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Research Article

Genetic Relationships among Sorghum [*Sorghum bicolor* (L.) Moench] Lines and Correlation between Genetic Distance and Hybrid Performance

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Abstract: *In certain cases the improved and released sorghum varieties are not being used by Ethiopian farmers because of lack of farmers preferred traits in the hybrids developed so far. This study was conducted to identify the genetic potential of selected sorghum inbred lines involving landraces, introduced and improved lines and its effect on combining ability and hybrid performance to use for hybrid development. Genetic diversity of 37 inbred lines used for the test hybrids was assessed using 7339 SNP markers. The genotypes were grouped into four clusters, with the Ethiopian landraces depicting clear distinction from the introduced and breeding lines. The result implies that efforts might be needed to bring the best genes from the landraces for the hybrid breeding. Genetic distance between inbred lines estimates based on SNP markers ranged from 0.02 to 0.358 with an average of 0.247, indicating the very narrow genetic distance for this study. The regression analysis indicates that grain yield showed an increment as the genetic distance among parental lines increased. Generally, the genetic distance between lines and regression analysis played vital role in determining which lines showed yield increment. Thus, sorghum lines with desirable trait of interests were properly identified for future sorghum breeding program.*

Keywords: Cluster, Genetic diversity, Molecular marker, SNP, Regression analysis

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1. Introduction

Wide diversity is found within and among the sorghum (Sorghum Bicolor (L.) Moench) cultivars at both phenotypic and genotypic levels (Kebede et al., 2025). To date, several studies have been conducted on sorghum germplasm in Ethiopia using limited numbers of molecular markers (Ayana et al., 2000; Geleta et al., 2006; Desmae, 2007) and have shown the extent of genetic diversity within the landrace collections of the different agro-ecologies. Improvement by selection within traditional cultivars or by selecting progeny from crosses between similar traditional cultivars has generally not been promising in enhancing yields (House, 1995). The success of a crop improvement program highly relies on the power and efficiency of capturing and managing genetic variability (Crouch and Ortiz, 2004). According to Geleta and Labuschagne (2005) and Mehmood et al. (2008), morphological biochemical and molecular markers are the methods used for measuring genetic diversity among crop species. In studying genotype performance under normal growing conditions morphological traits provide a simple way of measuring genetic diversity but are influenced by environmental factors (Tuinstra et al., 1996; Beuningen and Busch, 1997; Abdi et al., 2002; Fufa et al., 2005; Graner et al., 1994). Thus, molecular markers are invaluable for understanding the genetic make-up of crops. They differ from morphological traits in that they usually occur in greater numbers, they can be distinguished without relying on the complete development of the plant, and their expression is not altered by the environment (Jeya Prakash et al., 2006; Tabbasam et al., 2006; Mehmood et al., 2008; Abu Assar et al., 2009).

Knowledge of genetic diversity not only generates a better understanding of germplasm sampling but also has implications concerning the choice of parents for crosses and gene introgression from distantly related germplasm. Both morphological and molecular markers analyses are informative tools for estimating genetic distances (Vieira et al., 2007). According to Smith and Smith (1992), the use of molecular markers, particularly DNA-based polymorphisms which detect variation at DNA sequence level, has become an increasingly useful and powerful tool in the assessment of genetic similarity and manipulation of important agronomic traits in breeding populations (Lee, 1995). Furthermore, classical or conventional plant breeding is time-consuming and highly dependent on environmental conditions. The application of molecular markers is crucial and more effective and efficient for the selection of traits of interest in breeding programs as well as to assess genetic diversity (Fufa et al., 2005; Geleta et al., 2006; Shehzad et al., 2009)

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In the determination of genetic diversity and relationships, molecular markers play a vital role. Attempts have been made to estimate genetic diversity among Ethiopian sorghum gene bank accessions (Alemu et al., 2020). Different kinds of markers have been used in many studies of sorghum and showing genetic diversity in sorghum gene-pool high degree of correspondence between sorghum racial classification and marker-based grouping (Shehzad et al., 2009; Brown et al., 2011; Ramu et al. 2013). For example, restriction fragment length polymorphism (RFLPs) (Ahnert et al., 1996), random amplified polymorphic DNA (RAPDs) (Iqbal et al., 2010), simple sequence repeats (SSRs) (Bucheyeki et al., 2009) and amplified fragment length polymorphism (AFLPs) (Menz et al., 2004; Ritter et al., 2007) were widely applied in this process. These molecular markers have been used efficiently to characterize and estimate the genetic diversity among sorghum germplasm. Similarly, studies have also been conducted on sorghum germplasm from Ethiopia using limited numbers of molecular markers, e.g. with RAPD markers (Ayana et al., 2000), SSR and AFLP markers (Geleta et al., 2006), and SSR and ISSR marker Goyals (Desmae, 2007), and have shown the extent of genetic diversity within the landrace collections of the different agro-ecological adaptation zones. Sometimes, farmers are not interested to use improved and released sorghum varieties due to lack of certain trait of interest. To fill such gap, a modern breeding approach using molecular marker is mandatory in order to get a parent with the required trait of interest. Thus, the present study was aimed to investigate the effect of genetic distance between the parents on GCA, SCA and hybrid performance to use genetic distance as a tool for predicting hybrid performances in sorghum research.

2. Materials and Methods

2.1. Description of the study area

Sorghum hybrids and lines were evaluated at Sheraro and Meiso sub-centers trial site. Whereas, the laboratory work was done at The University of Queensland (UQ), primarily through the Queensland Alliance for Agriculture and Food Innovation (QAAFI) and the Centre for Crop Science, focuses on improving yield, drought tolerance, and nutritional value.

2.2. Plant materials

The plant materials involved in this study were a total of 37 sorghum genotypes classified as improved lines from the Ethiopian national sorghum pedigree breeding program bred using landraces and introduced genotypes as parents for adaptation to the dry lowland agro-ecologies of the country. Of which 24 genotypes were from Ethiopia (landraces and improved) and 13 were introduced inbred lines being used as hybrid parents in the breeding program (Table 1). The inbred lines were used to develop F1 hybrids by crossing with three seed parents (A lines) to evaluate hybrid performance, GCA and SCA.

Table 1: Sorghum inbred lines used for molecular study

Origin	Number of Genotype	Agro-ecology
Ethiopian landraces	10	Dry lowland
Ethiopian improved	14	Dry lowland
Introduction	13	Dry Lowland
Total	37	

2.3. Genomic DNA extraction and SNP genotyping

The total DNA was extracted from two-week-old seedlings as described by DArT PLC (Paterson et al., 2009). The samples were sent for sequencing using DArT Seq platform in Australia along with sorghum genotypes being used in the national sorghum improvement program (DArT, www.diversityarrays.com). The common SNP filtering criteria were used which aims to remove potential false positives or low-quality data points (Roshyara et al., 2014). Thus, which includes:

•Call Rate (CR): Percentage of samples with a valid genotype call for a SNP.
Threshold: Often >90-99%
•Minor Allele Frequency (MAF): Frequency of the less common allele.
Threshold: Typically >1-5% to ensure sufficient statistical power and avoid rare, potentially erroneous calls.
•Hardy-Weinberg Equilibrium (HWE): Checks if allele/genotype frequencies in a population are as expected by chance.
Threshold: A high p-value (e.g., >10-4 or >10-6) is desired; SNPs deviating significantly (low p-value) are removed.
•Read Depth/Coverage: Number of sequence reads covering a SNP position.
Threshold: Often a minimum of 4-10 reads per genotype to ensure accuracy.
•SNP Quality Score (QUAL): A measure of genotype quality.
Threshold: Often >30 (for Illumina platforms).
•Distance to Indels: Filtering out SNPs located too close (e.g., <35 bp) to insertions or deletions (indels).
•Mendelian Errors: Checking for inheritance inconsistencies in family data.

The sequence data generated were then aligned to the sorghum reference genome sequence (Paterson et al., 2009) to properly identify SNP markers.

2.4. Data scoring and statistical analysis

The analysis of molecular variance (AMOVA) was carried out using the formula:

$$\text{F Statistic} = \frac{\text{Variance of the group means}}{\text{Mean of within group variances}}$$

The SNP markers were selected based on the polymorphism and a total of 7339 SNP markers were filtered to be used for the analysis. Statistical analyses were performed after converting the adjusted allele calls into binary format. The analysis was conducted using a combination of SNP data and genotype origin (landraces, released and introduced) and adaptation to allocate the sorghum genotypes into groups and assess the relative genetic distance of these groups. Unweighted neighbor-joining cluster analysis, using DARwin version 6.0 statistical software (Perrier and Jacquemoud-Collet, 2006), was then used to identify the pattern of genetic differentiation within and between the groups of sorghum genotypes. To estimate the discriminatory power of each SNP marker, the polymorphic information content (PIC) was calculated as per the formula developed by Anderson et al. (1993), which assumes homologous alleles as:

$$PIC_i = 1 - \sum_{j=1}^{n} xij^2$$

Where, PICi = the polymorphism information content value of the ith marker, Xij = the frequency of the ith allele for the jth marker and summed over n alleles. Besides, major allele frequency (MAF) and expected heterozygosity (He) were also calculated for all the SNPs using Power Marker 3.25 software (Lui and Muse, 2005). Analysis of molecular variance (AMOVA) and pair-wise population diversification analysis was conducted using GenAlEx version 6.5 statistical software (Peakall, R. and Smouse P.E. 2012).

Observed heterozygosity (Ho) for each primer was calculated by dividing the number of heterozygous genotypes at a given locus by the total number of genotypes analyzed (Hormaza, 2002).

3. Results and Discussion

3.1. Analysis of Molecular Variance (AMOVA)

The analysis of molecular variance assigns 84% of genetic variation to the differentiation among individuals and 16% to differences within individuals in a population. It revealed a highly significant genetic poly 2011), and Kenya (Mutegi et al., 2012) reported similar levels of genetic diversity between cultivated and wild sorghum, which they attributed to crop-to-wild gene flow. As observed by Chakauya et al., (2006), differences in the genetic diversity could be attributed to traditional farming systems with agronomic, economic and cultural considerations that foster high levels of genetic diversity.

Table 2: AMOVA for 37 sorghum inbred lines calculated according to Weir and Cockerham, (1996)

Sources of variation	Df	Variance components	Percentage of variation	P-value	F_{ST}
Among individuals	2	4.896	84.00	<0.001	0.84
Within individuals	34	3.375	16.00		
Total	36				

3.2. Grouping pattern of sorghum genotypes

The neighbor-joining tree generated using the genome-wide SNPs grouped the genotypes into four main clusters (Figure 1) These four clusters were identified at the 0.05 similarity level. The first cluster (Group I) is composed of the genotypes coming from various localities which comprise 13 sorghum genotypes. This cluster constituted almost exclusively of Ethiopian landraces containing 11 (78.5%) of the landrace genotypes. The previous findings by Mindaye et al. (2015) showed similar groupings and accentuated the limited use of the landraces for inbred line development. The majority of genotypes clustered here are Ethiopian landraces except for containing one improved genotype called Birmash. Cluster II consisted of the breeding lines by the national sorghum improvement program with the exception of the variety Gobiye, a released variety from introduced lines for striga infested areas. Cluster III consisted of the largest genotypes where the majority of the locally improved and introduced sorghum genotypes clustered in this group. The cluster containing 8 (80%) improved and 6 (46%) of introduced sorghum genotypes with few landraces. Finally, cluster IV has as a single genotypes ICSV9304 which is among the introduced genotypes. These results reflect the use of common gene pool of the sorghum breeding program and the introduction characterized by early maturity and short plant statur. The use of local sorghum germplasms in the inbred line and hybrid development is thus required due to attention to enhance adoption of the derived hybrids.

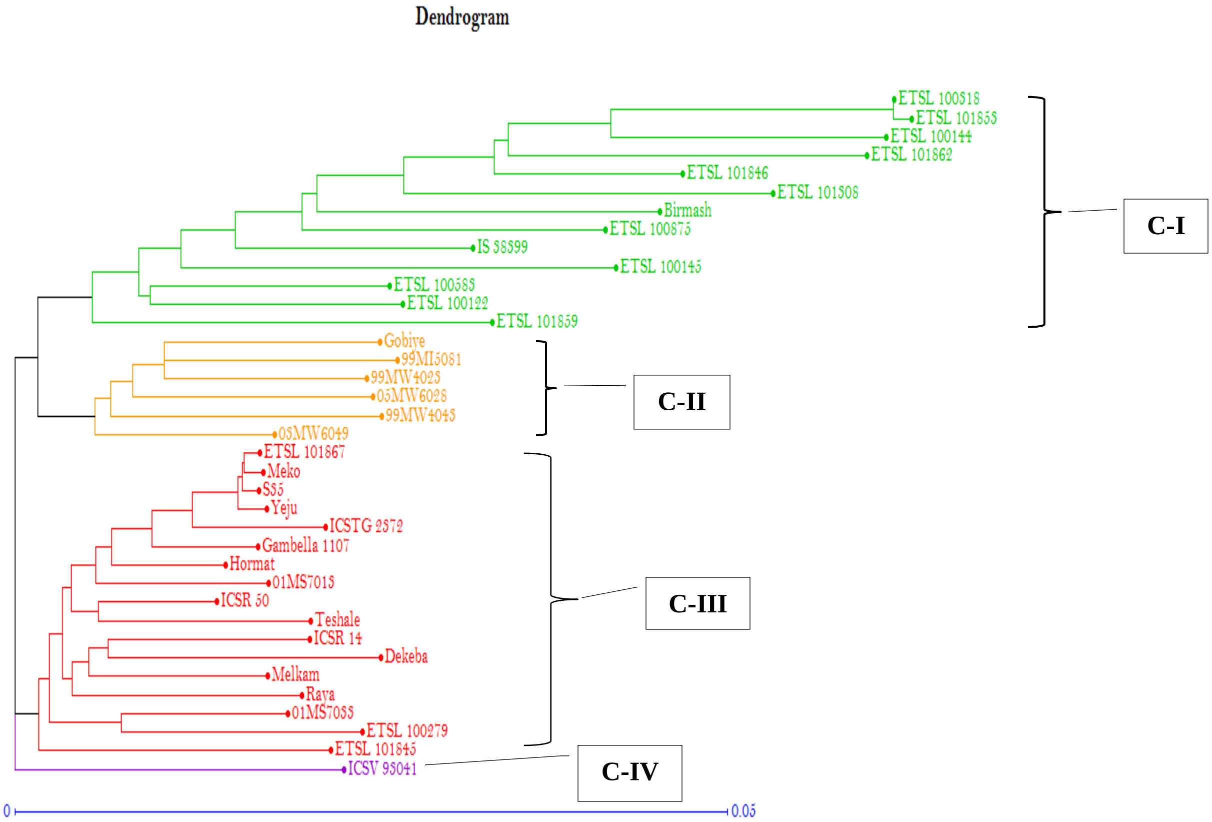

Figure 1. Genetic relationships among 37 sorghum lines (improved landrace and introduced) SNP markers.

The polymorphic information content (PIC) of markers ranged from 0.08 to 0.38 with an average of 0.33 across the three groups (landraces, improved, and introduced). Botstein et al. (1990) classified a marker with a PIC value of more than 0.5 as highly informative, between 0.25 and 0.5 as informative, and less than 0.25 as slightly informative. According to Botstein et al. (1990), the results revealed a good level of polymorphism but a moderate level of diversity based on the average polymorphic information content values (0.33). The summary statistics for major allele frequencies (MAF), expected heterozygosity (He), and the polymorphic information content (PIC) is presented in Table 3. Low expected heterozygosity (0.07) was observed with the SNP marker (SNP_150) that has the high major allele frequency (0.93) whereas the highest heterozygosity is 0.52. Low heterozygosity implies that little genetic variability among populations. This result is in agreement with the previous finding by Mindaye et al., (2015). The mean of expected heterozygosity was 0.42 and that of the major allele frequency was 0.68. The allele frequencies of all the markers were greater than their corresponding expected heterozygosity values. The results revealed a good level of polymorphism but a moderate level of diversity based on the average polymorphic information content values (0.33). Similar findings were also observed by different investigators; Brown et al., (1996); Dean et al., (1999); Dje et al., (2000); Grenier et al., (2000); Uptmoor et al., (2003) who reported that landraces from other world sorghums were sampled over different geographic regions, therefore our data is comparable to another molecular marker-based research. The present study confirms the previous observations showing that sorghum germplasm collections are structured genetically, with about 84% of the genetic diversity occurring among genotypes (Dje et al., 2000).

Table 3. Genetic diversity parameters of 7339 SNPs in 37 sorghum accessions.

Parameter	**Min.**	**Max.**	**Mean**	**Std. Dev.**
MAF	0.51	0.91	0.68	0.166
PIC	0.06	0.39	0.33	0.142
Heterozygosity	0.07	0.52	0.42	0.078
Gene diversity	0	1.00	0.28	0.184

MAF, major allele frequency; PIC, polymorphism information content.

3.3. Genetic distance among genotypes

Genetic distance (GD) in sorghum, particularly when measured using molecular markers like SNPs or RFLPs, generally shows a positive correlation with both Specific Combining Ability (SCA) and hybrid yield, indicating that more genetically diverse parents tend to produce higher-yielding hybrids. However, this relationship is often weak to moderate and depends heavily on the genetic background of the parents and the specific population being studied (Silva et al., 2020).

In the present study, genetic distance estimates based on genotyping-by-sequencing SNP data ranged from 0.075 to 0.358 with an average of 0.247 (Table 4). The lowest genetic distance (0.075) was obtained between the inbred lines L3 and L6, whereas the highest genetic distance (0.358) was observed between the inbred lines L4 and L8. Genetic variation was observed within R-lines in the subset of elite germplasm that facilitated the selection of parents for use in hybrid combinations with varying degrees of genetic divergence. An average genetic similarity between B-lines and R-lines of 0.73, with a range of 0.61 to 0.99 using genome-wide SNP markers, or an average genetic similarity of 0.584, ranging from 0 to 0.886 using a series of AFLP and SSR markers as shown in a previous sorghum study by (Mindaye et al., 2015) and (Menz et al., 2004) respectively. The estimates of genetic similarity in the present study were similar, but the range of genetic similarity was lower. This implies that the less the population the little genetic variation while the more the population results lots of genetic variation.

Genetic distance among the parents based on SNP markers ranged from 0.097 for cross MARC6A/ETSL101576 to 0.324 for cross TX623A/IESV23010DL (Figure 4) with an average of 0.238. Two crosses that had a combination of high yield (>4.0tha-1), high SCA (>0.9), and high genetic distance (= 0.3) were TX623A/ETSL101576 and TX623A/IESV23010DL. These crosses consisted of lines that were probably most divergent in this set of germplasm. In previous study Ceballos et al., (2016) observed weak associations between the GD and SCA effects, which presented r 2 values ranging from 0.00 to 0.28. in another investigation by Ndhlela et al., (2015) and Dreisigacker et al., (2005) found no explicit correlation between GD and heterosis. Cress (1966) proposed that the extent of GD between parents is necessary for significant heterosis but is not sufficient to guarantee it. On the other hand, Betrán et al., (2003) suggested that heterosis can be better predicted only when GD is smaller than a certain threshold.

Table 4. Genetic distance (GD) matrix among the 12 sorghum inbred lines based on SNP analysis.

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12
L1	-	0.266	0.234	0.172	0.168	0.253	0.175	0.113	0.236	0.090	0.119	0.264
L2		-	0.284	0.284	0.095	0.160	0.146	0.276	0.286	0.134	0.272	0.088
L3			-	0.240	0.260	***0.075***	0.324	0.099	0.197	0.340	0.321	0.155
L4				-	0.253	0.192	0.095	***0.358***	0.218	0.265	0.099	0.303
L5					-	0.249	0.252	0.300	0.249	0.308	0.304	0.297
L6						-	0.149	0.280	0.287	0.320	0.322	0.299
L7							-	0.297	0.284	0.266	0.314	0.182
L8								-	0.243	0.228	0.322	0.166
L9									-	0.326	0.341	0.283
L10										-	0.293	0.327
L11											-	0.186
L12												-

As illustrated in Figure 2 regression analysis indicates that grain yield showed an increment as the genetic distance among parental lines increased. According to the previous study, there was no discernable relationship to grain yield between Finlay and Wilkinson's (1963) regression coefficients (bi). Their performance over environments cannot be predicted. However, high and positive deviations from regression at some environments may also reveal that the genotype has higher adaptability to these specific growing conditions than the average of the whole material (Nurminiemi and Rognili, 1996). Likewise, specific combining ability (SCA) of the selected hybrids increased as the genetic distance increases (Figure 3). Similar conclusions were drawn by Finlay (1964) who analysed the values of bi in barley and observed large significant estimates for general combining ability (GCA) and no significant values for specific combining ability (SCA). This is in agreement with several studies with maize (Eberhart and Russell, 1969; Dhillon and Singh, 1977; Geiger et al., 1987).

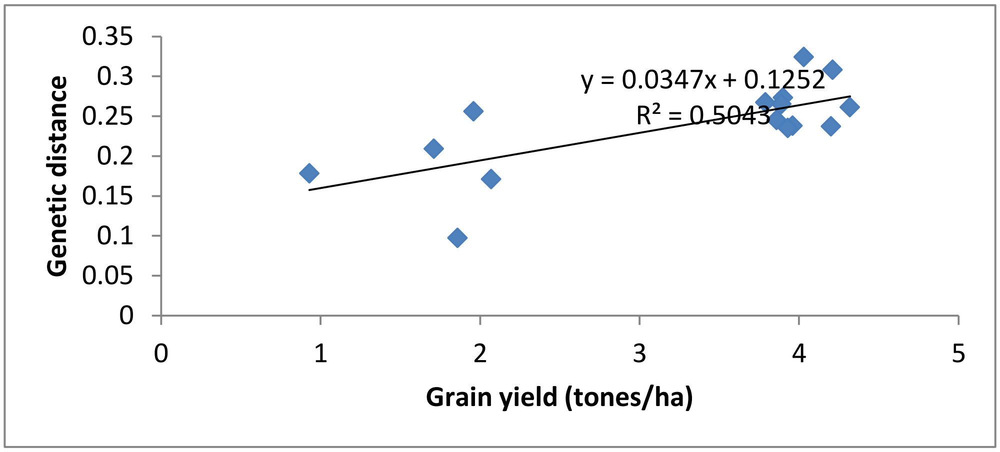

Figure 2. Regression of genetic distance on grain yield in selected hybrids

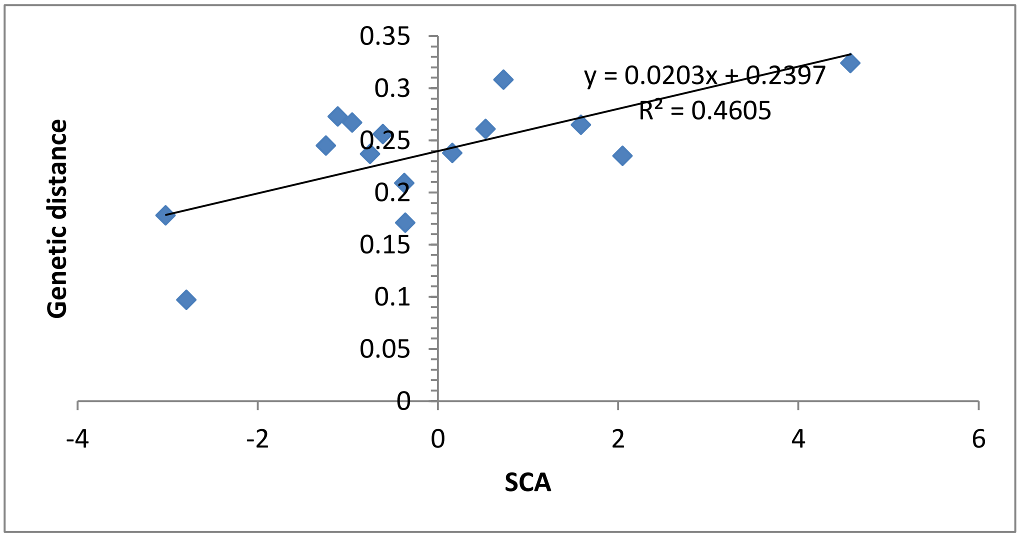

Figure 3. Regression of genetic distance on specific combining ability on selected hybrids

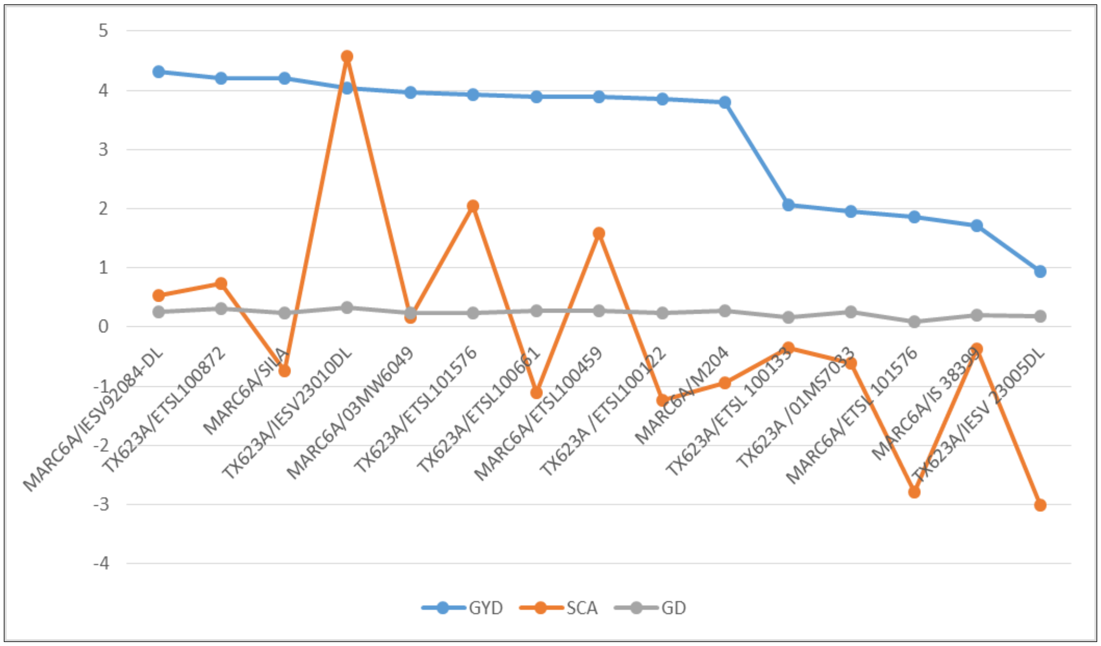

Figure 4. Grain yield (GYD), specific combining ability (SCA) effects and genetic distances (GD) determined by SNP markers for the top 10 and bottom five crosses

4. Conclusion

Identified genotypes and genetic distance thresholds are critical in modern, efficient plant breeding, allowing for the prediction of hybrid performance and heterosis before conducting expensive field trials. By classifying inbred lines into heterotic groups based on genetic distance, breeders can optimize parental selection to maximize heterosis (hybrid vigor) and prevent inbreeding depression. In the current study, clustering grouped by neighbor-joining tree generated from SNP data showed the four distinct sorghum classifications. On the other hand, some of the hybrids obtained from the crosses among selected lowland adapted landraces and introduced once showed superior performances than their parental inbred lines.

The grouping of lines using SCA estimates, F1 hybrids, and genetic distance with markers was fairly consistent and dependent on relatedness and/or pedigree of the source of populations. Thus, based on the results obtained, it can be conclude that there is an opportunity to develop commercial hybrid sorghum with superior grain yield. The cross combinations such as MARC6A*IESV92084-DL, TX623A*ETSL100872, and TX623A*IESV23010DL were identified as promising hybrids with highly significant SCA effects and positive heterosis for grain yield. Moreover, this result might be useful as an indication of the variations among sorghum inbred lines in both landraces, improved, and introduction once and it requires further validation under field conditions.

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Conflict of interest

The author declared no conflict of interest associated with this work.

Data availability statement

The data used and analyzed during the current study are available from the corresponding author on reasonable request.

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