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Molecular genetic diversity and its relationship with heterosis for grain yield and related traits in Sorghum [*Sorghum bicolor* (L.) Moench]

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ABSTRACT

The parental diversity has been proposed as a possible predictor of heterotic potential in many crops. Advances in molecular markers have generated considerable interest in assessing genetic diversity and predicting hybrid performance. The present study was aimed to assess the genetic diversity among parents at molecular level and investigate the extent of correlation between marker heterozygosity and hybrid heterosis using Random amplified polymorphic DNA (RAPDs) markers. Twenty four hybrids along with their parents (six lines x 4 testers) were evaluated for ten agronomic important traits including in a replicated field trial. The RAPD analysis revealed 48.4 per cent of polymorphism among the 10 genotypes. The genetic dissimilarity index obtained from the simple matching coefficient had a range of 0.390 (IS 3504 and IS 3541) to 0.938 (CO 28 and CO 27). As a whole, 10 genotypes were grouped into five clusters. The two fodder genotypes IS 3504 and IS 3541 originated from Sudan were grouped in the same cluster indicating the molecular similarly within them. Correlation coefficients computed between the genetic distances among parents and relative heterosis of their hybrids revealed a positive and significant association for leaf breadth (r=0.467), panicle length (r=0.438), panicle weight (r=0.405) and grain yield per plant (r=0.383) and positive and non significant relationship with leaf length. Results indicated that molecular markers like RAPDs may be useful for predicting heterotic potential of the hybrids based on the parental diversity.

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Key words : Random amplified polymorphic, DNA analysis, *Sorghum bicolor*, Heterosis, Genetic distance

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench], a traditional crop in much of Asia and Africa has an ability to tolerate drought and temperature extremes more effectively than the cereals. It has wide range of adaptation from sea level to an altitude of 3000 meters and from 40^0S to 45^0N latitude (Chopra, 2001). It plays a very important role in providing nutrition to human race along with wheat, rice and maize (Mohanraj *et al.,* 2006). Sorghum is fourth in worldwide economic importance among cereal crops and is grown throughout the arid and semi-arid tropics (Smith and Frederiksen, 2000). In India, sorghum ranks third in area and production after rice and wheat. Certainly sorghum, with average yield far below its potential has the opportunity with input from genetics and management comparable to rice, wheat and maize to become the global grain of the future.

Most of the national sorghum breeding programs in India focus on development of high yielding varieties and hybrids suitable for both grain and fodder production. Heterosis is an important component of hybrid yield performance and it has been shown to exist in a wide variety of crops for various yield and agronomic traits. In sorghum, heterosis for yield has been reported to range from 39 to 80% (Quinby, 1962). The level of genetic diversity between the parents has been proposed as a possible predictor of hybrid performance and heterotic potential in many crops. Development of heterotic hybrids involves extensive field crossing and evaluation and is time consuming. If a simple, reliable and efficient method to assess heterotic potential of F_1 hybrids prior to field testing is developed, much of the field work associated with field crossing and multi environment testing would be eliminated (Xiao *et al*., 1996).

In recent years, use of DNA-based markers for the genetic analysis and manipulation of important agronomic traits has become an increasingly useful tool in plant breeding. DNA markers have the potential to enhance the operation of a plant breeding program through a number of ways, ranging from finger printing of elite genetic stocks, assessment of genetic diversity, increasing the efficiency of selection for difficult traits, to making environment-neutral selection possible (Weising *et al.,* 2005). However, advances in molecular markers have generated considerable interest in assessing genetic diversity and predicting hybrid performance in crop breeding programmes (Krystkowiak *et al*., 2009).

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The introduction of PCR based molecular markers like Random Amplified Polymorphic DNAs (RAPDs), Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphisms (AFLPs) etc., constituted a new milestone in the field of DNA fingerprinting in plants. SSRs and RAPDs have been used to characterize genetic diversity in sorghum (Ayana *et al.,* 2000; Agrama and Tuinstra, 2003). Large number of studies has been conducted in various crops to investigate the relationship between marker genotype divergence of parents and performance of hybrids. This suggests that marker system could be used to survey the parental diversity and hybrid performance.

Though many have investigated the relationship between molecular diversity of the parents and heterosis of the hybrids, similar studies involving sorghum is limited. Hence, the present study was intended with following objectives (i) to assess the genetic diversity among parents at molecular level using RAPDs (ii) to assess the extent of relationship between marker heterozygosity and hybrid heterosis in order to explore whether such marker system were useful in predicting hybrid heterosis in sorghum.

MATERIALS AND METHODS

Parental lines and crosses:

The present experiment was carried out with ten morphologically diverse genotypes of sorghum [*Sorghum bicolor* (L.) Moench]. The ten genotypes consisted of six lines *viz*., IS 1003, IS 3504, IS 3541, IS 6354, CO 27 and CO 28 and four testers *viz*., IS 0030, AKSS 5, GSSV 1 and TNS 5 selected from the germplasm maintained at Department of Millets, Tamil Nadu Agricultural University, Coimbatore, based on their grain cum stover yield (Table 1). Each of the lines was crossed with four testers in a line x tester mating design and 24 cross combinations were generated.

Field evaluation and data collection:

Twenty four hybrids along with their parents were raised in a Randomized Block Design with three replications by adopting a spacing of 45 x 15 cm in a row of 4m length at Department of Forage crops, TamilNadu Agricultural University (TNAU), Coimbatore, India. Recommended agronomic and plant protection measures were followed to maintain the crop stand. In each line, five competitive plants were selected at flowering stage at random and were tagged for recording data. Ten agronomically important traits *viz*., plant height (cm), leaf number, leaf length (cm), leaf breadth (cm), leaf stem ratio, stover yield (g), panicle length (cm), panicle weight (g), 100 seed weight (g) and grain yield per plant (g) were recorded in both parents and hybrids.

Table 1 : Details of the parents used

Sr. No.	Variety / Accession	Origin	Particulars
1.	IS 1003	India	Fodder genotype selected from ICRISAT germplasm
2.	IS 3504	Sudan	Fodder genotype selected from ICRISAT germplasm with high leaf/stem ratio
3.	IS 3541	Sudan	Fodder genotype selected from ICRISAT germplasm
4.	IS 6354	India	Fodder genotype selected from ICRISAT germplasm with broad leaves
5.	CO 27	India	A derivative of *Sorghum halepense* x CO 11 with open panicle and thin stem
6.	CO 28	India	A derivative of CO 25 x SPV 924 and non lodging
7.	IS 0030	India	Sorghum genotype with sweet stem
8.	AKSS 5	India	Sorghum genotype with high grain yield
9.	GSSV 1	India	Sorghum genotype with high grain yield
10.	TNS 52	India	Sorghum genotype with high grain yield, bold grain and sweet stem

DNA extraction and electrophoresis:

To perform the molecular analysis, genomic DNA was isolated from 15 days old seedlings grown in pots under glass house condition following the method described by Gawel and Jarret (1991). The RAPD analysis was performed by using 55 decamer random primers. PCR reactions were performed in a volume of 18µl and amplified through 35 cycles of 1 min at 94^0C, 1 min at 37^0C, 1 min at 72^0C, followed by final extension at 72^0C for 5 min in model Gene Amp. PCR system 2400, Perkin Elmer, Norwalk, CT, USA.

Data analysis:

Plant amplified DNA fragments detected after electrophoretic separation was scored for the presence (1) or absence (0) of a particular DNA fragment in each accession. Only the reproducible fragments were considered for analysis and faint fragments were omitted. Similarity matrix was generated based on similarity coefficient (simple matching) determined between each pair of accessions using 'simqual' sub programme for the

software NTsys – PC version 2.02 (Numerical taxonomy System) (Rohlf, 1992). Similarity coefficients were used for cluster analysis of the varieties using the 'SHAN' sub programme of NTsys– PC and also to build dendrograms by the Unweighted Pair Group Method with Arithmetic Average (UPGMA) (Sneath and Sokal, 1973).

Relative heterosis (mid-parent heterosis) was estimated as a percentage of deviation from the mid-parent value (Fonesca and Patterson, 1968). Simple correlation coefficients between the genetic distances among the parents estimated based on the RAPDs and relative heterosis in hybrid combinations were estimated.

RESULTS AND DISCUSSION

The results of the present study as well as relevant discussion have been presented under the following sub heads:

Extent of polymorphism revealed by RAPD markers:

Parents used in the line x tester mating design were subjected to RAPD analysis for assessing genetic diversity among them and an attempt was made to find out the relationship between the genetic distances with hybrid vigour (Relative heterosis) in sorghum. Among the 55 decamer primers used for DNA amplification 20 did not produce clearly scorable bands. The remaining 35 primers generated a total of 192 scorable RAPD markers of which, 93 (48.4 %) were polymorphic among the genotypes. The range of markers produced by different primers varied from 3 to 11 with an average of 5.4.

Genetic distances among parents:

Dissimilarity indices for all pair wise combination among the genotypes were estimated and are presented in the Table 2. The genetic dissimilarity index obtained from the simple matching coefficient had a range of 0.390 (IS 3504 and IS 3541) to 0.938 (CO 28 and CO 27).

Clustering of parents:

The dendrogram constructed by the Unweighted Paired Group Method (UPGMA) is shown in Fig 1. As a whole, 10 parents were grouped into five clusters at 41 per cent of similarity level as Cluster I (IS 1003 and IS 0030), Cluster II (IS 3504 and IS 3541), Cluster III (IS 6354), Cluster IV (CO 27, GSSV 1 and TNS 52) and Cluster V (CO 28 and AKSS 5).

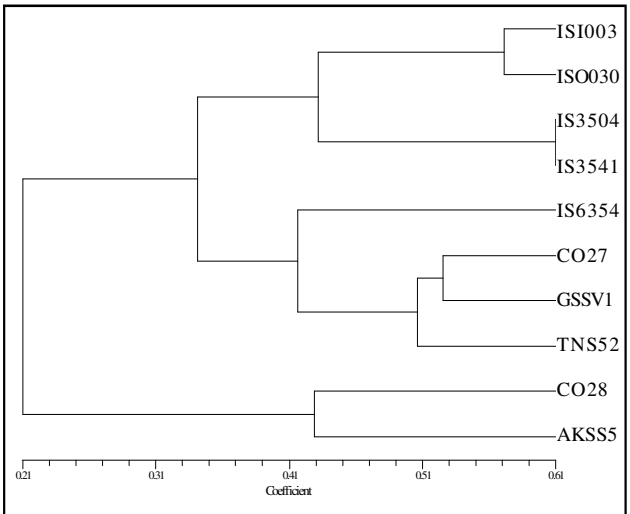

Fig. 1 : Dendrogram of ten parental lines based on unweighted paired group method with arithmetic average (UPGMA)

Relationship of marker based diversity with heterosis:

To assess the relationship between the genetic distances among parents estimated based on RAPD and

Table 2 : Genetic dissimilarity indices among ten parents as computed from RAPD data

Parents	IS 1003	IS 3504	IS 3541	IS 6354	CO 27	CO 28	IS 0030	AKSS 5	GSSV 1	TNS 52
IS 1003	0.000									
IS 3504	0.550	0.000								
IS 3541	0.500	0.390	0.000							
IS 6354	0.541	0.744	0.681	0.000						
CO 27	0.643	0.705	0.725	0.528	0.000					
CO 28	0.818	0.732	0.667	0.909	0.938	0.000				
IS 0030	0.429	0.690	0.535	0.622	0.744	0.775	0.000			
AKSS 5	0.789	0.789	0.714	0.800	0.929	0.571	0.771	0.000		
GSSV 1	0.681	0.735	0.600	0.619	0.475	0.809	0.689	0.684	0.000	
TNS 52	0.545	0.578	0.615	0.605	0.500	0.792	0.702	0.732	0.489	0.000

heterosis, simple correlation coefficient were computed and presented in the Table 3. Simple correlation coefficient (r) ranged from –0.331 (Stover yield) to 0.467 (leaf breadth). Positive and significant correlation between genetic distance and relative heterosis was observed for leaf breadth, panicle length and panicle weight and grain yield per plant. Positive but non-significant correlation was observed between genetic distance among parents and relative heterosis for leaf length.

Table 3 : Correlation coefficients between parental dissimilarity indices and heterotic vigour (Relative heterosis) in sorghum

Trait	Correlation coefficient (r)
Plant height	-0.092
Number of leaves	-0.049
Leaf length	0.091
Leaf breadth	0.467*
Leaf/stem ratio	-0.05
Stover yield	-0.331
Panicle length	0.438*
Panicle weight	0.405*
100 seed weight	-0.152
Grain yield per plant	0.383*

* indicates significance of value at P=0.05)

Genetic diversity among the parents may be estimated based on morphological and molecular markers (Diers *et al*., 1996). The diversity estimated using morphological traits may not be a real one because of influence of environment on the expression of the traits. Plant breeders have always been looking for traits, which directly relate to genes without superimposed effects for many morphological characters, which may be influenced by environmental effects. Molecular markers are independent of environmental conditions and they show a wide range of polymorphisms. Moreover, they are promising tools for estimating genetic diversity in various crop plants (Ayana *et al.,* 2000; Agrama and Tuinstra, 2003). Because of the low cost and simplicity of the technique, RAPDs is being used in wide range of plant breeding programs (Bhutta *et al*., 2006). In the present study, RAPD markers have been used to detect genetic diversity within parental genotypes. Thirty five RAPD primers altogether revealed 48.40 per cent polymorphism but lower than that reported by Menkir *et al*. (1997) in cultivated races of sorghum.

The results of the parental diversity revealed that, the two high yielding forage genotypes IS 3504 and IS 3541 were grouped in the same cluster indicating the molecular similarly within them and both were originated from Sudan. The highest genetic distance was observed between varieties Co 28 and Co 27 though they had same geographic origin. This may be due the fact that the genotype Co 27 was a derivative of a cross between wild *Sorghum halapense* (L.) Pers. and Co 11 while Co 28 was derived from a cross between CO 25 and SPV 924 both of which come under *Sorghum bicolor* (L.) Moench. a cultivated species. A similar result was observed by Xiao *et al*., (1996) in rice for assigning germplasm into appropriate subspecies or variety groups. This demonstrates the usefulness of RAPDs to differentiate species or variety groups in germplasm characterization. The genotype IS 6354 formed a single solitary cluster indicating its diverging nature from other genotypes. Even with 35 arbitrary primers, the present study could reveal a fair degree of genetic divergence as seen from the five clusters into which the parental genotypes fall. RAPDs was utilized as a tool to study genetic diversity in sorghum also by Menkir *et al*. (1997), and Uptmoor *et al*. (2003).

One of the objectives of this study was to determine whether there was a relationship between genetic distances among parents based on molecular markers and the level of relative heterosis of the hybrids. The results demonstrate that genetic diversity was positively correlated with heterosis for leaf breadth, panicle length, panicle weight and grain yield per plant. Such significant and positive correlations between genetic distance and relative heterosis were observed for yield potential in sorghum by Jordan *et al*. (2003), in rapeseed by *Riaz et al*. (2001) and in bread wheat by Corbellini *et al*. (2000). However, this was not the case with respect to plant height, number of leaves, leaf/stem ratio, stover yield and 100 seed weight in dual purpose sorghum. Similarly Xiao *et al*. (1996) reported positive correlation between genetic distance and heterosis for yield while negative correlation for spikelets per panicle within *indica* subspecies in rice.

Conclusion:

Genetic distances measured by 35 RAPD decamer primers showed significant relationship with grain yield per plant (r=0.383*) in sorghum indicating the usefulness in identifying potential parents for crossing which will reduce the field work associated in making crosses and testing.

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