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RESEARCH ARTICLE

# Genetic analysis for morphological, quality and biofuel related traits in sweet sorghum [*Sorghum bicolor* (L.) Moench]

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## Abstract

The present investigation was aimed to study the relationship between different methods of diallel data analysis of ethanol yield traits in sweet sorghum to select the relevant superior genotypes. Half-diallel hybrid analysis revealed that both general and specific combining abilities were significant for all the traits studied but the specific combining ability (SCA) was higher than the general combining ability (GCA), suggesting that the non-additive gene action is more important than additive one in controlling the studied traits. Graphically, traits of juice extraction percent, juice yield, and stripped stalk yield were most correlated with ethanol yield. Griffing and GT-biplot analyses produced equivalent results for GCA and SCA and were useful in estimating gene action by identifying the superior crosses across all the correlated traits and comparing the genotypes based on multiple traits. For the ethanol yield trait, genotype, Rio showed good GCA and was considered the best combiner parent, while crosses, Sugar Drib× Ramada and Sugar Drib × Honey may be used for the selection of the relevant traits in sorghum breeding programs to improve ethanol yield trait.

**Keywords**: Bio-ethanol, sweet sorghum, half diallel, heterosis, combining ability.

## Introduction

Sweet sorghum [*Sorghum bicolor* (L.) Moench] is one of the most common crops for syrup and bio-fuel, food and feed or fodder (green/dry) and therefore, it is considered as a multi-purpose crop. It is also considered as one of the most cost-effective sources of bio-fuels because its short duration crop (short growth period), high tolerance to soil water shortage, higher biological productivity with limited inputs, and higher tolerance to environmental stresses with high adaptability to a wide range of environments (Gielen et al. 2019). Sweet sorghum is classified as one type of the bio-energy sorghum group (Rooney et al. 2007). Its stem juice contains high concentration of soluble sugars (53–85% sucrose, 9 to 33% glucose, and 6 to 21% fructose) that can easily be converted to bio-ethanol with high octane, low sulfur, and low biological content (Appiah-Nkansah et al. 2019; Zhou and Thomson 2009). The stem juice yield with high biomass produces higher fermentable sugars (upto 78% of the total biomass), obtaining a higher bio-ethanol yield (Tesfamichael et al. 2015). Bio-ethanol production depends on environmental conditions, genotypes genetic groups, harvesting stage, and genotype resources.

Breeding programs aim to utilize genetic variability in sorghum to improve with higher sorghum yield and its components under limited water and low fertility land resources in Egypt. The diallel analysis is the mating design widely used to evaluate the combining ability of the hybrids between suitable parents. Estimation of GCA and SCA are indicators for the nature of gene action (Sarker et al. 2002; Rashid et al. 2007). GCA is due to genes that are additive in nature, while SCA is due to genes with non-additive effects (dominance or epistatic effects). Griffing's analysis was used to evaluating the parents and their hybrids by partitioning the total genetic variance to GCA and SCA and determining the best parents and the best crosses. Meanwhile, heterosis expression levels are also useful to help breeders choose the best hybrid combinations that serve suitable

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materials for selecting superior genotypes. The breeders uses heterosis as the best genetic parameter for judging the parents and their crosses that have good combining ability and possess the ability to pass favorable genes to their progeny (El Hosary 2020; Marisol et al. 2020; Sedhom et al. 2021; Turk et al. 2021). Gene action measured the attributes' proportion of total genetic variance. Broad-sense coefficient of determination is defined as the proportion of phenotypic variation attributed to total genetic (additive and non-additive) variance (Falconer and Makay 1996; Babu et al. 2012; Abu-Ellail et al. 2023). On the other hand, the relatively narrow-sense coefficient of determination measured a proportion of phenotypic variation attributed to only additive gene variance. Therefore, the objectives of the present investigation were to use diallel design to evaluate some sweet sorghum genotypes with different origins for yield and bio-ethanol production via studying different sorghum traits, to determine the nature of gene action influencing traits and select the best genotypes and to explain the relationships among different yield and biofuel-related traits.

## Materials and methods

### *Plant material and the experimentation*

The current experiment was carried out in two seasons (2021 and 2022) at Giza Experimental Agriculture Research Station, (30º 02' N latitude and 31º 13' E longitudes with an altitude of 22.50 meters above sea level), Giza Governorate, Egypt. The following plant material consisted of five sorghum genotypes imported from the United States of America (USA) to strengthen the breeding program to improve the sorghum were used in the study.

| S. No. | Name of the genotype | Origin |
|---|---|---|
| P1 | Brands | Mississippi, USA |
| P2 | Sugar drib | Oklahoma, USA |
| P3 | Ramada | Mississippi, USA |
| P4 | Honey | Mississippi, USA |
| P5 | Rio | Texas, USA |

The parents were crossed in a diallel fashion to obtain all possible combinations, excluding reciprocals during the first summer season of 2020. In the summer seasons of 2021 and 2022, the five parents and their respective ten $F_1$ hybrids were evaluated at Giza Agricultural Research Station. The genotypes were arranged in a randomized complete block design (RCBD) with three replications. Each plot consisted of 5 rows x 5 meters long and 60 cm wide and plants were spaced at 20 cm within a row (the plot size is 15 $m^2$). The plots were informally fertilized at the rate of 120 kg of nitrogen per feddan (1 feddan = 4200 $m^2$) given before the first and second irrigations. Cultural, agricultural practices of sweet sorghum growing, such as land preparation, hoeing, thinning, fertilization, irrigation, etc., were applied properly as the Ministry of Agriculture and Land Reclamation recommended.

### *Observations and measurements*

At harvest time (120 days from sowing), ten guarded plants were randomly selected from each plot for each genotype, and data regarding different plant traits were recorded during the cropping season, then immediately crushed through 3 roller labs. Mill and the obtained raw juice were filtered, weighed, and the traits, namely, days to 50% flowering (FL), number of days from the sowing date to 50% of the plants showing their heads, plant or stem height (SL) in cm from the soil surface to the top of the panicle, stem diameter in centimeters (SD) measured at the mid stalk, total soluble solids percent (TSS%) determined with a hand refractometer, Sucrose percentage (SU%) of clarified juice determined by using an automated saccharimeter according to A.O.A.C. (2005), purity (PUR%) that was calculated as: [(Sucrose / Brix) x 100], Juice extraction percent (JEP%) as per the formula, juice weight/stalk weight x100, Juice yield per ton/fed (JY) = stripped stalk yield × JEP /100, Stripped-stalks yield per ton/fed (SSY), was calculated on a plot basis kg/fed and then converted to ton/fed and Theoretical ethanol yield (EY) was calculated according to Smith and Buxton (1993) were measured.

### *Statistical analysis*

The collected data of studied traits were subjected to analysis of variance for each season. According to Gomez and Gomez (1984), a combined analysis of variance was performed after proving the homogeneity of error mean squares across seasons by Levene's test (1960). Data obtained from the 15 $F_1$ hybrids and six parents were subjected to analysis by Griffing (1956) method II, model 1. Combining ability was analyzed to determine the general combining ability (GCA) effects of parents and specific combining ability (SCA) effects of hybrids and to separate some components of genetic variances, such as additive and non-additive gene action under certain assumptions. Heterosis was estimated as outlined by Foohad and Bassiri (1983), then the significance of deviation of the $F_1$s from the mid-parent values was tested by "t-test" (Wynne et al. 1970). The variance component method determined heritability and genetic parameters (Breese 1972). Predicted genetic advance as mean percent from selection (GA %) was calculated according to Johanson et al. (1955) at 5% selection intensity.

The ($\sigma^2_{gca}/\sigma^2_{sca}$) ratio was used for approximate estimations of gene effects (Verma and Srivastava 2004). Broad and narrow sense coefficients of genetic determination were calculated as the following Abney et al. (2000) equation:

Narrow Sense Coefficient of Genetic Determination (NSCGD) ~ $h^2 = 2\sigma^2gca / (2\sigma^2gca + \sigma^2sca + \sigma^2e)$. Broad Sense Coefficient of Genetic Determination (BSCGD) ~ $H^2 = (2\sigma^2gca + \sigma^2sca) / (2\sigma^2gca + \sigma^2sca + \sigma^2e)$.

The principal component (PC) analysis was performed on collected data to enhance interpretability through summary indices; the first two PCs were used to group the genotypes, whose values were used to generate the biplots (PC1 was used on the horizontal axis, whereas PC2 was used on the vertical axis) as described by Yan and Rajcan (2002). Bi-plot was performed using a computer software program Minitab v.19.

## Results and discussion

The genetic knowledge of yield traits helps in formulating a meaningful breeding strategy to improve genotypes with desirable traits.

### *Analysis of variance*

The obtained combined analysis of variance across two seasons is presented in Table 1, which was performed to test the significance of seasons and genotypes then, the variance was partitioned and the significance of differences among the effect of the genotype to the variance of general and specific combining ability (GCA, SCA); and so as the interactions across seasons for each trait. Results of the combined analysis of variance showed significant or highly significant differences among both seasons for all the traits except for SL and PUR%, indicating the differences between the two seasons. Various researchers have observed similar differences in sorghum and maize (El Hosary 2020; Marisol et al. 2020; Sedhom et al. 2021; Turk et al. 2021).

The combined analysis of variance for combining the ability all the traits is also presented in Table 1. Significant differences among the parents and hybrids for all the studied traits were displayed, providing evidence of the high considerable amount of genetic variability. Consequently, both GCA and SCA were highly significant for all traits, suggesting that additive and non-additive gene actions were responsible for controlling these traits in both seasons. In addition, significant or highly significant differences were detected only for the EY trait in genotypes*year and both GCA and SCA*year. Meanwhile, JY and SSY traits recorded significant differences for genotypes* year; but only SSY for SCA*year. These results indicated the presence of variability in natural gene action among the studied genotypes. Working on maize, El Hosary (2020) and Turk et al. (2021) also reported significant differences among parents and hybrids with respect to combining ability assessed over the seasons.

Baker's ratio or the relative importance of both types of combining ability (GCA/SCA) was used in determining the gene action type to utilize the performance of cross progeny. The closer the baker's ratio was to the GCA with greater variance than SCA one, registering the larger for FL (0.73), SL (0.89), and SSY (0.70) and suggesting the importance of additive genetic control. Then, FL (1.37), SL (4.16), and SSY (1.18) traits with high GCA/SCA ratios (more than unity) indicated the large part of the total genetic variability associated with these traits that had additive and additive by additive gene effect (Girma et al. 2010). On the other hand, all ratios were moderate to low for other traits, indicating the preponderance of non-additive gene effects in their expression (Baker 1978). Similar results were obtained by Zaghloul et al. (2022) and Al-Aaref et al. (2016) who used the relative importance of both types of combining ability to measure types of gene action.

### *Mean performance*

The obtained data presented in Table 2 indicates highly significant differences among mean performances for all measured traits in sorghum genotypes across two seasons. The mean days to 50% flowering (FL) ranged from P1 (62.80

**Table 1.** Mean squares of Griffing for some studied traits over two seasons

| Source | d.f | FL | SL | SD | TSS% | SU% | PUR% | JEP % | JY | SSY | EY |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | 1 | 36.56$^{**}$ | 73.44 | 0.28$^{**}$ | 18.51$^{**}$ | 6.41$^{**}$ | 12.19 | 187.11$^{**}$ | 15.71$^{**}$ | 35.77$^{**}$ | 30325.58$^{**}$ |
| Rep/Y | 4 | 15.90 | 151.12 | 0.04 | 0.61 | 2.34 | 104.02 | 20.47 | 0.47 | 0.40 | 405.64 |
| Genotype | 14 | 85.60$^{**}$ | 10637.40$^{**}$ | 0.38$^{**}$ | 13.10$^{**}$ | 6.83$^{**}$ | 236.45$^{**}$ | 269.00$^{**}$ | 11.65$^{**}$ | 29.42$^{**}$ | 57867.76$^{**}$ |
| Gen*Y | 14 | 0.08 | 0.03 | 0.02 | 0.04 | 0.05 | 0.52 | 0.39 | 0.13$^{*}$ | 0.78$^{*}$ | 22808.20$^{**}$ |
| GCA | 4 | 35.31$^{**}$ | 7751.60$^{**}$ | 0.04$^{*}$ | 1.44$^{**}$ | 1.45$^{**}$ | 75.31$^{**}$ | 26.80$^{**}$ | 2.27$^{**}$ | 10.99$^{**}$ | 15523.87$^{**}$ |
| SCA | 10 | 25.34$^{**}$ | 1863.48$^{**}$ | 0.16$^{**}$ | 5.54$^{**}$ | 2.61$^{**}$ | 80.22$^{**}$ | 114.82$^{**}$ | 4.34$^{**}$ | 9.33$^{**}$ | 20795.41$^{**}$ |
| GCA/Y | 4 | 0.001 | 0.012 | 0.001 | 0.001 | 0.010 | 0.11 | 0.032 | 0.004 | 0.212 | 4014.11$^{**}$ |
| SCA/Y | 10 | 0.017 | 0.010 | 0.001 | 0.003 | 0.010 | 0.19 | 0.160 | 0.020 | 0.274$^{*}$ | 9038.17$^{**}$ |
| Error | 56 | 1.65 | 13.53 | 0.014 | 0.07 | 0.057 | 2.84 | 0.180 | 0.02 | 0.13 | 44.65 |
| Baker ratio |  | 0.73 | 0.89 | 0.33 | 0.34 | 0.53 | 0.65 | 0.32 | 0.51 | 0.70 | 0.60 |
| GCA/SCA |  | 1.37 | 4.16 | 0.25 | 0.26 | 0.55 | 0.94 | 0.23 | 0.53 | 1.18 | 0.75 |

*, ** Significant at 0.05 and 0.01 probability levels, respectively

FL= Days to flowering, SL= Stalk length, SD = Stalk diameter, TSS% = Total soluble solids percent, SU% = Sucrose percent, PUR% = Purity percent, JEP % = Juice extraction percent, JY = Juice yield (ton/fed), SSY = Stripped stalk yield and EY = Ethanol Yield (l/fed)

**Table 2.** Combined mean performances for sorghum studied grain yield of the crosses over two seasons

| Crosses | FL | SL | SD | TSS% | SU% | PUR% | JEP% | JY | SSY | EY |
|---|---|---|---|---|---|---|---|---|---|---|
| P1xP1 | 62.80 | 158.73 | 1.98 | 20.35 | 9.37 | 46.04 | 39.81 | 6.24 | 15.98 | 513.53 |
| P1xP2 | 66.30 | 177.41 | 1.98 | 19.06 | 6.37 | 33.46 | 34.50 | 4.27 | 11.87 | 365.78 |
| P1xP3 | 69.30 | 145.11 | 2.03 | 19.57 | 6.70 | 34.27 | 22.46 | 2.46 | 10.77 | 240.32 |
| P1xP4 | 73.30 | 164.06 | 2.15 | 17.79 | 6.84 | 38.48 | 24.98 | 3.00 | 11.98 | 330.18 |
| P1xP5 | 73.63 | 248.48 | 1.60 | 17.96 | 7.42 | 41.36 | 29.71 | 4.08 | 14.04 | 390.06 |
| P2xP2 | 75.80 | 230.44 | 2.28 | 19.96 | 8.77 | 44.05 | 27.77 | 4.15 | 14.76 | 293.42 |
| P2xP3 | 73.30 | 165.43 | 1.75 | 16.76 | 6.99 | 41.79 | 40.10 | 5.44 | 13.05 | 490.14 |
| P2xP4 | 73.97 | 150.73 | 2.01 | 19.33 | 7.43 | 38.46 | 32.97 | 4.96 | 15.70 | 480.71 |
| P2xP5 | 71.30 | 244.06 | 1.62 | 16.00 | 7.98 | 49.97 | 24.31 | 3.63 | 15.25 | 374.65 |
| P3xP3 | 66.14 | 164.46 | 1.68 | 21.00 | 8.77 | 41.77 | 30.29 | 5.57 | 18.37 | 386.55 |
| P3xP4 | 70.34 | 125.73 | 2.06 | 16.92 | 9.25 | 54.70 | 26.88 | 3.83 | 14.03 | 290.20 |
| P3xP5 | 71.34 | 143.69 | 1.79 | 17.29 | 8.12 | 46.97 | 35.63 | 5.44 | 15.38 | 408.79 |
| P4xP4 | 64.80 | 168.01 | 1.41 | 18.31 | 9.83 | 53.78 | 35.02 | 5.66 | 16.81 | 414.39 |
| P4xP5 | 72.34 | 146.36 | 2.20 | 20.12 | 8.32 | 41.37 | 25.53 | 4.28 | 17.18 | 372.44 |
| P5xP5 | 70.47 | 248.75 | 2.08 | 18.86 | 8.75 | 46.39 | 45.41 | 7.89 | 17.36 | 624.96 |
| LSD 0.05 | | | | | | | | | | |
| Year | 2.33 | NS | 0.12 | 0.46 | 0.89 | NS | 2.64 | 0.40 | 0.37 | 11.79 |
| Genotype | 2.58 | 7.37 | 0.24 | 0.53 | 0.48 | 3.37 | 0.86 | 0.27 | 0.74 | 13.39 |
| Y*Gen | NS | NS | NS | NS | NS | NS | NS | 0.38 | 1.05 | 18.93 |

*, ** Significant at 0.05 and 0.01 probability levels, respectively

FL= Days to flowering, SL= Stalk length, SD = Stalk diameter, TSS% = Total soluble solids percent, SU% = Sucrose percent, PUR% = Purity percent, JEP % = Juice extraction percent, JY = Juice yield (ton/fed), SSY = Stripped stalk yield and EY = Ethanol Yield (l/fed)

days) to P2 (75.80 days). The earliest crosses were (P1xP2) and (P1xP3), which had 66.30 days and 69.30 days, while the latest cross was (P2xP4), recording 73.97 days. However, genotype P5 and cross (P1xP5) had the tallest stalk length (SL), recording (248.75 and 248.48 cm, respectively) while the cross (P3xP4) was the shortest (125.73 cm) stalk length. P2 genotype gave the highest stalk diameter (SD) with (2.28 cm) whereas P4 showed the lowest value (1.41 cm).

The genotype Ramada (P3) showed the highest values of total soluble solids percent (21%), but cross (P2xP5) revealed the lowest value for this trait (16%). Results also showed that parent Honey (P4) and cross P3xP4 possessed the highest percentage of sucrose (SU%) and purity percent (PUR %), scoring (9.83%) and (54.70%), respectively, whereas the lowest value (6.37%) and (33.46%) was scored for the cross (P1xP2). In the stripped stalk yield (SSY), the genotype P3 produced the best value (18.37), while; cross (P1xP3) revealed the lowest value (10.77).

The genotype Rio (P5) produced the profuse values of juice extraction percent (JEP%), juice yield (JY), and ethanol yield (EY), registering (45.41%, 7.89 ton/fed and 624.96 l/fed, respectively), whereas the cross P1xP3 was inferior in respect to the mentioned traits, recording 22.46%, 2.46 tons/fed, and 240.32 l/fed, respectively. These findings are in harmony with those reported previously by other workers (Zaghloul et al. 2022; Sally Chikuta et al. 2017; Al-Aaref et al. 2016; Abu-Ellail et al. 2023) who found significant differences among sorghum genotypes in respect of yield-related traits.

### *Correlation and bioethanol related traits*

The simple correlation coefficient among the bioethanol yield (EY) and other related traits over the two seasons is presented in Table 3 and Fig. 1. Results showed a highly significant positive correlation between ethanol yield and each of juice extraction percent (JEP%), juice yield (JY) and stripped stalk yield (SSY), recording coefficient values = 0. 87**, 0.88** and 0.51**, respectively. It is evident from the results that the selection for these traits would improve the bioethanol productivity of sorghum because of their positive and significant association with yield. The present findings are in concurrence with those reported earlier (Rani and Umakanth 2012; Kanbar et al. 2019; Naoura et al. 2019; Shukla et al. 2017 in sorghum.

The bioethanol related traits exhibited various trends of association among themselves. Stripped stalk yield was

**Table 3.** Correlation coefficients for bioethanol yield and related traits evaluated across two years (n=45)

| Traits | FL | SL | SD | TSS% | SU% | PUR% | JEP% | JY | SSY |
|---|---|---|---|---|---|---|---|---|---|
| SL | 0.25 | | | | | | | | |
| SD | 0.29 | -0.11 | | | | | | | |
| TSS% | -0.30* | -0.13 | 0.27 | | | | | | |
| SU% | -0.25 | -0.02 | -0.05 | 0.10 | | | | | |
| PUR% | -0.08 | 0.04 | -0.18 | -0.44** | 0.85** | | | | |
| JEP% | -0.26 | 0.13 | -0.11 | -0.04 | 0.22 | 0.15 | | | |
| JY | -0.30* | 0.17 | -0.15 | 0.19 | 0.49** | 0.32* | 0.89** | | |
| SSY | -0.20 | 0.13 | -0.17 | 0.31* | 0.66** | 0.43** | 0.33* | 0.72** | |
| EY | -0.16 | 0.26 | -0.15 | 0.03 | 0.19 | 0.13 | 0. 87** | 0.88** | 0.51** |

*, ** Significant at 0.05 and 0.01 probability levels, respectively

positively associated with the juice yield (0.72**), juice extraction percent (0.33*), purity percent (0.43**), sucrose percent (0.66**), and total soluble solids percent (0.31*). However, juice yield was positively correlated with juice extraction percent (0.89**), sucrose percent (0.49**), and purity percent (0.32*). However, total soluble solids percent had a significant and positive correlation value with sucrose percent (0.85**), while the significant and negative value was recorded with days to flowering (-0.30*). Also, the negative and significant association between total soluble solids percent (TSS %) and each purity percent (-0.44**) and days to flowering (-0.30*) was detected. On the other side, the observed insignificant associations for each of the stalk length (SD) and stalk diameter (SD) with bioethanol and other related traits indicated that these traits may be independent of genetic behavior among tested genotypes.

In this investigation, many sugar-related traits like juice extraction percent, juice yield, and stripped stalk yield were important in ethanol production and prediction of bioethanol yield. These results were in agreement with those reported by Kanbar et al. (2021); Rani and Umakanth (2012); Kanbar et al. (2019); Naoura et al. (2019); Shukla et al. (2017).

Graphically correlation interrelationships among the bioethanol-related traits across evaluated sorghum genotypes were illustrated by the principal component (PC) biplot graph in Fig 1. Both PC1 and PC2 loadings were plotted in the horizontal and vertical axes. The two traits of days to flowering and stalk diameter are located on the left side of the graph, indicating a negative correlation with the other studied traits (Table 3). However, the traits on the graph's right are more correlated. Especially juice extraction percent (JEP%), juice yield (JY), and stripped stalk yield (SSY) to ethanol yield (more correlated) followed by sucrose percent (SU%) and purity percent, indicating their importance in ethanol yield development.

### *Combining ability effects*

Results of both general (ĝi) and specific (ŝij) combining ability effects of five parental genotypes and their 15 crosses for 10 traits across two sites are given in Table 4. Positive values would be desirable and useful for all mentioned traits except days to flowering, where negative effects would be interest from the breeder. Estimates of general combining ability effects (ĝi) revealed the comparison between GCA effects associated with each parent. The genotype Brands (P1) showed a positive significant GCA effect only for total soluble solids percent (TSS%) but it had a desired negative significant GCA effect for days to 50% flowering. However, the Sugar Drib (P2) displayed a significant or highly significant desirable GCA effect for stalk length (SL) only, while it showed an undesirable GCA effect for other cases. The parent Ramada gave a negative but highly significant and undesirable GCA effect for all cases. Moreover, the parental genotype (P4) gave significant desirable GCA for sucrose percent (SU%), purity percent (PUR%), and stripped stalk yield (SSY). Also, the stalk diameter (SD) trait had a boor effect value under all parents. The parental genotype Honey proceeded as a good general combiner for stalk length (SL), for purity percent (PUR%), leaves juice extraction percent

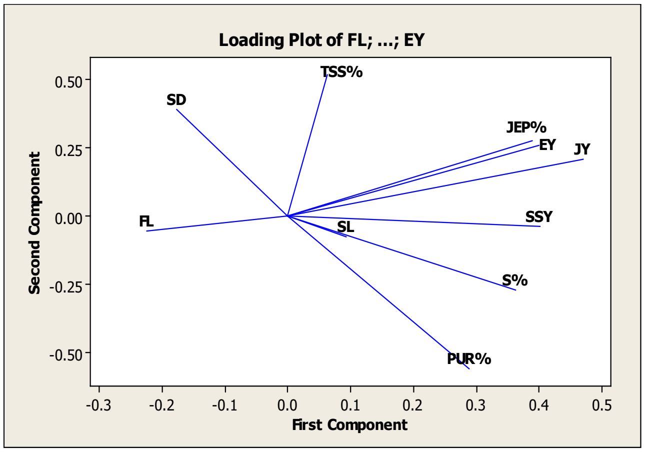

**Fig. 1.** Biplot graph, illustrating the correlation relationships among the bioethanol related traits evaluated across studied sorghum genotypes.

**Table 4.** Combined estimates of general and specific combining ability of the studied parents over two seasons

| Parent | FL | SL | SD | TSS% | SU% | PUR% | JEP% | JY | SSY | EY |
|---|---|---|---|---|---|---|---|---|---|---|
| P1 | $-0.99^{**}$ | $-1.43^{*}$ | ns | $0.24^{**}$ | $-0.16^{**}$ | $-1.53^{**}$ | ns | $-0.15^{**}$ | $-0.60^{**}$ | $-2.65^{*}$ |
| P2 | $1.03^{**}$ | $8.99^{**}$ | ns | ns | $-0.15^{**}$ | $-0.67^{*}$ | $-0.19^{*}$ | $-0.13^{**}$ | $-0.26^{**}$ | $-6.60^{**}$ |
| P3 | -0.39 | $-11.69^{**}$ | ns | ns | ns | ns | $-0.32^{**}$ | ns | ns | $-13.42^{**}$ |
| P4 | -0.18 | $-10.69^{**}$ | ns | ns | $0.22^{**}$ | $1.39^{**}$ | $-0.70^{**}$ | $-0.07^{*}$ | $0.25^{**}$ | $-6.29^{**}$ |
| P5 | $0.54^{*}$ | $14.82^{**}$ | ns | $-0.19^{**}$ | 0.07 | $0.81^{*}$ | $1.13^{**}$ | $0.35^{**}$ | $0.54^{**}$ | $28.96^{**}$ |
| LSD $g_{i\ 5\%}$ | 0.47 | 1.35 | 0.04 | 0.10 | 0.09 | 0.62 | 0.16 | 0.06 | 0.14 | 2.44 |
| LSD $g_i\text{-}g_{j\ 5\%}$ | 0.69 | 1.97 | 0.06 | 0.14 | 0.13 | 0.90 | 0.23 | 0.08 | 0.20 | 3.58 |
| Crosses | Specific combining ability (SCA) effects | | | | | | | | | |
| $P_1 \times P_2$ | $-2.06^{*}$ | $-8.24^{**}$ | ns | ns | $-0.53^{**}$ | $-2.83^{**}$ | $1.52^{**}$ | ns | $-0.62^{**}$ | ns |
| $P_1 \times P_3$ | ns | ns | ns | ns | $-0.53^{**}$ | $-3.10^{**}$ | $-4.37^{**}$ | $-0.98^{**}$ | $-1.50^{**}$ | $-62.98^{**}$ |
| $P_1 \times P_4$ | $2.65^{**}$ | $4.77^{*}$ | ns | $-0.59^{**}$ | $-0.67^{**}$ | $-2.37^{*}$ | $-2.74^{**}$ | $-0.65^{**}$ | $-1.08^{**}$ | $-25.17^{**}$ |
| $P_1 \times P_5$ | $2.10^{*}$ | $21.47^{*}$ | $-0.17^{*}$ | $-0.38^{*}$ | ns | ns | $-2.20^{**}$ | $-0.52^{**}$ | ns | $-30.49^{**}$ |
| $P_2 \times P_3$ | ns | ns | ns | $-0.94^{**}$ | $-0.40^{**}$ | ns | $4.72^{**}$ | $0.48^{**}$ | $-0.70^{**}$ | $65.88^{**}$ |
| $P_2 \times P_4$ | ns | $-12.32^{**}$ | ns | $0.47^{**}$ | $-0.39^{*}$ | $-3.25^{**}$ | $1.53^{**}$ | $0.31^{**}$ | ns | $54.04^{**}$ |
| $P_2 \times P_5$ | ns | $8.83^{**}$ | $-0.17^{*}$ | $-1.08^{**}$ | ns | $3.08^{**}$ | $-4.63^{**}$ | $-0.77^{**}$ | ns | $-34.24^{**}$ |
| $P_3 \times P_4$ | ns | ns | ns | $-0.84^{**}$ | $0.35^{*}$ | $4.19^{**}$ | $-1.39^{**}$ | $-0.37^{**}$ | $-0.72^{**}$ | $-34.39^{**}$ |
| $P_3 \times P_5$ | ns | $-20.66^{**}$ | ns | $-0.54^{**}$ | ns | ns | $1.16^{**}$ | ns | ns | $-10.35^{*}$ |
| $P_4 \times P_5$ | ns | $-20.33^{**}$ | $0.17^{*}$ | $1.00^{**}$ | ns | $-3.27^{**}$ | $-3.52^{**}$ | $-0.50^{**}$ | ns | $-35.65^{**}$ |
| LSD $S_{ij\ 5\%}$ | 1.59 | 4.55 | 0.15 | 0.33 | 0.30 | 2.08 | 0.53 | 0.19 | 0.46 | 8.26 |
| LSD $s_{ij}\text{-}s_{ik\ 5\%}$ | 2.39 | 6.82 | 0.22 | 0.49 | 0.44 | 3.12 | 0.80 | 0.28 | 0.69 | 12.39 |
| LSD $s_{ij}\text{-}s_{kl\ 5\%}$ | 0.98 | 2.79 | 0.09 | 0.20 | 0.18 | 1.28 | 0.32 | 0.11 | 0.28 | 5.06 |

*, ** Significant at 0.05 and 0.01 probability levels, respectively

FL= Days to flowering, SL= Stalk length, SD = Stalk diameter, TSS% = Total soluble solids percent, SU% = Sucrose percent, PUR% = Purity percent, JEP % = Juice extraction percent, JY = Juice yield (ton/fed), SSY = Stripped stalk yield and EY = Ethanol Yield (l/fed)

(JEP %), juice yield (JY), stripped stalk yield (SSY), and ethanol yield (EY). Therefore, honey could be considered the best combiner parent in breeding programs to improve these traits, most importantly, the bioethanol yield-related traits.

The results on specific combining ability (SCA) effects are presented in Table 4, revealing the parental combinations at the combined analysis. The desirable effect values were performed among the studied crosses. Only one $F_1$ cross, P1×P2, gave negatively significant desirable SCA effects to the flowering trait for days. Three crosses, namely, P1×P4, P1×P5, and P2×P5 gave significant or highly significant and positive values for the stalk length (SL) trait, while the total soluble solids percent (TSS%) trait in two crosses, P2× P4 and P3× P5 highly significant and positive effects were obtained. Similarly, the crosses, P2× P4 and P3× P4 showed significant and positive SCA effects for the sucrose percent (SU%) trait, whereas the crosses, P2× P5 and P3× P4 showed highly significant and positive values for the purity percent (PUR%) trait. Moreover, highly significant and positive values were detected among four crosses viz., P1×P2, P2×P3, P2×P4, and P3×P5 for the juice extraction percent (JEP %) trait. Also the crosses, P2× P3 and P2× P4 expressed highly significant and positive desirable effects for both juice yield (JY) and stripped stalk yield (SSY) traits. These findings indicate that SCA may influence SCA effects on bioethanol yield for their related traits and therefore these crosses may be important in sorghum breeding programs towards bioethanol yield production.

The results also revealed that GCA effects, for some traits, were related to several SCA values of their corresponding crosses. The two parents, honey (P4) and Rio (P5) displayed positive and significant GCA effects for most ethanol yield traits. Also, the crosses, P2× P3 and P2× P4 enjoyed the best and most desirable SCA effects for these traits. Thus the present findings may suggest that additive and non-additive genetic effects in the crosses may act in the same direction in maximizing the traits. These findings are in agreement with those of Zaghloul et al. (2022); Sally Chikuta et al. (2017); Al-Aaref et al. (2016) in sorghum.

**Table 5.** Significant mid-parent heterosis (%) for the studied bioethanol yield and related traits in sorghum

| Crosses | FL | SL | SD | TSS% | SU% | PUR% | JEP% | JY | SSY | EY |
|---|---|---|---|---|---|---|---|---|---|---|
| $P_1 \times P_2$ | -4.33$^{*}$ | ns | -7.04$^{**}$ | -5.45$^{**}$ | -29.76$^{**}$ | -25.73$^{**}$ | 2.13$^{**}$ | -17.73$^{**}$ | -22.75$^{**}$ | ns |
| $P_1 \times P_3$ | 7.49$^{**}$ | ns | 10.93$^{**}$ | -5.36$^{**}$ | -26.13$^{**}$ | -21.94$^{**}$ | -35.90$^{**}$ | -58.31$^{**}$ | -37.26$^{**}$ | -46.60$^{**}$ |
| $P_1 \times P_4$ | 14.88$^{**}$ | ns | 26.52$^{**}$ | -7.98$^{**}$ | -28.77$^{**}$ | -22.90$^{**}$ | -33.22$^{**}$ | -49.63$^{**}$ | -26.92$^{**}$ | -28.83$^{**}$ |
| $P_1 \times P_5$ | 10.50$^{**}$ | 21.96$^{**}$ | -21.18$^{**}$ | -8.41$^{**}$ | -18.10$^{**}$ | -10.50$^{**}$ | -30.26$^{**}$ | -42.27$^{**}$ | -15.75$^{**}$ | -31.48$^{**}$ |
| $P_2 \times P_3$ | ns | -16.22$^{**}$ | -11.78$^{**}$ | -18.18$^{**}$ | -20.26$^{**}$ | ns | 38.17$^{**}$ | 11.89$^{**}$ | -21.20$^{**}$ | 44.17$^{**}$ |
| $P_2 \times P_4$ | 5.21$^{**}$ | -24.34$^{**}$ | 9.03$^{**}$ | 1.01$^{**}$ | -20.13$^{**}$ | -21.37$^{**}$ | 5.04$^{**}$ | ns | ns | 35.83$^{**}$ |
| $P_2 \times P_5$ | ns | ns | -25.84$^{**}$ | -17.58$^{**}$ | -8.87$^{**}$ | 10.50$^{**}$ | -33.54$^{**}$ | -39.66$^{**}$ | -5.00$^{**}$ | ns |
| $P_3 \times P_4$ | 7.44$^{**}$ | -24.37$^{**}$ | 33.41$^{**}$ | -13.92$^{**}$ | ns | 14.50$^{**}$ | -17.66$^{**}$ | -31.77$^{**}$ | -20.20$^{**}$ | -27.53$^{**}$ |
| $P_3 \times P_5$ | 4.44$^{*}$ | -30.45$^{**}$ | -4.96$^{**}$ | -13.26$^{**}$ | -7.34$^{**}$ | 6.55$^{**}$ | -5.84$^{**}$ | -19.24$^{**}$ | -13.91$^{**}$ | -19.17$^{*}$ |
| $P_4 \times P_5$ | 6.95$^{**}$ | -29.76$^{**}$ | 26.15$^{**}$ | 8.25$^{**}$ | -10.42$^{**}$ | -17.41$^{**}$ | -36.50$^{**}$ | -36.91$^{**}$ | ns | -28.33$^{**}$ |

* & ** significant at the 0.05 & significant at the 0.01 probability levels, respectively and ns insignificant.

### *Combined heterosis*

Heterosis as relative to mid-parents ($\overline{\mathrm{MP}}$) for bioethanol yield and related traits is given in Table 5. Days to flowering recorded a significantly negative percentage relative to ($\overline{\mathrm{MP}}$) for P1×P2 with a value of -4.33. Heterosis values were highly significant and positive percentage in the cross, P1×P5 with (21.96**%) for stalk length. Five crosses, P1×P3, P1×P4, P2×P4, P3×P4, and P4×P5 scored 10.93, 26.52, 9.03, 33.41 and 26.15%, respectively heterosis for stalk diameter, while two crosses, P2×P4 and P4×P5 gave significantly positive heterotic effects (1.01 and 8.25%, respectively) for total soluble solids percent. However, purity percent revealed highly significant and positive heterosis in crosses, P2×P4, P3×P5, and P4×P5 (10.50, 14.50, and 6.55%, respectively). In juice extraction, three crosses, P1×P2, P2×P3, and P2×P4 gave positive heterosis in order of 2.13, 38.17, and 5.04%, respectively. Heterosis was significant and positive in P2×P3 (11.89%) for juice yield, whereas it was significant and positive for P2×P3 (44.17%) and P2×P4 (35.83%) crosses for ethanol yield.

The data presented in Table 5 suggest that heterotic effects for bioethanol yield were associated with some related- yield traits in several crosses, such as purity percent, juice extraction percent and juice yield. Several researchers reported positive significant heterotic percentages for sorghum traits which varied according to the cross combinations and traits (Pagliosa et al. 2017; Dehghani et al. 2013). Therefore, selection could be made to develop specific traits to indirectly improve crop productivity. Significant mid-parent heterosis was reported for bioethanol yield and its related traits (Khakwani et al. 2020; Solomon et al. 2012). The selection based on juice extraction percent and juice yield may improve grain production.

### *Predicting of GCA and SCA by GGE-biplot method*

GGE-biplot was developed to the identification of the GCA and SCA effects and the best crosses. The biplot shown in Fig. 2 illustrated diallel analysis for the bioethanol yield. Graph of the ethanol yield data explained 68.89% (35.98 and 33.00% for PC1 and PC2, respectively) of the total variation of data (whole performance variation of the parental and the cross combinations). These results are similar to others obtained by Yan and Hunt (2002); Malla et al. (2010); Boćanski et al. (2011).

The polygon biplot view in Fig. 2 illustrates the interaction between the genotype (parent) and the tester cross by joining the outermost genotypes, which appear as the vertices of the polygon. Scatter lines drawn from the polygon origin to the sides divided the graph into different sectors. Any cross (s) located in a given sector is considered a superior cross demonstrating heterosis from the interaction between the tester and the parent vertex entry of the cross. The parental P3, P4, and P5 testers were allocated to the negative side in the same sector. Meanwhile, the parental tester Rio (P5) and vertex entry of genotype (g5) exist together in the same sector, and then the parent

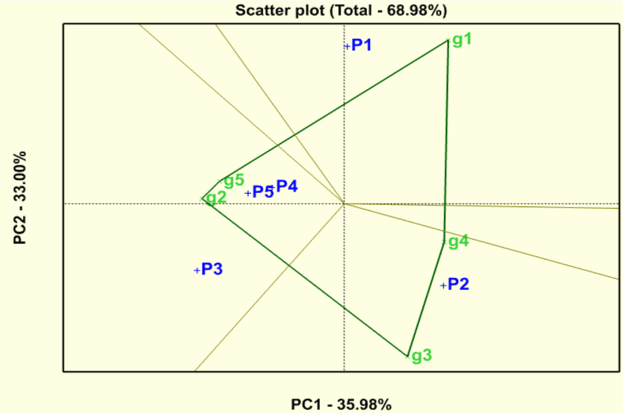

**Fig. 2.** GGE Biplot of five sorghum parents to show Diallel analysis: a) Best crosses for bioethanol yield (EY) and parental genotypes are labeled with ($P_i$) when viewed as tester entries and with ($g_i$) letters when viewed as parent/cross, and b) GCA and SCA for EY.

**Table 6.** Genetic parameters for the studied traits in sorghum combined data over two seasons

| Genetic parameters | FL | SL | SD | TSS% | SU% | PUR% | JEP% | JY | SSY | EY |
|---|---|---|---|---|---|---|---|---|---|---|
| E | 1.93 | 16.09 | 0.01 | 0.08 | 0.11 | 5.30 | 0.81 | 0.03 | 0.12 | 42.06 |
| D | 24.99$^{**}$ | 1764.14$^{**}$ | 0.11 | 1.13 | 0.13 | 15.94 | 50.14$^{**}$ | 1.94$^{**}$ | 1.77 | 10905.85$^{**}$ |
| F | 28.71 | -112.72 | 0.24 | 1.95 | 0.15 | 17.93 | 99.91$^{**}$ | 2.84$^{**}$ | 0.87 | 12768.49 |
| H1 | 45.50$^{**}$ | 3722.16$^{*}$ | 0.37 | 9.99$^{**}$ | 3.72$^{**}$ | 152.29$^{**}$ | 237.66$^{**}$ | 8.38$^{**}$ | 12.95$^{**}$ | 27455.72$^{**}$ |
| H2 | 36.22$^{**}$ | 3007.89$^{*}$ | 0.23 | 8.72$^{**}$ | 3.15$^{**}$ | 122.99$^{**}$ | 181.81$^{**}$ | 6.86$^{**}$ | 9.89$^{**}$ | 22438.68$^{**}$ |
| (H1/D)$^{\wedge 0.5}$ | 1.35 | 1.45 | 1.83 | 2.97 | 5.35 | 3.09 | 2.18 | 2.08 | 2.70 | 1.59 |
| H2/4H1 | 0.20 | 0.20 | 0.16 | 0.22 | 0.21 | 0.20 | 0.19 | 0.20 | 0.19 | 0.20 |
| KD/KR | 2.48 | 0.96 | 3.94 | 1.82 | 1.24 | 1.44 | 2.69 | 2.09 | 1.20 | 2.17 |
| Nh$^2$% | 20.00 | 63.00 | 7.00 | 9.00 | 23.00 | 27.00 | 6.00 | 15.00 | 43.00 | 22.00 |
| N-CD | 0.72 | 0.89 | 0.31 | 0.34 | 0.52 | 0.64 | 0.32 | 0.51 | 0.70 | 0.60 |
| Bh$^2$% | 86.00 | 98.90 | 86.00 | 97.00 | 91.00 | 89.00 | 98.00 | 99.00 | 97.00 | 99.20 |
| B-CD | 0.98 | 0.99 | 0.92 | 0.99 | 0.98 | 0.98 | 0.99 | 0.99 | 0.99 | 0.99 |

*and **: Significant at 0.05 and 0.01 level of probability, respectively.

must be superior to any hybrid formed with the vertex entry (Malla et al. 2010). Therefore, Rio (P5) was a good combiner (GCA effect= 28.96**) and all hybrids containing Rio (P5) recorded superior or significant crosses as presented in Table 4. However, on the positive side, the vertex entry of genotype (g4 and g3) allocated with other parental Sugar drib (P2) testers in the same sector, suggesting that it could be concluded the best crosses, P2× P3 and P2× P4 recording SCA effect (65.88** and 54.04**, respectively) for EY (Table 4). Therefore, based on the SCA values, the highest heterotic estimates (44.17** for P2× P3 cross) were obtained in P2 was placed in the utmost on the positive right side, but P3, which was plotted in the utmost on the negative left side recorded the highest negative heterosis (-46.60**) for P1× P3 cross.

### *Genetic parameters*

Separation of the total genetic variance to its components, additive, and dominant gene effects for the examined traits was shown in Table 6. Estimates of the additive (D) component of genetic variability were highly significant for FL: days to flowering, SL: Stalk length, JEP%: Juice extraction percent, JY: Juice yield, and EY: Ethanol Yield, suggesting the importance of additive variance for these traits and selection for them in segregating generations would be effective. Dominance components of variation ($H_1$ and $H_2$) differed from zero recording significant and highly significant in all traits except for SD: Stalk diameter. Also, $H_1$ was greater than $H_2$, indicating that positive and negative alleles at loci for these traits were not proportionally equal for parents. This result was reflected in (F) estimates of covariance of additive and dominance effects in JEP%: Juice extraction percent and JY: Juice yield traits. F recorded highly significant positive values, suggesting the excess of dominant alleles but SL: Stalk length had insignificant negative value and recessive alleles. Generally, both additive and non-additive (D and H) components scored important gene effects. Meanwhile, D values were lower than $H_1$, suggesting that additive genetic variance is less important than dominance (non-additive) variance.

The greater $H_1$ than $H_2$ indicated that the positive and negative allele frequencies at the loci for these traits were not equal in proportion in parents. These results were further established by the $H_2/4H_1$ ratio that recorded less than 0.25, confirming that positive and negative alleles were not equally distributed among the parents for all traits. The average degree of dominance $(H_1/D)1/2$ was more than one in all studied traits, indicating over-dominance. The ratio of the total number of dominant to recessive genes KD/KR was more than one in all studied traits except for SL: Stalk length, indicating the presence of more dominant genes in the parents than recessive ones. These results confirm that both additive and non-additive gene effects were the source of genetic variation for all the studied traits, but non-additive had the most effect and that selection applied in the late segregating generations could be effective. Similar results were obtained by Ocan et al. (2021); Abu-Ellail et al. (2023) who found that additive gene action was predominant.

Both heritability in broad (Bh$^2$) sense and narrow (Nh$^2$) sense values, with their broad and narrow sense coefficient of genetic determination (B-CD and N-CD) are presented in Table 6. Data revealed that broad sense heritability values were higher than narrow sense for all the studied traits. Heritability in the broad sense ($H_b{}^2$%) estimates were generally high for all the observed traits and recorded values ranging from (86.00%) for FL and SD to (99.20%) for ethanol yield. However, Considering B-CD, all the ethanol yield-traits recorded very high values ranging from 0.92 to 0.99. These results indicated that the proportion of total variance attributed to genetic variance (additive and non-additive) across the studied traits; also the environmental effect was low.

On the other hand, the narrow-sense heritability depending on the relative magnitude of the additive component to the total genetic variance was recorded and estimated to fall between 6% for juice extraction percent (63.00%) for stalk length. Whenever, N-CD estimates ranged between 0.31 for stalk diameter to 0.89 for stalk length. Also, the relatively high N-CD estimates indicated that the large proportion of genetic variation is due to the additive gene effect in these traits (stalk length and stripped stalk yield). Meanwhile, the relatively lower N-CD estimates suggested that these traits (juice extraction percent and stalk diameter) were controlled mostly with non-additive gene effects and more influenced by the environment. Accordingly, several researchers have previously reported different genetic parameters in various crops (Zaghloul et al. 2022; Oca et al. 2021; Babu et al. 2012; Abu-Ellail et al. 2023).

## Authors' contribution

Conceptualization of research (FFBA, ZEG); Designing of the experiments (FFBA, ZEG); Contribution of experimental materials (FFBA, AEA); Execution of field/lab experiments and data collection (FFBA); Analysis of data and interpretation (FFBA, AEA); Preparation of the manuscript (FFBA, ZEG, AEA)

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