Theor Appl Genet (1994) 87:816–820

Genetic diversity among elite *Sorghum* lines revealed by restriction fragment length polymorphisms and random amplified polymorphic DNAs

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Received: 14 June 1993 / Accepted: 28 June 1993

Abstract. The genetic diversity of sorghum, as compared to corn, is less well characterized at the genetic and molecular levels despite its worldwide economic importance. The objectives of this study were to: (1) investigate genetic diversity for restriction fragment length polymorphism (RFLPs) and random amplified polymorphic DNAs (RAPDs) in elite sorghum lines, (2) compare similarities based on molecular markers with pedigree relationships, and (3) examine the potential of RFLPs and RAPDs for assigning sorghum lines to the A/B (sterile) and R (restorer) groups. Using four restriction enzymes, polymorphism was detected with 61% of the RFLP probes used, compared to 77% of the random primers. One hundred and sixteen (64%) probe-enzyme combinations yielded multiple-band profiles compared to 98% of the random primers. RFLP profiles generated 290 polymorphic bands compared to 177 polymorphic RAPDs. Pair-wise comparisons of polymorphic RFLPs and RAPDs were used to calculate Nei and Jaccard coefficients. These were employed to generate phenograms using UPGMA and neighbor-joining clustering methods. Analysis of RFLP data with Jaccard's coefficient and neighbor-joining clustering produced the phenogram with the closest topology to the known pedigree.

Key words: Molecular markers – *Sorghum bicolor* – Phenetics – Genetic diversity

Introduction

Assessment of the genetic diversity in a crop species is fundamental to its improvement. Molecular markers such as RFLPs and PCR amplification products have proven to be useful in determining genetic diversity.

In maize (*Zea mays* L.) there is a high degree of molecular marker polymorphism and relationships based on these polymorphisms have been calculated for elite inbred lines (Smith et al. 1990; Melchinger et al. 1991; Messmer et al. 1991; Livini et al. 1992; Messmer et al. 1992) and hybrids (Smith and Smith 1991; Smith et al. 1992). The high degree of RFLP polymorphism observed in maize has led to the rapid construction of genetic maps (Helentjaris et al. 1986; Coe et al. 1988). The saturation of the RFLP genetic map allows for its practical application in the tagging and tracking of genes of agronomic importance. Examples include disease resistance (Bentolila et al. 1991; Louie et al. 1991) and the dissection of complex traits such as plant height (Beavis et al. 1991; Edwards et al. 1992) and heat tolerance (Ottaviano et al. 1991).

In contrast, *Sorghum bicolor*, belonging to the same tribe, Andropogoneae, as *Z. mays*, is less well characterized genetically and molecularly despite its worldwide economic importance. In an effort to apply molecular genetic technology to sorghum, several laboratories have initiated the construction of RFLP maps using maize RFLP probes. Data from these studies indicate that maize RFLP probes hybridize sufficiently well to sorghum DNA that the construction of sorghum RFLP maps based on maize RFLP probes is entirely feasible. Hulbert et al. (1990) analyzed the cosegregation patterns of 55 individuals in an F_2 sorghum population and described eight linkage

Communicated by H. F. Linskens
Contribution of the College of Agricultural Sciences, Texas Tech University, Journal No. T-4-365
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groups encompassing 37 markers. Binelli et al. (1992) analyzed 149 F_2 individuals and described five linkage groups containing 35 markers. Comparison of the sorghum RFLP maps to the maize RFLP map showed some colinearity but several rearrangements had evidently occurred. Neither study surveyed elite breeding material for molecular marker polymorphism. Recently, Whitkus et al. (1992) have reported the linkage relationships among 85 maize low-copy-number DNA probes in an F_2 population derived from a cross between *S. bicolor* ssp. *bicolor* and *S. bicolor* ssp. *arundinaceum*. They defined 13 linkage groups, many of which were conserved between the maize and sorghum genomes.

The objectives of the present study are to: (1) investigate genetic diversity for RFLPs and RAPDs in elite sorghum lines, (2) compare similarities based on molecular markers with pedigree relationships, and (3) examine the potential of RFLPs and RAPDs for assigning sorghum lines to groups.

Materials and methods

Plant material

In this study, 18 sorghum inbred lines (coded as SL 1–18), including six public lines (SL5 = B35, SL6 = Tx 378, SL9 = Tx 7000, SL13 = Tx 2737, SL14 = Tx 430 and SL17 = Tx 2783) and 12 private lines from Cargill Hybrid Seeds breeding materials, were used. Maize lines Mo17 and B73 were employed as controls in the RFLP work. DNA was extracted from ground leaf tissue taken from a bulk of 30–50, 2- to 3-week-old plants (Saghai-Maroof et al. 1984).

RFLP analysis

Genomic DNA was individually digested with *Bam*HI, *Eco*RI, *Eco*RV, and *Hin*dIII restriction endonucleases. Electrophoresis was carried out in an 0.9% agarose gel in 10 mM Tris-acetate EDTA. Southern blots were made by alkaline capillary transfer to MSI magnagraph nylon membranes and hybridized with random-prime labeled (Feinberg and Vogelstein 1983) maize-DNA insert probes. All maize probes were kindly provided by Dr. Ed Coe at the University of Missouri-Columbia. Probes were hybridized on the membranes overnight at 60 °C (Helentjaris et al. 1986). Post-hybridization treatment consisted of a total of 2 h of washes with 0.1 × SSC and 0.1% SDS at 65 °C. After autoradiography, profiles were scored for each probe/enzyme combination by assigning a letter to each band according to its relative migration.

RAPD analysis

Amplification reactions contained 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ 0.001% gelatin, 2×10^{-5} M tetramethylammonium chloride, 200 μM dATP, dCTP, dGTP, and dTTP, 60 ng random 10-mer primer (Operon Technologies, Calif.), 100 ng genomic DNA, and 0.5 units *Taq* DNA polymerase for 43 cycles of 1 min at 95 °C, 1 min at 37 °C and 1.5 min at 72 °C (Vierling and Nguyen 1992). Products were electrophoresed in 1.4% agarose gels and visualized under UV light by ethidium bromide staining and scored the same as RFLP profiles.

Phenetic analyses

RFLP and RAPD data were analyzed separately. Jaccard (1908) and Nei (1978) coefficients were calculated for all possible combinations of lines. Both average linkage analysis (UPGMA) and neighbor-joining (Saitou and Nei 1987) analyses were performed with the matrices of Jaccard and Nei coefficients based on RFLP and RAPD data using NTSYS (Rohlf 1989).

Results

RFLP analysis

Of the 75 maize probes tested, only six did not hybridize to sorghum DNA while another 11 probes hybridized only very weakly. The remaining 58 maize probes hybridized as strongly, or nearly as strongly, to sorghum DNA as they did to maize DNA. One such autoradiogram from the hybridization of sorghum genomic DNAs from 18 lines with maize clone UMC 112 is shown in Fig. 1. Most probes detected more than one locus but generally one locus hybridized more strongly than the other. Of the 58 maize probes that hybridized strongly to sorghum DNA, 46 detected polymorphism (61% of total) in at least one of the four restriction enzyme digests. Nearly all of the polymorphic probes were simultaneously polymorphic for more than one restriction enzyme.

Scoring of 180 polymorphic probe/enzyme combinations detected a total of 574 bands, of which 290 (51%) were polymorphic. Sixty-four (36%) probe/enzyme combinations yielded single-band RFLP profiles. The number of RFLP patterns for polymorphic, single-banded profiles ranged from two to four with an average of 2.3. One hundred and sixteen (64%) probe/enzyme combinations yielded multiple-band, RFLP profiles. The number of RFLP patterns for multiple-banded profiles ranged from two to eight with the average being 3.6.

RAPD analysis

Genomic DNA was amplified with 73 random primers, and 70 of these primers produced amplifiction prod-

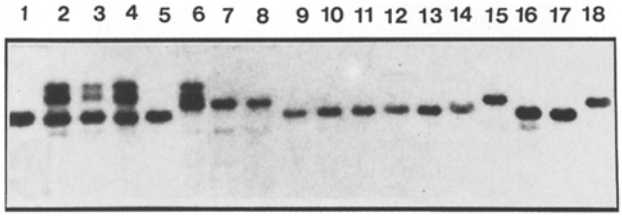

Fig. 1. Autoradiogram from a hybridization between a maize clone, UMC 112, and genomic DNAs from 18 sorghum lines (SL1–SL18, designated by *1 to 18*) digested with the restriction enzyme *Eco*RI

ucts. Only 57 primers produced clear amplification profiles that were easily scoreable, and 56 (77% of total) of these produced polymorphic products. Figure 2 shows one such RAPD pattern using primer P-13 and 18 sorghum lines. A total of 194 amplification products were produced of which 177 (91%) were polymorphic.

Only one primer yielded a single-band profile, which was polymorphic and exhibited two patterns. The other 55 primers produced multiple-band profiles. The number of RAPD patterns for multiple-band profiles ranged from two to ten with the average being 3.5.

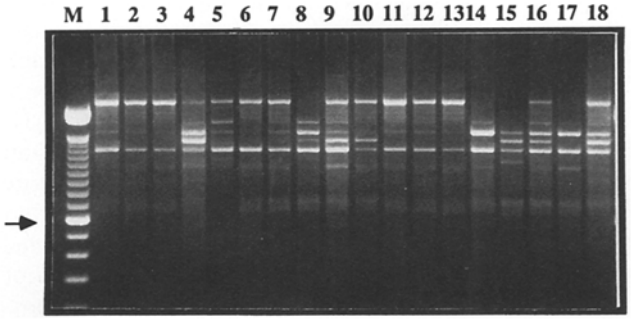

Fig. 2. Amplification products (RAPDs) from genomic DNAs of 18 sorghum lines (SL1–SL18, designated by *1 to 18*) using the primer P-13 from Operon Technologies. The molecular weight marker (100-bp ladder) is shown in the left lane (*M*) and the 600-bp band is indicated by an *arrow*

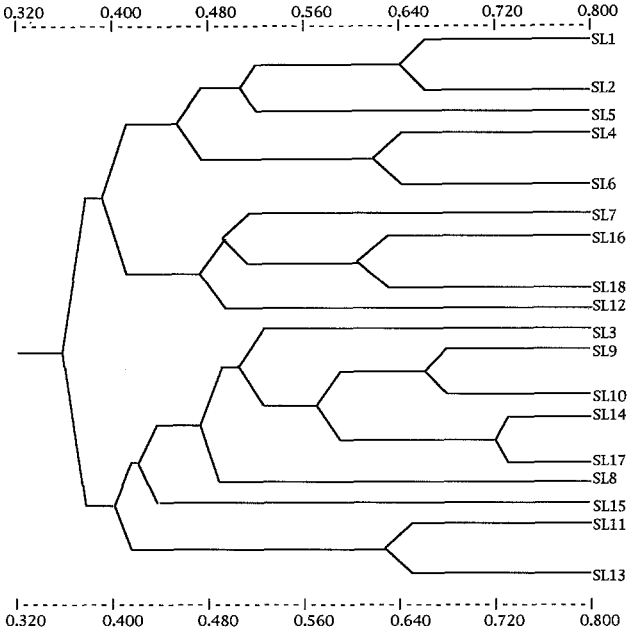

Fig. 3. Phenogram of genetic relationships, based on the UPGMA method, between 18 lines of sorghum. The scale indicates the similarity index

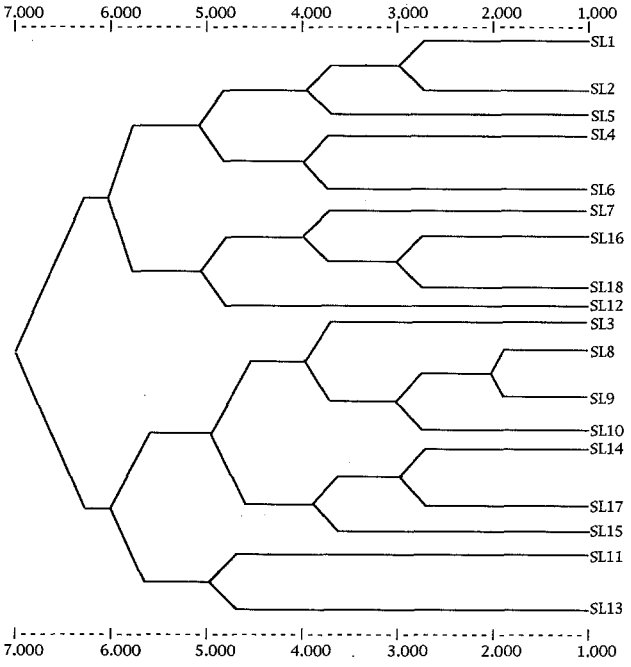

Fig. 4. Phenogram of genetic relationships, based on the neighbour joining method, between 18 lines of sorghum. The scale indicates the similarity index

Phenetic analysis

The phenograms generated using the Jaccard matrix derived from RFLP data were the ones whose topologies were the most similar to the pedigree dendrogram. Both the UPGMA and neighbor-joining phenograms clustered the lines into two fairly distinct groups that corresponded to the pedigree relationships (Figs. 3 and 4). The phenograms from the Nei matrix from RFLP data did not correspond to the pedigree dendrogram. None of the phenograms generated from RAPD data were similar to the pedigree dendrogram (data not shown).

Discussion

Molecular marker polymorphism

As in previous studies (Hulbert et al. 1990; Binelli et al. 1992; Whitkus et al. 1992), we found heterologous maize probes to be effective for determining polymorphism in sorghum. In the present study, 61% of the probes tested were polymorphic. This is higher than the percentages reported previously, probably due to the greater number of lines examined. As in the three previous studies, we found that the RFLP profiles in both sorghum and maize were similar.

Thirty-six percent of the polymorphic probe/enzyme combinations detected single-band profiles in sorghum. This is less than the percentages (40–47%) detected in maize (Livini et al. 1992; Messmer et al.

1992). The average number of RFLP patterns detected per single-band profile was 2.3, which is almost half that observed in maize. For multiple-band profiles, the average number of patterns was 3.6, which is well below those observed in maize (Livini et al. 1992; Messmer et al. 1992).

Like RFLP markers, RAPD markers were effective in determining polymorphism between sorghum lines. In our study, 77% of the random primers tested amplified polymorphic bands. This is greater than the percentage (61%) found using RFLP probes. In contrast to the RFLP data, only one primer produced a single-band RAPD profile and it exhibited two patterns. The remaining primers produced multiple-band profiles with an average of 3.5 patterns. Even though profiles from random primer amplification were nearly always multiple-band, the average number of patterns was the same in both RAPD and RFLP profiles. In addition to being more polymorphic, random primers nearly always (98%) produced multiple-band profiles, whereas only 64% of the RFLP probes produced multiple-band profiles.

Phenetic analysis

Since we were interested in describing molecular marker diversity, we used a phenetic approach to data analysis. Both unbiased (Nei) and biased (Jaccard) coefficients were generated for both RFLP and RAPD data. Jaccard's coefficient is biased in that it omits consideration of negative matches. All matrices were clustered using both neighbor-joining and UPGMA. UPGMA and neighbor-joining are conceptually related but the major difference in that neighbor-joining removes the assumption that the data are ultrametric. UPGMA uses sequential clustering, where local relationships are identified. The two most similar entries are clustered and subsequently treated as a composite entry and then clustered with the next most similar single or composite entry. Neighbor-joining identifies sequential pairs and connects neighbors that are connected by an internal node. Connecting neighbors in this manner will limit the total length of the tree and generate only a single tree. The resulting phenograms were compared to the known pedigree of the lines.

Although sorghum heterotic groups are not yet as clearly defined as in corn, hybrid crosses are generally derived from crosses of A/B (sterile) and R (restorer) lines. The only phenograms that successfully divided the lines into two groups were those derived from clustering the Jaccard, RFLP matrix. There were a few exceptions, but these were generally due to a specific heterotic tendency and not to the sterility/restorer relationship. As shown in Figs. 3 and 4, public B lines, B35 and Tx 378, were grouped similarly and near Cargill B lines, while public R-lines, Tx 2737, Tx 430, and Tx 2783, were also grouped similarly and near Cargill R-lines. As predicted, clustering the Jaccard, RFLP matrix with UPGMA and neighbor-joining generated phenograms with very similar topology. But comparing them to the pedigree dendrogram revealed that the neighbor-joining phenogram had a more similar topology than the UPGMA phenogram. This does not in any way invalidate the UPGMA phenogram since the dendrogram is derived from empirical data. Both phenograms had the same topology in the A/B line cluster, but there were slight differences within the R-line cluster.

Clustering the Nei, RFLP matrix with both UPGMA and neighbor-joining did not correctly cluster the lines. There was a small cluster of two A/B and one R-line and a large cluster of 15 A/B and R-lines. Again, both clustering methods generated phenograms with similar topology.

In contrast to the analysis of the RFLP data, analysis of the RAPD data yielded four phenograms with similar topology. There was a cluster of three A/B lines and two R-lines, an A/B outlier, and the remaining lines were clustered. Though all the phenograms derived from RAPD data had similar topology, their topology was not similar to the topology of the dendrogram. Unlike the RFLP probes, we do not know the chromosomal location of the random primers. Therefore, we are not certain that we successfully amplified regions throughout the entire genome. This may be one explanation for not generating any phenograms with a topology similar to the pedigree.

In conclusion, both RFLPs and RAPDs were successful in detecting polymorphism. There was a higher percentage of polymorphic random primers than there were RFLP probes. Random primers also produced a higher percentage of multiple-band profiles than RFLP probes. In this study, phenetic analysis indicated that RFLP data was more informative than RAPD data. The neighbor-joining clustering of the Jaccard coefficients derived from RFLP data produced the phenogram with the most similar topology to the dendrogram. It may be argued that the Jaccard coefficient is more appropriate because basing similarity on the mutual absence of a character is improper. It may also be argued that neighbor-joining is a more appropriate algorithm since it removes the assumption that the data are ultrametric. Since all lines are not selected under similar conditions, the data can be considered to be nonultrametric.

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