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Full Length Research Paper

Phylogenetic diversity and relationships among sorghum accessions using SSRs and RAPDs

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Two DNA-based fingerprinting techniques, simple sequence repeats (SSR) and random amplified polymorphic DNA (RAPD) analyses, were applied in sorghum germplasm analysis to compare suitability for quantifying genetic diversity. Twenty-two sorghum genotypes, representing an array of germplasm sources with important agronomic traits, were assayed for polymorphism using 32 RAPD primers and 28 sets of sorghum SSR primers. The results indicated that SSR markers were highly polymorphic with an average of 4.5 alleles per primer. The RAPD primers were less polymorphic with nearly 40% of the fragments being monomorphic. An analysis of genetic diversity among sorghum lines indicated that the genetic distances calculated from SSR data were highly correlated with the distances based on the geographic origin and race classifications. Based on the results of these studies, SSR markers appear to be particularly useful for the estimation of genetic similarity among diverse genotypes of sorghum.

Key words: cluster, diversity, polymorphism, RAPD, *Sorghum*, SSR.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is ranked the fifth most important cereal crop in the world. The United States, India, Nigeria, Mexico, Sudan, and China currently produce the most grain sorghum. More than half the world's sorghum is grown in the semi- arid tropics, where it is a staple food for millions of people in India and Africa; however, livestock feeding accounts for most of the U.S. sorghum usage.

Many studies have been devoted to assessing patterns of sorghum genetic variation based on morphology (Appa-Rao et al., 1996; Djè et al., 1998) or pedigree (Jordan et al., 1998). More recently, DNA-based techniques have been used successfully in DNA fingerprinting of plant genomes (Hongtrakul et al., 1997; Cervera et al., 1998) and in genetic diversity studies (Paul et al., 1997; Sonnate et al., 1997; Barrett and Kidwell, 1998; Chowdari et al., 1998b; Zhu et al., 1998; De-Bustos et al., 1999). Among them, random amplified polymorphic DNA (RAPD) analysis is quick (Colombo et al., 1998; Fahima et al., 1999) and well adapted for nonradioactive DNA fingerprinting of genotypes (Cao et al., 1999). However, problems with the reproducibility in amplification of RAPD markers and with data scoring have been reported (Jones et al., 1998). Although major bands from RAPD reactions are highly reproducible, minor bands can be difficulty to repeat due to the random priming nature of this PCR reaction and potential confounding effects associated with co-migration with other markers (Tessier et al., 1999).

SSR markers are attractive for DNA fingerprinting studies for several reasons. They are codominant and highly informative. They generally display high levels of

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Table 1. Country of origin, race classification, and other distinguishing characteristics for sorghum accessions used in this genetic diversity studies.

No	Germplasm Accession	GRIN Designation	Origin	Region	Race	Distinguishing Characteristics
1	P954035 (SC 33)	PI 534132	Ethiopia	1	D	Drought tolerance
2	B35 (SC 35)	PI 534133	Ethiopia	1	D	Drought tolerance
3	SC 1158	PI 597957	Ethiopia	1	D	Disease resistance
4	SC 326	IS 3758C	Ethiopia	1	C	Disease resistance
5	SC 414	PI 533831	Sudan	2	C	Disease resistance
6	SC599	PI 534163	Sudan	2	C	Drought tolerance
7	ShanQuiRed	-	China	7	B	Cold tolerance
8	SanChiSan	GRIF 620	China	7	B	Cold tolerance
9	PI 550590	PI 550590	Russia	8	B	Cold tolerance
10	12-26	-	Egypt	5	W	Wild sorghum
11	47-121	-	Kenya	1	W	Wild sorghum
12	PI 465483	PI 465483	Yemen	1	D	Large seed size
13	PI 559761	PI 559761	Yemen	1	D	Large seed size
14	Dorado	-	El-Salvador	6	M	Elite tropical variety
15	Malisor	-	Mali	3	M	Elite tropical variety
16	Sureno	-	Honduras	6	M	Elite tropical variety
17	Macia	-	Mozambique	4	M	Elite tropical variety
18	M91051	-	USA	6	M	Elite tropical variety
19	TX430	PL-140	USA	6	M	Elite U.S. pollinator
20	TX2737	GP-82	USA	6	M	Elite U.S. pollinator
21	TX2741	GP-86	USA	6	M	Elite U.S. pollinator
22	PL1	-	USA	6	M	Large seed size

Region: 1 = East Africa, 2 = Central Africa, 3 = West Africa, 4 = South Africa, 5 = Northern Africa, 6 = North America, 7 = Asia, 8 = Europe. Race: D = Durra, C = Caudatum, B = Bicolor, W = Wild, M = Breeding.

polymorphism (Beckmann and Soller, 1990; Brown et al., 1996; Senior et al., 1998) and are amenable to automated genotyping strategies. They also can be amplified by PCR and efficiently detect DNA polymorphism (Pejic et al., 1998). Finally, radioisotopes are not required in the detection of SSR markers, because sequence polymorphism usually can be detected by separation in agarose gels (Burr, 1994).

Although SSRs are well established for human and mammalian genetics, these markers have only recently become available in plant species. They have been identified in many plant genomes including those of maize (Senior and Heun, 1993; Shatuck-Eidens et al., 1990; Taramino and Tingey, 1996); soybean (Akkaya et al., 1992; Morgante and Olivieri, 1993); *Brassica* spp. (Poulsen et al., 1993); rice (Wu and Tanksley 1993); barley (Saghai-Maroof et al., 1994); pearl millet (Chowdari et al., 1998a); *Arabidopsis* (Depeige et al., 1995); tomato (Broun and Tanksley, 1996); conifers (Tsumura et al., 1997); and sorghum (Brown et al., 1996; Taramino et al., 1997; Dean et al., 1999). The results of studies using SSR markers in these species suggest that they may provide an outstanding new tool for genetic analysis of plant species.

Harlan and DeWet (1972) classified cultivated sorghum based on agronomic and morphological characteristics. The utilities of isozymes (Morden et al., 1989; Aldrich et al., 1992), RFLP (Aldrich and Doebley, 1992), and RAPD (de Oliveira et al., 1996; Menkir et al., 1997; Ayana et al., 2000) markers have been used to study genetic diversity in sorghum germplasm. Several efforts have been made to utilize SSR markers in plants to study genetic diversity, characterize germplasm, and evaluate population dynamics (Zhang et al., 1997; Liu and Wu 1998; Senior et al., 1998; Struss and Plieske, 1998). A comparison of RAPD and SSR marker techniques in sorghum is timely, even though the utility of different molecular markers for corn (Smith and Helentjaris, 1996), soybeans (Powell et al., 1996) and barley (Russell et al., 1997) germplasm already has been reported. The objectives of the present study were to: (1) compare the application and utility of RAPD and SSR marker techniques for analysis of genetic diversity among sorghum genotypes, (2) compare genetic similarity quantified by molecular markers with regional and race information.

MATERIALS AND METHODS

Plant Materials

Twenty-two sorghum accessions including landraces, improved lines, and wild accessions were evaluated in this study (Table 1). Most of these accessions represent landrace varieties and are described in detail in the USDA-ARS Germplasm Resources

Information Network available at http://www.ars-grin.gov/npgs. Less information is available on wild accessions and improved varieties that were obtained from plant breeders from different parts of the world.

DNA extraction and SSR and RAPD markers

Genomic DNA was extracted from etiolated hypocotyls 5- to 7-day-old plants of each genotype according to the method of Djè et al. (2000). Initially the five individual plants of each accession were assayed for RAPDs or SSRs using 5 primers. No polymorphisms were detected between individuals within a genotype.

Seventeen SSR markers described by Brown et al. (1996) and 11 described by Taramino et al. (1997) were used for genotyping assays (Table 2). Eighty different RAPD primers obtained from OPERON Technologies (Kits A-D) were used to generate markers as described by Tao et al. (1993). Thirty-two primers that generated clear and reproducible fragments were used to fingerprint the 22 sorghum genotypes. The SSR and RAPD reaction products were evaluated for polymorphisms on 3% Metaphor agarose gels (FMC Products, Rockland, ME, USA) and 1.6% agarose gels, respectively. Gels were stained with 1 $\propto$g mL^{-1} ethidium bromide for 30 to 60 min.

Band scoring and cluster analysis

The SSR and RAPD gel images and marker data were processed using Quantity One Software v. 4.0.1 (Bio-Rad Laboratories, Hercules, CA USA). The bands were sized and then binary coded by 1 or 0 for their presence or absence in each genotype. The assay efficiency index was calculated as described in detail by Pejic et al. (1998). Nie's genetic diversity (Nei, 1972) was computed from the binary data for all pairwise combinations of sorghum genotypes. Cluster analysis was based on similarity matrices obtained with the unweighted pair-group method using the arithmetic average to estimate the phenogram. Race and region information was scored as 1 and 0 and then analyzed using the simple matching coefficient of the SimQual method. All the data analyses were performed using the software package NTSYS-pc (Rohlf, 1993). Polymorphism information content (PIC) for each SSR primer set was determined as described in Smith et al. (1997). Senior et al. (1998) reported that PIC is synonymous with the term “gene diversity” as described by Weir (1996). The correlations of pairwise distances among all pairs of genotypes for SSRs were compared to RAPDs and origin and for RAPDs were compared to origin cluster.

RESULTS

The 22 sorghum genotypes evaluated in this study were differentiated uniquely using the 28 SSR markers and 32 RAPD primers. The SSR primer was considered to be polymorphic when the most abundant allele in the population has frequency lower than 95.4%. The analysis of SSR products in this study indicated fragment lengths that were slightly different from those previously reported (Table 2). PIC values for SSR loci range from 0 (monomorphic) to 1 (very highly discrimitive, with many alleles in equal frequencies). The PIC values were quite high and ranged from 0.23 to 0.81 (Table 2). The average PIC value for SSR markers was 0.622. The SSR markers containing dinucleotide repeats had PIC values that averaged 0.634. These results were in agreement with the results of Smith et al. (1997) who reported that the PIC of SSR dinucleotide repeats had the highest values. A few of the SSR primers amplified more than one band per genotype, indicating residual heterogeneity within lines. RAPD markers were less polymorphic than SSR markers.

Table 2. SSR marker used to diverse the 22 sorghum accessions.

No.	SSR Markers[1]	No of alleles	Size range in bp	PIC value
1	SBKAFGK1	5	310-325	0.60
2	ZMADH2N	2	170-175	0.23
3	Sb1-1	3	255-310	0.61
4	Sb1-10	4	365-385	0.74
5	Sb4-15	3	155-170	0.66
6	Sb4-22	3	300-320	0.38
7	Sb4-32	7	195-210	0.66
8	Sb4-121	7	185-210	0.81
9	Sb5-85	2	210-230	0.40
10	Sb5-206	4	145-155	0.71
11	Sb5-214	2	190-245	0.29
12	Sbf-236	4	160-190	0.43
13	Sb6-36	4	160-190	0.65
14	Sb6-57	6	290-320	0.79
15	Sb6-84	5	180-195	0.76
16	Sb6-325	3	125-150	0.63
17	Sb6-342	2	265-305	0.35
18	SbAGA01	5	105-120	0.68
19	SbAGE01	7	195-235	0.65
20	SbAGB02	4	130-155	0.68
21	SbAGD02	4	115-135	0.67
22	SbAGG02	5	185-200	0.80
23	SbAGB03	7	120-155	0.72
24	SbAGE03	3	130-160	0.59
25	SbAGF06	6	135-165	0.71
26	SbAGF08	6	145-175	0.72
27	SbAGH04	7	130-160	0.71
28	SvPEPCAA	7	235-265	0.79

[1]Primers 1- 17 from Brown et al. (1996) and primers 18-28 from Taramino et al. (1997).

The 32 OPERON primers generated a total of 213 RAPD bands. Of these bands, only 125 were polymorphic across the 22 sorghum accessions. A band (locus) was considered as plymorphic if the band differentiates at least any 2 of the 22 genotypes. The number of amplification products per primer varied from 3 to 11, with a mean of 6.66. These primers produced fragments varying from 225 to 2600 bp in size. Although SSR primers amplify PCR products from only one locus per assay, an average of 4.5 alleles per locus was detected in this study, indicating a large degree of genetic

diversity among accessions. The RAPD primers amplified dominant markers that were scored as two alleles per locus. An average of 3.9 polymorphisms was amplified for each RAPD primer tested in this study. The assay efficiency was higher for SSR marker reactions than for RAPD marker reactions.

The SSR markers provided the most powerful assay for discriminating genetic diversity among sorghum accessions. Similarity matrices constructed based on shared allele analysis revealed that the average genetic similarity between genotypes was lowest when it was estimated using SSR markers (0.437). Genetic similarity among entries was higher when it was determined using RAPD markers (0.612). These results indicated that RAPD markers provide less resolving power than SSR markers. Molecular assays were much more powerful at discriminating genetic diversity than estimates based on geographical and race classification, which revealed high levels of genetic similarity among accessions (0.951).

Genetic similarity among sorghum lines based on SSR, RAPD, and geographical and race data are represented in Figures 1A, B and C, respectively. The UPGMA dendrogram based on SSR and RAPD marker data clearly discriminated among genotypes (Figures 1A and B). The cluster analysis generated using country of origin and race information was not able to differentiate among all entries because of the limited number of class variables (Figure 1C). Some consistency in classification was observed among clusters. The two Chinese genotypes (Shan Qui Red and San Chi San) and two wild types (12-26 and 47-121) are fully conserved across the three clusters. The genotypes SC35 and SC1158, which originate from Ethiopia and represent race Durra, also were grouped together. The breeding line Sureno was classified as a lone outlier using both RAPD and SSR markers.

Genetic diversity of sorghum measured using SSR and RAPD markers exhibited highly significant association with geographic origin and race classification ($P<0.01$). The correlation of pairwise distances between all pairs of genotypes for SSRs compared to geographical and race was $r = 0.51$; the correlation for RAPDs with geographical and race data was $r = 0.43$. The correlation of pairwise distances among all pairs of sorghum genotypes for SSRs compared to RAPDs was $r = 0.79$.

DISCUSSION

Sorghum SSR markers revealed higher levels of genetic polymorphism than did RAPD markers in this study. These results are in agreement with studies in other species (Morgante and Olivieri, 1993; Powell et al., 1996; Wu and Tanksley, 1993). The higher level of polymorphism associated with SSR markers may be a function of the unique replication slippage mechanism responsible for generating SSR allelic diversity (Pejic et

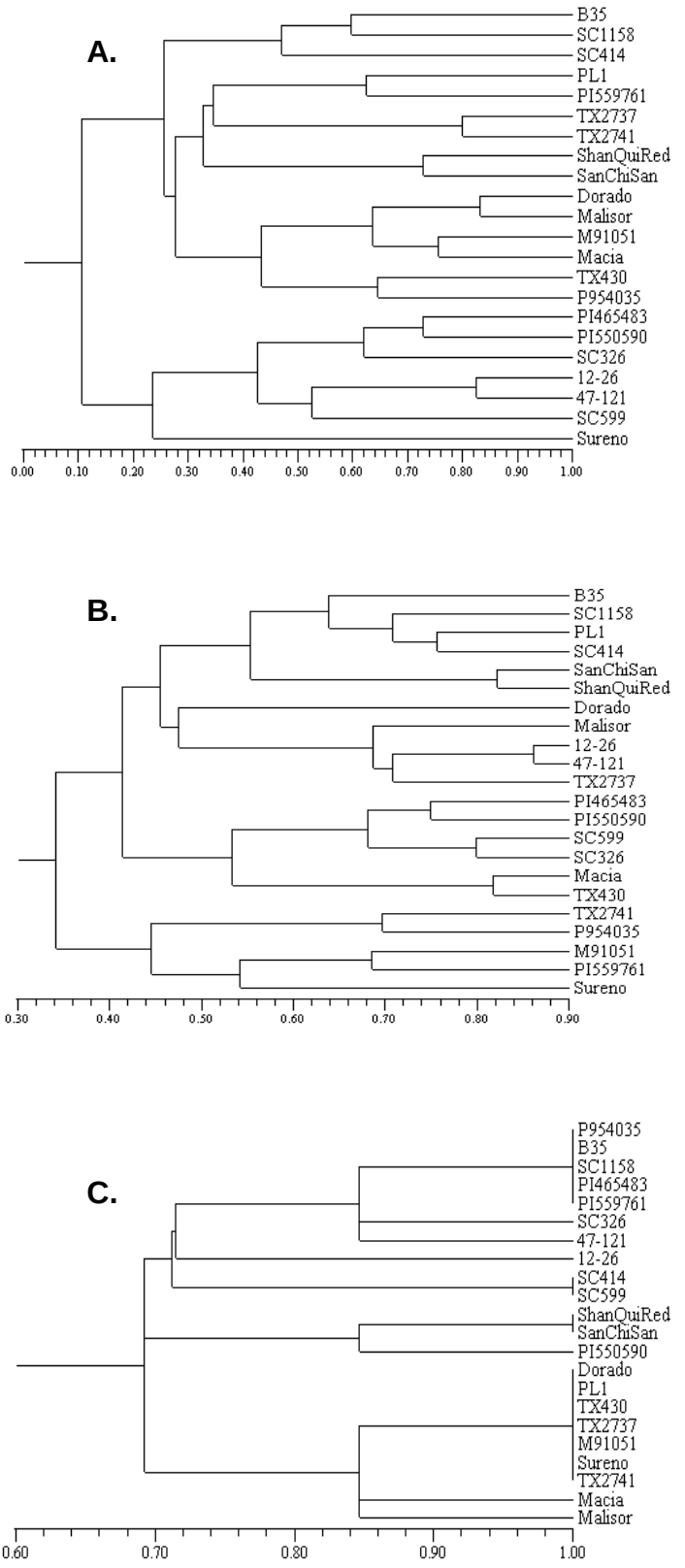

Figure 1. Genetic similarity among sorghum genotypes revealed by UPGMA cluster analysis based on (A) SSR, (B) RAPD, and (C) country origin and race data with the simple matching coefficient of SimQual method.

al., 1998). The PIC value of SSR markers was in the range reported by Senior et al. (1998). However, higher PIC values have been reported (Smith et al., 1997), but this difference may be associated with the use of acrylamide gels for allele detection in their study. Acrylamide gels have greater resolving power than agarose gels. The increased resolution of acrylamide over agarose gel separation could result in the detection of larger number of alleles per locus. This may be particularly important for SSR loci containing dinuleotide repeats whose amplification products are in the 130 to 200 bp range, because PCR products differing by two base pairs cannot be resolved with agarose gels.

The mean number of RAPD bands per primer obtained in this study is low compared to that reported by Tao et al. (1993) and Ayana et al. (2000) and is comparable with most of the previous studies in sorghum using RAPDs (Vierling et al., 1994; de Oliveira et al., 1996; Menkir et al., 1997). The level of RAPD polymorphism observed in the study is higher than those reported in previous studies (Tao et al., 1993; Vierling et al., 1994; Yang et al., 1996; Menkir et al., 1997).

The high levels of allelic diversity of SSR and RAPD markers observed in this study probably were associated with the extensive range of genetic diversity represented in the panel of sorghum genotypes. We took into account not only differences in geographical range but also results of morphological variation. These results are in agreement with previous observations in soybeans, barley and corn (Powell et al., 1996; Russell et al., 1997; Pejic et al., 1998). The highest degree of polymorphism was associated with SSR markers. The presence of many unique alleles may be explained by the relatively high rate of mutation in SSR loci (Henderson and Petes, 1992). Such alleles are important, because they may be diagnostic for particular regions of the genome specific to a particular type of sorghum.

Differentiation among sorghum genotypes was much higher for molecular markers than for geographical and race classification. These results are consistent with morphological, isozyme, and RFLP studies (DeWet et al., 1970; DeWet et al., 178; House, 1985; Aldrich et al., 1992; Cui et al., 1995). These studies indicated that sorghum has an unusual amount of diversity for a predominately self-pollinating species. The levels of polymorphism found for sorghum in this study were similar to those reported for other plant species including maize and barley (Cui et al., 1995). These results may reflect the fact that the 22 genotypes examined were chosen deliberately to include racial and geographic diversity. Multiple origins for domesticated sorghums, cross-pollination between selected races, and outcrossing between domestic cultivars and highly variable wild species all are considered to be factors contributing to the extensive genetic diversity observed in sorghum (Doggett, 1988). The relatively high frequency of SSR polymorphism should be helpful in phylogenetic analyses to better understand these relationships.

RAPD variation showed weak association with regional and racial diversity. Several studies have reported limited regional differentiation for world collection of sorghum using allozyme data (Moeden et al., 1989), RFLP (Cui et al., 1995) and RAPD data (Tao et al., 1993; Menkir et al., 1997; Djè et al., 1998; Ayana et al., 2000). The results of Fahima et al. (1999) showed no association with geographic distance between emmer wheat population sites of origin and RAPD markers. Considering that many of the accessions evaluated in this study were breeding lines of mixed pedigree, SSR markers exhibited a remarkably strong association with genetic origin and race.

Both SSR and RAPD markers have unprecedented utility for analysis of population genetics and phylogenetic diversity of sorghum. Because neither requires radioactive isotopes, these methods can be used efficiently by researcher in developing countries (Udupa et al., 1998). The use of SSRs potentially could remove most, if not all, of the limitations in revealing polymorphisms and in obtaining more complete genomic coverage for plants, as has been achieved already for the human genome (Smith and Helentjaris, 1996). The utility of PCR-based markers such as SSRs for measuring diversity, for assigning genotypes to heterotic groups, and for genetic fingerprinting should prove valuable for sorghum breeding programs.

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