contrasting as compare to morphological and biochemical markers. DNA markers are infinite in numbers and are not exaggerated by environmental factors and the developmental stages of the plant (Ovesna *et*

Table 1:**List of genotypes used for the analysis**

Accession ID	Characteristics	Source
AKR-504-A	Restorer	SRU, Dr. PDKV, Akola
30-A	Male sterile line	SRU, Dr. PDKV, Akola
AKMS-30B	Maintainer line	SRU, Dr. PDKV, Akola
CSH-35	Hybrid	SRU, Dr. PDKV, Akola

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Table 2: List of SSR Primers used in sorghum DNA fingerprinting

SN	PRIMERS		Nucleotide sequence	Tm (ºC)	Base pairs
1.	MsbCIR 223	F	CGTTCCAATGACTTTTCTTC	53.2	20
		R	GCCAATGTGGTGTGATAAAT	53.2	20
2.	MsbCIR 240	F	GTTCTTGGCCCTACTGAAT	54.5	19
		R	TCACCTGTAACCCTGTCTTC	57.3	20
3.	MsbCIR 246	F	TTTTGTTGCACTTTTGAGC	52.2	19
		R	GATGATAGCGACCACAAATC	55.3	20
4.	XCUP 02	F	GACGCAGCTTTGCTCCTATC	59.4	20
		R	GTCCAACCAACCCACGTATC	59.4	20
5.	XTXP 012	F	AGATCTGGCGGCAACG	54.3	16
		R	AGTCACCCATCGATCATC	53.7	18
6.	XTXP 057	F	GGAACTTTTGACGGGTAGTGC	59.8	21
		R	CGATCGTGATGTCCCAATC	56.7	19
7.	gpsb 089	F	ATCAGGTACAGCAGGTAGG	56.7	19
		R	ATGCATCATGGCTGGT	49.2	16
8.	MsbCIR 248	F	GTTGGTCAGTGGTGGATAAA	55.3	20
		R	ACTCCCATGTGCTGAATCT	54.5	19

Table 3: List of microsatellites markers screened for sorghum DNA fingerprinting

SN	Primer	Location (chromosome)	Alleles (bp)	AKR-504-A	30-A	AKMS-30B	CSH-35	PIC value
1.	Gpsb089	Chr 1	200	1	1	1	1	0.7031
			300	1	1	1	1	
			400	1	1	1	1	
			450	1	1	1	0	
2.	Msbcir223	Chr 2	100	0	0	0	1	0.0036
			130	1	1	0	0	
			200	0	0	0	1	
3.	Xtxp012	Chr 4	230	1	1	1	1	0.8167
			250	1	1	1	1	
			300	0	1	1	1	
			400	1	1	1	1	
			500	1	1	1	1	
			650	1	1	1	1	
			700	1	1	0	0	
4.	Msbcir 248	Chr 5	100	1	1	1	1	0.7674
			310	1	0	0	1	
			400	1	0	0	1	
			450	1	1	1	1	
			2000	1	1	1	1	
5.	Xtxp057	Chr 6	100	1	1	1	1	0.768
			200	1	1	1	1	
			290	1	1	1	1	
			500	1	1	1	1	
			1200	1	1	1	1	
6	Msbcir 246	Chr 7	100	0	0	1	0	0.0366
			110	1	1	0	0	
			200	0	0	0	1	
			450	0	0	1	0	
7	Msbcir 240	Chr 8	100	0	0	1	0	0.0366
			110	1	1	0	0	
			200	0	0	0	1	
			450	0	0	1	0	
8	Xcup 02	Chr 9	100	0	0	1	0	0.0366
			110	1	1	0	0	
			200	0	0	0	1	
			450	0	0	1	0	

al., 2002, Saker *et al*., 2005). The genetic markers arise from different classes of DNA mutations such as substitution mutation, rearrangements or DNA damage in replication of tandemly repeated DNA. Crossing over and mutation are likely to be occurred more frequently at the distant locations from the centromere position of a chromosome and therefore, enhance the possibility of forming more alleles and heterozygosity of a particular locus (Rahman *et al*., 2008). As the overall genetic variability estimate is very less among the parents and hybrids, in present study genetic recombination

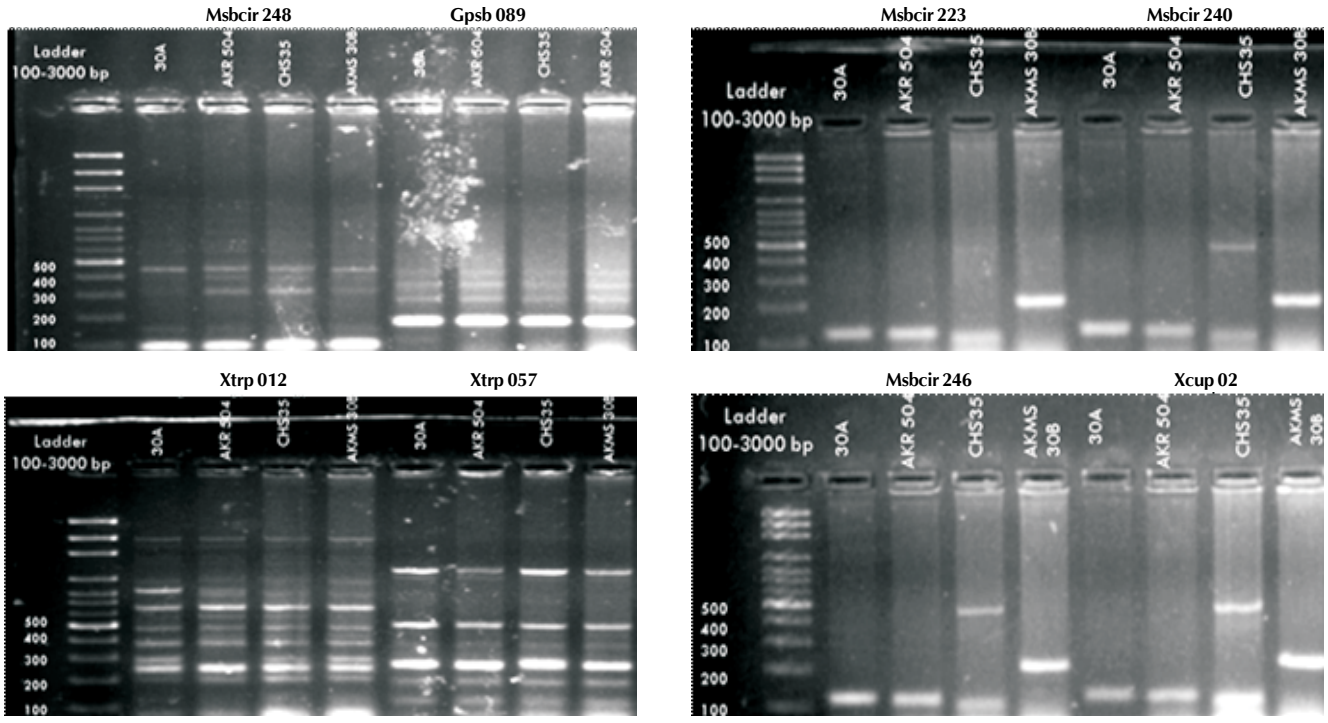

Figure1: DNA profiles generated by 8 SSR markers used among the sorghum hybrid CSH-35 and parental lines.

Table 4: Genotype specific SSR markers

Accession ID	Genotype specific SSR markers
AKR-504-A	Gpsb089, Msbcir 248, Xtxp057
30-A	Gpsb089, Xtxp057
AKMS-30B	Gpsb089, Xtxp057, Xcup02
CSH-35	Msbcir 248, Xtxp057, Xcup02

does not occur due to lack of variation among the parents.

Eight primers produced a total of 36 scorable markers/alleles among the genotypes. The size of amplified products ranged from 200 bp to 2000 bp. The number of scorable markers produced per primer ranged from 3 to 7. The total number of polymorphic markers was 16 and out of 144 and the percentage of polymorphism is 11.11%. It indicates that the hybrid produced by the parents AKR-504-A, 30-A, AKMS-30B is closely related to the parents and the parents and hybrid CSH-35 are closely related to each other.

The PIC values are dependent on the genetic diversity of the cultivars chosen and this investigation had the 8 primers from each chromosome of sorghum which would have the effect of increasing the PIC values. It is important to indicate that the selection by breeders have increased the frequency of the alleles or allelic combination with favorable effects at the expense of the others, eventually eliminating many of them (Cao *et al*., 1998). The markers in the specific chromosome and the allele size along with their frequencies and PIC values have been shown in the Table 3. The PIC values of eight primers was ranging from 0.0036 (Msbcir223, chr-2) to 0.8167 (Xtxp012, Chr-4). Lower PIC value may be the result of closely related genotypes and higher PIC values might be the result of diverse genotypes. From the PIC value (0.0036) the alleles produced by Msbcir223 are not helpful for the discrimination among the hybrids and parents. The primers of (Xtxp012, Chr-4) produced maximum variation among the parents and hybrid CSH-35 as its PIC value is maximum (0.8167) among all the primers under study. Xtxp012 is widely distributed in the sorghum genome as it produced 7 alleles maximum among all the primers. The SSR markers Msbcir 246, Msbcir 240, Xcup 02 are equally distributed in all genotypes including parents and hybrids as the PIC value (0.0366) of these markers is same for all four genotypes including hybrid. The SSR marker Xtxp057 is uniformly distributed in parents and hybrid as it produces the DNA fragments of same length in all genotypes.

Some SSR markers are specific to cultivars for cultivar identification which is the primary moto of DNA fingerprinting. Such type of SSR markers are given in Table 4. For identification of hybrid CSH-35 the Xcup02, Msbcir240, Msbcir 246 were produces an unique band (200bp) which is absent in parents. Also the profile produced by these three markers is similar as shown in Table 4.

REFERENCES

Ayesha, Z., Zabta, K. S., Tariq, M. 2011. Molecular markers assisted genetic characterization of some selected wild *poaceae* species *Pak. J. Bot*. **43(5):** 2285-2288.

Botstein, D., White, R. L., Skolnick, M., Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphism. *Am. J. Hum. Genet*. **32:**314-33.

Cao, T, Duprez, E., Borden, K. L. B., Freemont, P. S. and Etkin, L. D. 1998. Ret finger protein is a normal component of PML nuclear bodies and interacts directly with PML. *J. Cell Sci*. **111:** 1319-1329.

Chakravarthi, B. K. and Naravaneni, R. 2006. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa* L). *African J. Biotech*. **8:** 684- 688.

Chen, S., Liu, X., Zeng, L., Ouyang, D., Yang, J., Zhu, X. 2011. Genetic analysis and molecular mapping of a novel recessive gene *xa*34 (t) for resistance against *Xanthomonas oryzae pv. oryzae*. Theor. Appl. Genet. **122(7):**1331-8.

FAOSTAT, 2011. FAO statistical data base for food. Food and Agriculture Organization of the United Nations, Rome, Italy.

Godwin, I. D. and S. J. Gray. 2000. Overcoming Productivity and Quality Constraints in Sorghum: The Role for Genetic Engineering. In: Transgenic Cereals, O'Brien, L. and R.J. Henry (Eds.). *American Association of Cereal Chemists*, St. Paul, MN., pp. 153-177.

House, L. R. 1985. A Guide to Sorghum Breeding. 2nd Edn., ICRISAT Patancheru, India.

Jain N. and Yadav R. 2009. Genetic diversity analysis among pre-green revolution, post-green revolution era cultivars, and wheat landraces as revealed by microsatellite markers. Crop and Pasture Science. **60:** 373-381.

Komal, K., Bhargava, R., Yadav, P. K. and Karun Gurjar 2015. Molecular studies of pomegranate (*Punica granatum* L.) genotypes using RAPD markers. *The Ecoscan*. **(7):** 253-258. 2015.

Kumar, P. N., Sujatha, K., Laha ,G. S., Rao, K. S., Mishra, B., Viraktamath, B. C., Hari, Y., Reddy, C. S., Balachandran, S. M., Ram, T., Madhav, M. S., Rani, N. S., Neeraja, C. N., Reddy, G. A., Shaik, H. and Sundaram, R. M. 2012. Identification and fine-mapping of *Xa33*, a novel gene for resistance to *Xanthomonas oryzae* pv *oryzae*. Phytopathology. **102(2):**222-228.

Murray,M. G. and Thompson,W. F. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 10. **8(19):** 4321-4325.

Nandakumar, N., Singh, A. K., Sharma, R. K., Mohapatra, T., Prabhu, K. V., Zaman, F. U. 2004. Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. *Euphytica*. **136(3):** 257-264.

NAS, 1996. Lost Crops of Africa: Grains. National Academy Press, Washington, DC.

Ovesna, J., Polakova, K. and Lisova, L. 2002. DNA analyses and their applications in plant breeding. *Czech. J. Genet. Plant Breed*. **38:** 29-40.

Rahman, L., Islam, M. N., Rahmam, M. S. and Islam, M. S. 2008. PLANT VARIETIES OF BANGLADESH: Morphological and Molecular Characterization. Published by Seed Wing, Ministry of Agriculture, Government of the Peoples' Republic of Bangladesh, Vol. 2, P.300.

Saker, M. M., Youssef, S. S., Abdallah, N. A., Bashandy, H. S and El Sharkawy, A. M. 2005. Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP. *African J. Biotechnol.* **4(9):** 882-890.

Sarao, N. K., Vikal, Y., Singh, K., Joshi, M. A. and Sharma, R. C. 2010. SSR marker-based DNA fingerprinting and cultivar identification of rice (*Oryza sativa* L.) In: *Punjab state of India Plant Genet. Res.* **8:** 42-44.

Smith, J. S. C., Chin, E. C. L., Shu, H., Smith, O. S., Wall, S. J., Senior, M. L., Mitchell, S. E., Kresovich, S and Zeigle, J. 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisions with data from RFLPs and pedigree. *Theoretical and Applied Genetics*. **95:** 163-173.

Tautz, D. 1989. Hyper variability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res*. **17:** 6463-647.

Vikram Jeet Singh, Srihima Gampala, S. K. ChakrabortI AND A. K. Singh. 2015. molecular characterization and genetic diversity analysis of rice varieties and landraces based on SSR markers. *The Ecoscan*. **9(1and2):** 363-368, 2015.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J and Kuiper, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res*. **23(21):** 4407-14.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., Tingey, S.V. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*. **18:** 6531-6535.