

Evaluation of Heterosis in Biomass Related Traits in Sorghum [*Sorghum bicolor* **(L.) Moench] F1 Reciprocal Hybrids**

Birgül GÜDEN* , Bülent UZUN

Akdeniz University, Faculty of Agriculture, Department of Field Crops, Antalya, TÜRKİYE

Abstract: The global focus on enhancing sorghum [*Sorghum bicolor* (L.) Moench] for biomass-related traits is increasing due to its potential contribution to the growth and sustainability of the ethanol and biogas production chain. Heterosis has been widely used in sorghum breeding, especially in improving biomass yield using efficient crossing and selection methods. The objective of this study was to assess the heterosis potential of elite sorghum accessions. Ten hybrids were established using five reciprocal crosses of seven elite breeding accessions. The hybrids and the parental lines were significant of great variation for plant height (PH), panicle length (PL), number of leaves (NL), and stem diameter (SD). Most hybrids had high positive mid-parent heterosis for biomass-related traits, while better parental heterosis ranged from -7.90 to 31.16 for PH, 17.14 to 79.59 for PL, -39.68 to 13.20 NL, and -19.19 to 104.23% for SD. Four hybrids (P6×P4, P4×P6, P6×P5, and P5×P6) exhibited plant heights greater than the best parent (P5:322.33 cm). Reciprocal cross effects had a significant impact on PH and SD, with a wide range of -10.23 to 39.35% and -37.50 to 30.55%, respectively. The results indicated that heterosis could be come true for the characters of plant height, panicle length, and number of leaves, and stem diameter that contributes great impact on having high biomass.

Keywords: *Sorghum bicolor*, number of leaves, panicle length, plant height, stem diameter

1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cultivated crop that exhibits a broad tolerance to various agroecological environments. It has been classified as the fifth most significant food crop, behind wheat, rice, maize, and barley, and is extensively cultivated in semi-arid regions. Sorghum has many advantages as a potential bioenergy source, including its high biomass productivity and short growth cycle (Bollam et al., 2021). The high biomass of sorghum produces a significant amount of plant material, which provides an abundant source of feedstock for the generation of biofuels (Yang et al., 2021). For instance, the leaves and stems of the plant contribute to the synthesis of cellulose, which is a crucial element in the production of biofuel and bioethanol (Yang et al., 2021). Therefore, the biomass and related traits of sorghum have considerable potential for energy production.

Plant height, number of leaves, and stem diameter are the main contributing traits affecting biomass production in sorghum. Plant height and stem diameter greatly influence the biomass yield of sorghum, directly enhancing overall biomass production (Madhusudhana and Patil, 2013; Göler and Özyazıcı, 2024). Moreover, sorghum biomass refers to the vegetative parts of the plant, including the leaves. The plants with a greater number of leaves exhibit higher photosynthetic activities, which may result in increased biomass production (Jain et al., 2010). Along with high biomass production, increasing grain yield is another focus of many sorghum breeding studies (Von Pinho et al., 2022). Moreover, panicle length has the strongest relationship with grain yield in sorghum as well as addition to total biomass (Goma et al., 2021).

Heterosis, or hybrid vigor, is the occurrence in which progeny have enhanced characteristics compared to one or both parents. In crop breeding, this often describes the higher yield, better resistance to pests and diseases, and improved tolerance to abiotic stresses of F1 hybrids compared to both inbred parents (Paril et al., 2023). Moreover, heterosis among genetically different types of sorghum, like other plants, helps find favorable combinations for important agricultural traits (Mohammed et al., 2015). For many years, several crops have been used for heterosis as a technique to enhance biomass production. In sorghum, Blum et al. (1990) grew two grain sorghum hybrids and their parental lines in the greenhouse with varying temperatures under two water levels and identified significant heterosis for biomass. Packer and Rooney (2014) assessed the biomass hybrids resulting from 13 different biomass pollinators and 4 grain sorghum seed parents in 4 various conditions to quantify the possible heterosis in biomass production, and they identified high-parent heterosis with a mean of 24.8% across all the environments. Duraes et al. (2021) and Mangena et al. (2022) also reported high heterosis for biomass and related traits.

Reciprocal crosses are considered another source of genetic variability for heterosis (Seitz et al., 1995). Reciprocal effects arise from the genetic influences of both parents, including maternal and paternal effects, cytoplasmic effects, and parent-oforigin effects (Gonzalo et al., 2007). This provides information about which of the two parents should be used as a mother or father. Recently, only a limited number of researchers have reported differences in reciprocal effects in sorghum; for shoot fly resistance (Mohammed et al., 2016), days to 50% flowering, plant height, and 100-seed weight (Mohammed et al., 2015).

Research on the heterosis of sorghum biomass is very valuable for creating effective breeding strategies to produce sorghum hybrids as a bioenergy source. The objectives of this study were to evaluate the heterosis performance of sorghum hybrids in reciprocal crosses for biomass-related traits.

2. Materials and Methods

2.1. Plant material

Seven sorghum accessions, namely BSS 47, BSS 85, BSS 332, BSS 424 and BSS 532 and two cultivars, namely Öğretmenoğlu (grain sorghum) and Erdurmuş (sweet sorghum) were used as parents in the reciprocal crossing for F_1 carried out in the study (Table 1). BSS 47, BSS 85, BSS 332, BSS 424, BSS 532, Öğretmenoğlu and Erdurmuş were coded as P1, P2, P3, P4, P5, P6 and P7 throughout the study, relatively. Parents were selected based on evaluations from two locations for their high plant height (Guden et al., 2020).

2.2. Crossing

All the parents were grown in the greenhouse of Akdeniz University, Antalya, Türkiye in 2017. At the time of the flowering period, when the glumes open just before or just after sunrise, three anthers were emasculated from each female. Pollens from the fully bloomed flower of the male parent were harvested, and these were then used to pollinate the emasculated female and finally each one was bagged with kraft paper. The seeds from each of the crosses were collected after 15-20 days. According to mating design, seven parental lines were crossed to produce five F_1 hybrid seeds and their reciprocal seeds.

2.3. Experimental design and observations

All 17 sorghum materials, including 7 parents and 10 hybrids, were planted with a randomized complete blocks design of three replications in the experimental field of Akdeniz University, Antalya, Türkiye in 2018. For each line of each replication, plants were grown in a row of 3.0 m, at a spacing of 0.7 m between rows and a plant spacing of 0.2 m. For each line, nine randomly selected plants were harvested manually, and plant height (PH), panicle length (PL), number of leaves (NL) and stem diameter (SD) were used to characterize plants. Plant height was measured as the distance from the ground to the top of the flag leaf once the panicle had fully emerged (cm). Panicle length was

Accession name Accession ID Origin Gene banks BSS 47 PI175919 Türkiye USDA BSS 85 PI641835 United States of America USDA BSS 332 IS3121 United States of America ICRISAT BSS 424 IS 20697 United States of America ICRISAT BSS 532 IS 30466 China China ICRISAT Erdurmuş Released variety Türkiye * Öğretmenoğlu Released variety Türkiye *

Table 1. The origin and accession ID of sorghum plant material

*: The Western Mediterranean Agricultural Research Institute of Türkiye (WMARI) has registered as cultivars, USDA: United States Department of Agriculture, ICRISAT: The International Crops Research Institute for the Semi-Arid Tropics

manually measured as the distance from the beginning to the top of the panicle by ruler (cm). Number of leaves was counted along the main stem. Stem diameter was measured at the third node from the ground of the main with a digital caliper and Stainless Electronic Ruler Micrometer (Clockwise Tools DCLR-0605 Electronic Digital Caliper) (cm).

Mid-parent heterosis, better-parent heterosis and reciprocal crossings advantages or effects were calculated using the equations.

Reciprocal crossing advantages or effects (R) were identified by Equation 1 (Bulant et al., 2000).

$$
R = \frac{RC - NC}{NC} \times 100\tag{1}
$$

In equation, R represents the advantage percentage in reciprocal crossings, RC represents the mean of reciprocal crosses, and NC represents the mean of normal crosses.

Mid-parent heterosis (MPH) was identified by Equation 2 and 3.

$$
MPH = \frac{F_1 - MP}{MP} \times 100
$$
 (2)

$$
MP = \frac{P_1 + P_2}{2} \times 100
$$
 (3)

Where F_1 is the mean of the F1 hybrid performance; P1 and P2 are the means of the two parents.

Better parent heterosis (BPH) was identified by Equation 4.

$$
BPH = \frac{F_1 - BP}{BP} \times 100\tag{4}
$$

Where BP is the mean of the best parent.

2.4. Statistical analysis

Correlation analyses and analyses of variance (ANOVA, PROC GLM) were performed with SAS 9.0 software.

3. Results

The ANOVA results showed that F_1s and parents were of significant differences for all the traits studied except NL for F_1s (Table 2). Table 3 presents the correlation analysis of the traits for F1s and their parents. Correlation analysis identified that PL was significantly and positively correlated with SD and PH, with correlation coefficients of 0.37 and 0.62, respectively.

3.1. Hybrids performance

It was evaluated PH, PL, NL, and SD characters for ten F_1 s and their parents, and Table 4 reports the mean performance of all F₁s and their parents.

P6×P5 recorded the highest plant height at 370.00 cm, while P3×P7 recorded the lowest at 221.67 cm, with a mean of 296.83 cm for the F_1 s. Among the parents, PH ranged from 137.33 (P2) to 322.33 (P5), with a general mean of 276.70 cm. The hybrids P4×P6 and P5×P6 had the highest PL (37.66 cm), while $P1\times P6$ and $P3\times P2$ had the lowest PL (19.33 cm). P5 recorded the maximum PL of 28.00 cm, while the mean PL for the parents was 20.52 cm. The mean NL for F_1s and parents were 13.80 and 2.89, respectively. The maximum and minimum NL were identified in P2×P3 (17.66) and $P1\times P6$ (11.00), respectively, for F₁s. The highest SD of the F1s was 4.33 cm (P6×P4), and the mean of the F1s was 3.41 cm. Significant variability was observed in SD for the parents, ranging from 1.20 to 3.86 cm with the general mean as 3.20 cm (Table 4).

Table 2. Analysis of variance (*F* value) for some morphologic traits in F_1s , parents, as well as F_1s and parents

Source of variation	PH	PL	NL	SD.	
F_{1S}	$5.84***$	$10.39**$	1.80	$6.81***$	
Errors	0.0005	0.0001	0.1310	0.0002	
Parents	27.68**	$32.23***$	$6.00**$	$32.69**$	
Errors	0.0001	0.0001	0.0028	0.0001	
$F1s$ and parents	$11.78***$	$19.02**$	$3.14***$	$14.08***$	
Errors	0.0001	0.0001	0.0025	0.0001	
alanda a control \cdot \sim					

**: Significant at p≤ 0.01

Table 3. Pearson correlation coefficients between traits in F1s and parents

Traits	SD	PН			
PН	0.21				
PL.	$0.37***$	$0.62***$			
NL	-0.07	0.22	0.09		
**: Significant at $p \le 0.01$					

3.2. Heterosis estimation

The estimation of mid-parent heterosis and better-parent heterosis was conducted by evaluating the hybrids and parents in adjacent experiments. Sorghum hybrids exhibited significant heterosis in several aspects, with the degree of improvement varying considerably between the mid-parent and better-parent.

Significant differences were detected in midparent heterosis for all traits except NL and were presented in Table 5. All hybrids had positive midparent heterosis for all the traits, with the exception of hybrids P4×P6 for NL, P6×P5 for NL and SD, and P5×P6 for SD. The highest mid-parent heterosis values for PH, PL, NL, and SD were 98.85, 109.52, 44.68, and 152.63%, respectively. Hybrids P6×P1 and P1×P6 had positive mid-parent heterosis for all four traits, all above 40% (Table 5).

	Sorghum	PH (cm)	PL (cm)	NL	SD (cm)
	P ₁	291.00	23.33	15.66	3.43
	P ₂	137.33	13.66	14.66	1.20
	P ₃	240.67	16.33	15.66	1.96
	P ₄	314.33	27.66	15.00	3.23
Parents	P ₅	322.33	28.00	21.00	3.30
	P ₆	284.00	23.00	12.66	3.26
	P7	146.00	11.66	10.00	3.86
	Means	247.95	20.52	14.95	2.89
	LSD	$44.52***$	$3.54***$	$4.15***$	$0.50**$
	$P3 \times P2$	315.67	19.33	16.33	2.50
	$P2 \times P3$	227.33	29.33	17.66	4.00
	$P3 \times P7$	221.67	29.33	13.33	3.66
	$P7 \times P3$	247.67	27.33	14.00	4.00
Hybrids	$P6 \times P4$	366.00	35.33	15.66	4.33
	$P4 \times P6$	327.33	37.66	13.00	3.33
	$P6 \times P1$	273.33	19.33	11.33	3.00
	$P1 \times P6$	289.33	23.33	11.00	3.66
	$P6 \times P5$	370.00	29.66	13.00	3.00
	$P5 \times P6$	330.00	37.66	12.66	2.66
	Means	296.83	28.83	13.80	3.41
	LSD	65.65**	$6.19**$	4.72	$0.69**$
General means		276.70	25.41	14.27	3.20

Table 4. Mean performance of some morphologic traits for F₁ sorghum hybrids and their parents

P1: BSS 47, P2: BSS 85, P3: BSS 332, P4: BSS 424, P5: BSS 532, P6: Erdurmuş, P7: Öğretmenoğlu

**: Significant at p≤ 0.01, P1: BSS 47, P2: BSS 85, P3: BSS 332, P4: BSS 424, P5: BSS 532, P6: Erdurmuş, P7: Ögretmenoğlu

The average better parental heterosis was predominantly positive for PH, PL, and SD, while for NL was negative. Significant differences were detected in better parental heterosis values for all the traits (Table 6). Better parental heterosis for plant height ranged between -7.90 and 31.16%, with an average of 5.17%. The highest rate was identified in P3×P2 with 31.16%. The better parent heterosis of PL was the strongest. Out of the nine hybrids showing positive better-parent heterosis, seven (P3×P2, P2×P3, P3×P7, P7×P3, P6×P4, P4×P6 and P5×P6) had values over 10%. The highest rates were observed in P2×P3 and P3×P7, with 79.59% for PL. For NL, better parent heterosis ranged between -39.68 and 13.20%. The variation in SD was the largest, ranging from −19.19 to 104.23%. Four F1s were identified showing positive better

parental heterosis in SD, all above 10%, with the highest being 104.23% in P2×P3 (Table 6).

3.3. Evaluation of reciprocal effect

Significant differences were identified in the reciprocal effect for PH and SD, whereas they were not significant for PL and NL (Table 7). It was identified the mean relative reciprocal cross as negative for PL (-12.98%) and SD (-3.72%) and positive for PH (10.23%) and LN (6.00%). The maximum reciprocal cross advantages for PH were P3×P2 (39.35%), followed by P6×P5 (14.32%) and P6×P4 (12.32%), with an average of 10.23%. The reciprocal effect on SD ranged from -37.50 to 30.5%, with a mean of -3.72%. The maximum reciprocal effect for SD was identified as 30.55% in $P6\times P4$ (Table 7).

Hybrids	Traits				
	PH	PL	NL	SD	
$P3 \times P2$	31.16	18.37	6.62	28.04	
$P2 \times P3$	-5.54	79.59	13.20	104.23	
$P3 \times P7$	-7.90	79.59	-14.90	4.76	
$P7 \times P3$	2.91	67.35	-10.64	14.28	
$P6 \times P4$	16.44	27.71	4.44	34.02	
$P4 \times P6$	4.14	36.15	-11.48	3.09	
$P6 \times P1$	-6.07	-17.14	-27.66	-12.62	
$P1 \times P6$	-0.57	0.00	-29.79	6.79	
$P6 \times P5$	14.79	5.95	-38.10	-9.09	
$P5 \times P6$	2.38	34.52	-39.68	-19.19	
Means	$5.17*$	$33.20***$	$-14.80*$	$15.43***$	
LSD	21.02	26.20	33.23	29.31	

Table 6. Better parental heterosis for some morphologic traits of F_1 sorghum hybrids (%)

*: Significant at p≤ 0.05, **: Significant at p≤ 0.01, P1: BSS 47, P2: BSS 85, P3: BSS 332, P4: BSS 424, P5: BSS 532, P6: Erdurmuş, P7: Öğretmenoğlu

Table 7. The reciprocal effect on some morphologic traits for F_1 sorghum hybrids (%)

	Traits			
Reciprocal crosses	PH	PL	LN	SD
$P3 \times P2$	39.35	-33.45	-5.55	-37.50
$P3 \times P7$	-10.23	7.98	-1.78	-8.33
$P6 \times P4$	12.32	-3.01	29.16	30.55
$P6 \times P1$	-5.18	-15.67	4.87	-16.66
$P6 \times P5$	14.92	-20.73	3.33	13.33
Normal cross means	284.33	31.07	13.67	3.53
Reciprocal cross means	309.33	26.60	13.93	3.30
Reciprocal effect means (%)	$10.23***$	-12.98	6.00	$-3.72**$
LSD	22.44	30.74	62.51	21.97

**: Significant at p≤ 0.01, P1: BSS 47, P2: BSS 85, P3: BSS 332, P4: BSS 424, P5: BSS 532, P6: Erdurmuş, P7: Öğretmenoğlu

4. Discussion and Conclusion

Biomass production is a complex trait that derives from several agro-industrial characteristics (Chen et al., 2020). Direct or indirect methods can achieve sorghum biomass production, aiming to enhance several related characteristics (Habyarimana et al., 2020). Plant height, stem diameter, and number of leaves are some of the important biomass-related traits in sorghum. Moreover, panicle length is one of the important characteristics of seed yield (Von Pinho et al., 2022) and also contribute to total biomass. In this study, highly significant differences for biomass-related traits indicated the presence of considerable variation in the parents (Table 2). The F-values for all the characteristics analyzed suggested that the selected parents were appropriate for generating reciprocal crosses that were in parallel with the previous studies (Mohammed et al., 2015; Zhang et al., 2024).

The sorghum plant height, stem diameter, and number of leaves can range from 60.00 to 450.00 cm (Quinby and Karper, 1954), 1.63 to 2.30 cm (Lestari et al., 2021), and 4 to 24 (Derese et al., 2018), respectively. In this study, PH, NL, and SD varied from 221.67 to 370.00 cm, 11.00 to 17.66 cm, and 2.50 to 4.33 cm, respectively (Table 4). Especially four hybrids (P6×P4, P4×P6, P6×P5, and P5×P6) exhibited plant heights greater than the best parent (P5). These results suggest that it will aid in the indirect research strategy to develop hybrids with high biomass yields due to a strong correlation with plant height.

Understanding the relationship between heterosis and the phenotypic expression of biomass yield traits may assist in defining breeding strategies. A reasonable level of heterosis is crucial for achieving high biomass production and related traits in any hybrid breeding effort (Zhang et al., 2024). In this study, for all traits, the majority of the best-performing genotypes in terms of mid-parent heterosis were hybrids that showed positive heterosis, with a few exceptions (P4×P6 for NL, P6×P5 for NL and SD, and P5×P6 for SD) (Table 5). The same observation was also made by Mangena et al. (2022), who reported that most of the hybrids showed positive mid-parent heterosis for biomass and biomass-related traits. This occurrence may be attributed to heterosis, which is based on the idea that sorghum hybrids are generally superior to the majority of parental genotypes. On the other hand, Six, nine, three and seven hybrids had positive better-parent heterosis for PH, PL, NL and SD, respectively (Table 4). The

study findings indicated that some hybrids exhibited the mid-parent value, while others tended to have the better-parent value.

The level of heterosis varies by the genotypes being used, and the character studied. Duraes et al. (2021) identified heterosis of 21.4 and 4.7% for plant height and green biomass, respectively. Blum et al. (1990) also found significant heterosis in terms of biomass, grain yield per plant, and grain number per panicle. Zhang et al. (2024) reported mid-parent heterosis of 40.26 and 13.78% for plant height and panicle length, respectively. According to Wang et al. (2020), the effects of mid-parent and better parent heterosis on grain yield, grain weight per ear, and plant height were highest compared to other characteristics in sweet sorghum. In the present study, the average mid-parent and betterparent heterosis were the highest in PL, at 66.07% and 33.20%, respectively (Tables 5 and 6). The second trait was SD, and the average mid-parent and better parent heterosis were 47.33% and 15.43%, respectively. The same observation was also made by Mangena et al. (2022), who reported that of the hybrids tested, one of the highest values for better parent heterosis was in SD. Contrary to the study of Zhang et al. (2024), the average midparent and better parent heterosis for PH were relatively lower than others at 38.36% and 5.17%, respectively. These differences support the idea that the inheritance of the studied traits in sorghum is complex. They can also be explained by different levels of genetic variability in the parents used.

Reciprocal crosses provide valuable insights for breeders to comprehend the presence of variation caused by maternal effects and outbreeding depression (Sprague and Tatum, 1942; Griffing, 1956). If the cytoplasmic genomes of the two parents exhibit differences, the resulting reciprocal hybrids may display variations in their phenotypes (Gai and He, 2013). In this study, the effect of reciprocal crosses was defined as significantly important for PH and SD (Table 7), indicating that cytoplasmic variables may have a role in the inheritance of these traits. These findings are consistent with those of Mohammed et al. (2016), indicating that significant reciprocal effects for shoot fly resistance using reciprocal crosses in sorghum, are attributed to the influence of cytoplasmic factors. This effect may also be due to the genetic influences of the parents, which include both maternal and paternal effects, as well as cytoplasmic effects (Gonzalo et al., 2007). Zhang et al. (2016) identified 85 maternally expressed genes and 16 paternally expressed genes in reciprocal F1 hybrids. In this study, although the relative reciprocal cross advantages were negatively low on PL (-12.98%) and SD (-3.72%), and positively low

on PH (10.23%) and LN (6.00%) , the range was quite wide. For instance, Table 6 shows a wide range of variation in plant height for reciprocal cross advantages, from -10.23 to 39.35%, with the maximum value being significantly higher than Mohammed et al. (2015)'s (11.1%) determination. Moreover, these negative magnitudes in this study indicate that the inclusion of reciprocal crosses tends to shorten PL and narrow SD, while positive crosses tend to have taller PH and increase NL.

As a result, the study's successful crossings demonstrated the presence of cross-compatibility across all parental combinations and their reciprocal crosses. According to the evaluation of mid-parent heterosis, only number of leaves of the four traits studied did not exhibit any statistically significant differences. The hybrids P6×P4, P4×P6, P6×P5, and P5×P6, in particular, had plant heights higher than those of the superior parent (P5). The reciprocal cross effects had an important impact on plant height and stem diameter. More investigation is necessary to assess the performance of the hybrids that were tested in various conditions as a potential bioenergy source.

Ethical Statement

The authors declare that ethical approval is not required for this research.

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Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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