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DNA-BASED GENOTYPING OF SORGHUM HYBRIDS

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Abstract

Over the last two decades, DNA fingerprinting have been increasingly used to characterize germplasm of different organisms including crop plants, which was otherwise difficult with morphological and or isozyme markers because of paucity of genetic information at phenotypic level. In the present study, two hybrid samples of sorghum (*Sorghum bicolor* L.) received from Federal Seed Certification and Registration Department (FSC&RD), Islamabad Pakistan for DNA-based typing to prove their identity. A DNA fingerprinting technique, random amplified polymorphic DNA (RAPD) analysis was applied to find DNA polymorphisms using 20 different 10-mer primers. Out of these, 8 (40%) primers detected polymorphism between the hybrids. However, rest of the primers produced monomorphic pattern. Out of the 121 amplified loci, 33% were polymorphic. These polymorphic primers distinguished the sorghum hybrids. Our results revealed that RAPD is a useful tool in the identification of hybrids. In future, it can be utilized to estimate the extent of genetic diversity and warranting genome mapping studies in sorghum.
Keywords: *Sorghum, bicolor* L., hybrids, genotyping, RAPD

Introduction

Sorghum (*Sorghum bicolor* (L.) is ranked fifth after the four major grain crops (wheat, maize, rice, and barley) in the world, and is the second most important cereal crop (after maize) in sub-Saharan Africa. It was originated from Northeast Africa (the Ethiopia, Eritrea area), the center of diversity for the crop *(*Agrama & Tuinstra, 2004). Sorghum is cultivated mostly in the developing world (especially in Africa and Asia), although it has become an important industrial crop in many developed countries. Its value in arid climates is due to its ability to withstand dry conditions. In Pakistan, major usage of sorghum crop accounts for feeding livestock.

The continued release of new crop cultivars, particularly F_1 hybrids, by public and private breeders there is a need to test purity or identify duplicated crop varieties including hybrids. Traditionally, genetic purity determinations have been carried out by the evaluation of morphological or physiological traits expressed by seed, seedlings or mature plants. Seed observations and field-testing procedures are often inaccurate because environmental stress conditions during seed or seedling/plant development can mask the expression of specific morphological or physiological traits McDonald (1995).

DNA markers are being increasingly utilized for the identification of hybrid, breeding lines and clones (Paul *et al*., 1997; Sonnate *et al*., 1997; Barrett & Kidwell, 1998; De-Bustos *et al*., 1999; Asif *et al*., 2005). DNA markers are considered constant landmarks in the genome. Markers are identifiable DNA sequences found at specific locations of the genome and transmitted by the standard laws of inheritance from one generation to the next.

There are many different kinds of molecular markers including restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs),

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amplified fragment length polymorphisms (AFLPs), microsatellites, and single nucleotide polymorphisms (SNPs). There are merits and demerits of each type of marker, depending on the specific objectives of the particular study. Among these, RAPD analysis is quick (Colombo *et al*., 1998; Mukhtar *et al*., 2002) and well adapted for nonradioactive DNA fingerprinting of genotypes (Karp *et al*., 1997; Cao *et al*., 1999). RAPD markers (Williams *et al*., 1990; Welsh & McClelland, 1990) have been widely used for genotype identification in plants (Rahman & Zafar, 2001; Mukhtar *et al*., 2002), to study genetic variation in various organisms, including crop plants such as tomato (Klein-Lankhorst *et al*., 1991), wheat (Devos *et al*., 1992), potato (Quiros *et al*., 1993) and sorghum (Tao *et al*., 1993; de Oliveira *et al*., 1996; Menkir *et al.,* 1997; Ayana *et al.,* 2000), for genetic purity determination in seed testing (Hashizume *et al.,* 1993; McDonald *et al*., 1995), evaluation and characterization of germplasm (Bai *et al.,* 2003; Mohapatra *et al.*, 2003), verification of hybridity in interspecific crosses (Benedetti *et al*., 2000), tagging of genes (Martin *et al*., 1991) and marker-assisted selection (MAS) (Rahman, 2002; Sixin & Anderson, 2003) and gained importance due to its simplicity, efficiency and non-requirement of sequence information (Karp *et al*., 1997).

The objective of the present study was to determine the potential utility of RAPD technology for hybrid identification. The efficiency of the RAPD methodology in sorghum for seed purity testing was evaluated successfully, which would be beneficial for our sorghum breeding programs and seed industry.

Material and Methods

Seed Material: A total of two hybrid sorghum samples were received from Federal Seed Certification Department (FSCR&D), Islamabad, Pakistan. These were affixed as V_1 and V_2.

Total Genomic DNA Isolation: Total Genomic DNA was isolated separately from ≈6 g of pre-soaked seed of two hybrid sorghum samples using a modification of the cetyltrimethyleammonium bromide (CTAB) procedure (Personal communication Mehboob-ur-Rahman). After RNase treatment, the DNA concentration was determined both by DyNA Quant 200 and by comparison with standard DNA, electrophoresed on a 0.8% agarose gel and the DNA was diluted in 0.1 X TE buffer to a concentration of 15 ng/ul for PCR analysis.

PCR and Primers: For polymerase chain reaction (PCR), concentration of genomic DNA of two sorghum hybrids, 10 X PCR buffer without $MgCl_2$, gelatin, $MgCl_2$, dNTPs (dATP, dTTP, dGTP and dCTP), 10-mer random primer and *Taq* DNA polymerase were optimized. Ten-mer oligonucleotides were purchased from Operon Technologies Inc. Alameda, Calif. USA. *Taq* DNA polymerase together with 10 X PCR buffer, $MgCl_2$ and dNTPs were purchased from MBI Fermentas, and gelatin was of Sigma grade. PCR amplification was carried out in a 25μL reaction volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM $MgCl_2$, 0.1 mM each of dATP, dTTP, dGTP and dCTP, 30 ng of primer, 0.001% gelatin, 60 ng of genomic DNA and 0.2 unit of *Taq* DNA polymerase. A total of 20 random primers selected randomly from OPL and OPK series were used for PCR amplification. DNA amplification was performed using a thermal cycler programmed for first cycle of 5 min at 94 ^{o}C (initial strand separation); followed by 40 cycles of 1 min at 94 ^{o}C (denaturation), 1 min at 36 ^{o}C (annealing) and 2 min at 72 ^{o}C

(primer extension) followed by one cycle of 10 min at 72 °C (final extension). The tubes were kept at 20 °C until removed.

Analysis of RAPD Data: After amplification, PCR products were resolved on 1.2% agarose gel in 0.5 X Tris Borate EDTA (TBE) buffer, stained with ethidium bromide and photographed with Eagle Eye, gel documentation system. The amplified loci were scored as present/absent. The bivariate 1-0 data were used to estimate similarity on the basis of the number of shared amplification products (Nei & Li, 1979).

Results and Discussion

In the present study, RAPD analysis was applied to verify the identity of two sorghum hybrids. RAPD analysis has been successfully used for hybrid and parentage verification of other crop plants (Walker & Werner, 1997; Rahman *et al*., 2002; Asif *et al*., 2005). RAPD markers were also used to verify interspecific hybridization (Mei *et al*., 2004). However, reproducibility in amplification of RAPD markers is a major concern (Jones *et al*., 1998). In the present study, major/bright DNA fragments were scored to overcome the problem of reproducibility. Minor fragments, which are difficulty to repeat, were not scored because of random priming nature of the PCR reaction (Tessier *et al*., 1999).

Genomic DNA of the two samples was surveyed with 20 random 10-mer primers. A total of 8 primers detected polymorphism (Fig. 1), while, 12 primers produced monomorphic pattern. A total of 121 bands were amplified, with an average of 6 bands per primer. Out of these amplified products, 33% were polymorphic. The level of polymorphism was varied with different primers. Ten fragments were amplified with each of OPL-10 and OPL-15 primers, and a minimum number of one band was amplified with OPL-3 and OPL-17. The mean number of amplified RAPD loci by each of the primer is relatively low compared to the earlier studies conducted on other crop species (Tao *et al*., 1993; Ayana *et al*., 2000) and is comparable with most of the previous reports in sorghum using RAPDs (Vierling *et al*., 1994; de Oliveira *et al*., 1996; Menkir *et al*., 1997; Agrama & Tuinstra, 2004).

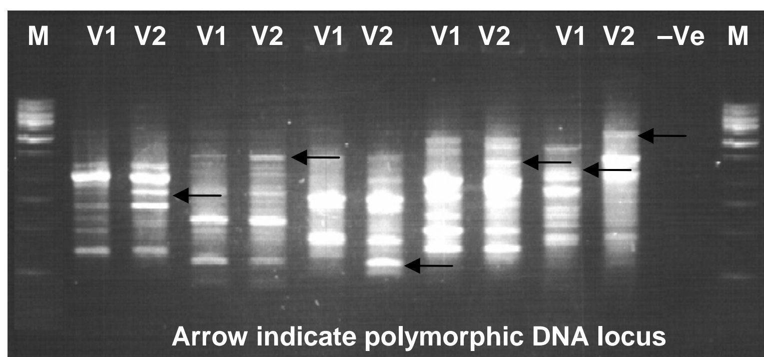

Fig.1. Amplification profile of two sorghum hybrids with primer OPL-7; OPL-10; OPL-14; OPK-19 & OPK-20. M=1kb ladder

Table 1. Polymorphic primers along with sequences and number of loci amplified.

Primer name	Sequence of the primer	No. of loci	Polymorphic loci	% age of polymorphism
OPL-07	AGGCGGGAAC	8	2	25
OPL-08	AGCAGGTGGA	5	3	60
OPL-10	TGGGAGATGG	10	1	10
OPL-14	GTGACAGGCT	7	5	71.42
OPL-15	AAGAGAGGGG	10	1	10
OPK-12	TGGCCCTCAC	8	4	50
OPK-19	CACAGGCGGA	5	4	80
OPK-20	GTGTCGCGAG	9	6	66.67

The size of DNA fragments varied with different primers. The approximate size of the largest fragment amplified was in the range of 2.5 to 3.0 kb and the smallest easily recognizable fragment amplified was approximately 0.2 kb. In another study, a total of 213 fragments were amplified with 32 Operon primers from 22 sorghum genotypes and these fragments were in the range of 225 to 2600 bp in size (Agrama & Tuinstra, 2004).

The findings of the present studies confirmed the efficiency of RAPD technique for verification of hybridity and purity identification. Because of the simple experimental procedures, the requirement of minimal amount of plant tissue and the possibility of automation (Terzi, 1997; Rahman *et al*., 2004), RAPD analysis would be very useful in breeding for rapid and early verification of hybridity in large seedling population and even purity testing of different seed lots, allowing the detection of true hybrids and verification of parentage of the hybrids and lines/cultivars. RAPD analysis has been successfully used for hybrid and parentage verification of other crop plants. In Cherokee rose, RAPD analysis allowed the identification of the erroneous classification of the hybrid 'Silver Moon' (Walker & Werner, 1997). RAPD markers were also used to verify interspecific hybridisation (Benedetti *et al*., 2000; Mei *et al*., 2004).

In the present study, 78.98 % genetic similarity was assessed between the two sorghum hybrids. The extent of genetic diversity is high compared to cotton (Abdallah *et al*., 2001; Rahman *et al*., 2002). The high level of genetic diversity observed was probably associated with the extensive range of genetic diversity represented in the panel of sorghum genotypes (Agrama & Tuinstra, 2004). Such commonalities have been observed in soybeans, barley and corn (Powell *et al*., 1996; Russell *et al*., 1997; Pejic *et al*., 1998).

It may be suggested that DNA-based markers such as RAPD have important implications for genotype identification and determination of hybridity and phylogenetic diversity of sorghum because of a requirement of simple agarose gel electrophoresis. RAPD analysis can be utilized efficiently in developing countries (Udupa *et al*., 1998; Agrama & Tuinstra, 2004). However, to have better understanding of genetic polymorphisms, it is imperative to survey sorghum genome with robust DNA marker tools; microsatellite analysis (SSR), amplified fragment length polymorphism (AFLP) and single nucleotide polymorphism (SNP) as has been achieved already for the human genome (Smith & Helentjaris, 1996).

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