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HETEROSIS AND INBREEDING DEPRESSION FOR YIELD AND OIL-RELATED TRAITS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) UNDER OPTIMUM AND WATER-DEFICIT CONDITIONS

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SUMMARY

Heterosis has been a key strategy in breeding sunflowers to improve sunflower yield and develop superior cultivars. Inbreeding depression, which is the loss of fitness and performance induced by increased homozygosity, is a major problem in the sunflower hybrids program. This research aimed to evaluate seven diversified genotypes (FMC-0046, Thatta, SH-3, A-2, Turkish, Mehran, and ST-2) and their 12 F_1 hybrids and F_2 populations for heterotic effects and inbreeding depression in earliness, yield, and oil content under normal and water-deficit conditions. The study used a split-plot design with three replications, carried out in 2023 at the Sindh Agriculture University, Tandojam, Pakistan. The analysis of variance revealed significant ($P \leq 0.01$) differences among F_1 and F_2 populations and genotype-by-environment interactions. Among the genotypes, FMC-0046, Turkish, and Mehran expressed the highest values for 1000 seed weight, head diameter, oil content, and seed yield plant^{-1}. Among the F_1 hybrids and F_2 populations, FMC-0046 x Mehran, Thatta x Turkish, A-2 x Mehran, SH-3 x ST-2, and A-2 x ST-2 performed better with desirable heterosis and desirable inbreeding depression for yield and oil traits. Hence, these populations received recommendations for inclusion in future breeding programs for the development of water stress-tolerant cultivars.

Keywords: Heterosis, inbreeding depression, yield and oil traits, water stress, sunflower (*Helianthus annuus* L.) genotypes

Key findings: Under water-stressed conditions, FMC-0046 x Turkish, FMC-0046 x Mehran, and Thatta x ST-2 performed well for traits of the number of seeds head^{-1} and head diameter, respectively. A-2 x Mehran produced desired results for 1000 seed weight in a stressed environment.

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INTRODUCTION

The area designated for sunflower cultivation in Pakistan is limited due to competition from other major crops. Sunflower holds considerable potential to enhance local oil production to meet the growing domestic demand for edible oil, confectionery, and bird feeds (Saif *et al.,* 2023). Its oil content ranges from 34.26% to 39.13% (Akhtar *et al.,* 2024). The primary challenge in achieving optimal sunflower oil yields is the absence of locally developed and widely adopted cultivars and hybrids (Hussain *et al.,* 2018; Sher *et al.,* 2022; Riasat *et al.,* 2025). Most seed requirements, met through imports, come from private seed companies.

Drought is a severe environmental challenge that inhibits the productivity of sunflowers and numerous other crops. Minimizing drought stress makes it crucial to identify phenotypic features that enable plants to maintain or enhance performance under water constraints. Likewise, it is critical to understand the complex physiological and genetic mechanisms involved (Tyagi and Dhillon, 2016; Rajper *et al.,* 2022).

Utilizing wild *Helianthus* species and potential inbred lines in breeding operations has become increasingly essential to improve yield components of sunflowers and develop a new optimum variety. However, the ability of hybrids to outperform their male and female inbred counterparts is vital for the successful development of sunflower hybrids. The improved performance of F_1 hybrids relative to their respective mid- and better-performing parents is known as heterosis.

Numerous studies have highlighted the significant heterotic effects on yield and its components in cross-pollinating crops like sunflowers. The documentation of this phenomenon has appeared in the literature (Karasu *et al.,* 2010). According to various studies (Tyagi and Dhillon, 2016), experts believe that heterosis results from a combination of nuclear and cytoplasmic factors, along with synergistic gene interactions. The key to developing an ideal hybrid model lies in identifying parental lines with favorable genes and recombining them to enhance their compatibility, resulting in superior F_1 hybrids. The widespread use of sunflowers as an oilseed crop is largely due to the hybrid vigor driven by heterosis. The ideal plant architecture for sunflower hybrids comprised traits such as a shorter maturity period, consistent performance, uniform plant stands, reduced plant height, and more leaves per plant. Other superior characteristics include larger head size, increased seed count per head, higher 1000-achene weight, greater seed yield, higher oil content, and resistance to lodging, insect pests, and diseases (Memon *et al.,* 2015; Kulundzic *et al.,* 2025).

Inbreeding depression, a typical occurrence in F_2 populations, is an outcome of increased homozygosity at numerous genetic loci, resulting in decreased vigor. It is interesting to note F_2 populations are more genetically diverse and heterogeneous than F_1 hybrids (Lande, 1981; Benowicz *et al*., 2020; Polat *et al*., 2025). This could lead to a greater flexibility and superior performance compared with their parents' inbred lines, sometimes even surpassing the F_1 hybrids (Acquaah, 2009). Mating systems, dispersal tactics, and general fitness can all attain significant effects from inbreeding depression, defined as decreased fitness and performance due to increased homozygosity. Therefore, inbreeding depression has been the subject of numerous empirical assessments (Scofield and Schultz, 2006). In regions with semi-arid climates and growing competition for water resources, such as water scarcity, the use of irrigation systems is crucial. Costa *et al.* (2007) stressed these approaches focused on preserving irrigation water while ensuring sufficient production.

The effects of water stress on sunflowers have gained extensive studies (Tahir and Mehdi, 2001; Angadi and Entz, 2002; Rajper *et al.,* 2022), but insufficient data about the impacts of well-irrigated and water-deficient settings on seed yield, seed oil content, and protein content of sunflowers exist. The conducted study sought to assess the heterotic effects and inbreeding depression in sunflower hybrids. This investigation aimed to determine how sunflowers react to water shortages for yield and oil-related traits in hybrid and self-pollinated environments. In addition to other traits—the number of seeds per head, head diameter, and 1000 seed weight—and other quantitative traits, such as the quantity of seed oil in sunflower hybrids, the study especially looks into how water-deficit conditions affect production.

MATERIALS AND METHODS

The experiment commenced in the spring and fall of 2023 at the Botanical Garden of Sindh Agriculture University, Tandojam. The layout of the experiment was a split-plot design with three replications. The main plots were two irrigation treatments, i.e., T1 = normal irrigation (five irrigations) and T2 = zero irrigation (single soaking dose), and the subplots had 19 genotypes. The genotypes comprised seven parental lines (FMC-0046, Thatta, SH-3, A-2, Turkish, Mehran, and ST-2). These also included 12 F_1 hybrids and F_2 populations from the crosses of FMC-0046 x Turkish, Thatta x Turkish, SH-3 x Turkish, A-2 x Turkish, FMC-0046 x Mehran, Thatta x Mehran, SH-3 x Mehran, A-2 x Mehran, FMC-0046 x ST-2, Thatta x ST-2, SH-3 x ST-2, and A-2 x ST-2. The conduct of two different seasonal experiments took place—with parents and F_{1s} grown in the spring and parents and F_{2s} grown in the fall of the same year.

Spacing between plants and rows remained at 25 and 60 cm, respectively. Each plot consisted of six rows, each 3 m long, with a total plot area of 3.0 m × 3.6 m (10.8 m^2). The first and last rows served as border rows, with the four middle rows used for data collection. Observations proceeded on 10 randomly selected plants for each treatment per replication. The entire crop cycle continued employing standard agronomic practices for sunflower cultivation. The soil texture was loamy. Data recording occurred for earliness, morphological characters, yield components (head diameter, seed number per head, seed yield per plant, and 1000 seed weight), and oil content. Oil extraction used the Soxhlet equipment, utilizing the siphon technique. Three grams of ground samples got placed into a Soxhlet extractor on a filter paper in the shape of a thimble. The oil extraction used petroleum ether as an extraction reagent that sustained heating to reflux. Afterward, the samples underwent drying before chilling in a desiccator and weighing with a metallic electric balance.

Statistical analysis

This research used the statistical factorial plot model of Gomez and Gomez (1984) to analyze the variance of the collected data. Fehr's (1987) approach served to calculate heterosis. By comparing F_2 populations to F_1 hybrid averages, inbreeding depression was calculated as a percentage drop in F_2 populations (Hallauer and Miranda, 1988).

RESULTS AND DISCUSSION

Analysis of variance

The mean squares from the analysis of variance expressed that F_1 hybrids and F_2 populations are significantly different for all traits under study. This included days to 90% maturity, head diameter, the number of seeds head^{-1}, seed yield plant^{-1}, 1000 seed weight, and oil content at 1% level of probability (Table 1). No genotype appeared nonsignificant for any of the attributes under study. On the other hand, for characteristics like days to first flowering, it produced nonsignificant results. As all other characteristics exhibited significant differences, oil content emerged as nonsignificant for the treatment × genotype interaction. Several studies, including Bakheit and El-Shimy (2014), Memon *et al.* (2015), Abdel-Moneam *et al.* (2022), Kumari *et al.* (2022), and Kaleri *et al.* (2023b), have discovered notable variations in the analysis of variance results for sunflower attributes.

Heterotic effects in F_1 hybrids

Earliness traits

Given the derivation and estimation of heterosis effects, it is preferable to attain minimal or negative heterosis for features associated with earliness to assure the shortest duration until maturity. SH-3 x Turkish exhibited commendable performance, identifying as an early maturing hybrid that required the least days to reach 90% maturity in a water-stressed environment. Additionally, it yielded the highest negative (-3.34%) mid-parent heterosis (MPH) and (-5.95%) better parent heterosis (BPH) (Table 2). Jadhao *et al.* (2023) and Kaleri *et al.* (2023a) noted a greater negative range of heterosis for days to maturity in certain sunflower hybrids.

Table 1. Mean squares from ANOVA for F_1 hybrids and F_2 populations for yield and oil-related traits in sunflower.

Traits	Source of variation					
	Replication (R)	Treatment (T)	Error (a)	Genotypes (G)	T x G	Error (b)
	d.f. = 02	d.f. = 01	d.f. = 02	d.f. = 18	d.f. = 18	d.f. = 72
F_1 hybrids						
Days to 90% maturity	14.22	1246.75**	2.48	191.27**	6.67**	2.14
Head diameter	0.03	99.30**	0.11	28.73**	17.71**	0.15
Number of seeds head^{-1}	325.90	33321**	109.60	35776	36928**	103.40
Seed yield plant^{-1}	0.32	444.08**	0.97	53.53**	26.52**	1.29
1000 seed weight	2.03	1450.21**	0.52	24.19**	13.08**	1.33
Oil content	4.37	105.63*	4.11	26.51**	1.47^{NS}	1.40
F_2 populations						
Days to 90% maturity	18.66	1213.89**	4.45	217.05**	5.91**	2.46
Head diameter	0.17	94.51**	0.26	28.53**	16.55**	0.28
Number of seeds head^{-1}	82.10	40044.40*	1083.80	37760.40**	36211.00**	300.80
Seed yield plant^{-1}	2.00	386.84**	0.94	54.81**	31.91**	2.42
1000 seed weight	1.25	1346.28**	0.65	40.85**	14.13**	2.27
Oil content	8.71	141.77*	3.03	29.18**	2.77^{NS}	2.04

^{NS},*,** = Nonsignificant, significant at 5%, and 1% level of probability, respectively.

Table 2. Heterotic effects of F_1 sunflower hybrids for days to 90% maturity and plant height grown under normal irrigation and water-deficit conditions.

F_1 hybrids	Days to 90% maturity				Head diameter			
	Normal condition		Water stressed		Normal condition		Water stressed	
	MPH (%)	BPH (%)	MPH (%)	BPH (%)	MPH (%)	BPH (%)	MPH (%)	BPH (%)
FMC-0046 x Turkish	8.02	1.59	5.36	0.28	-0.14	-13.04	2.76	1.12
Thatta x Turkish	-2.59	-5.29	-1.44	-3.40	-5.69	-16.56	3.55	-7.67
SH-3 x Turkish	-2.44	-4.76	-3.34	-5.95	-3.38	-15.25	16.15	9.85
A-2 x Turkish	-2.08	-6.35	0.59	-2.55	14.13	6.14	32.60	18.24
FMC-0046 x Mehran	12.76	11.44	11.01	8.36	8.18	-8.21	14.36	8.84
Thatta x Mehran	9.17	6.72	6.82	6.20	10.54	-4.76	35.20	28.15
SH-3 x Mehran	4.42	1.67	3.14	2.98	14.06	-2.54	46.20	44.82
A-2 x Mehran	8.45	7.83	5.40	4.77	29.84	17.39	62.00	53.55
FMC-0046 x ST-2	-1.98	-7.46	-1.04	-5.93	15.29	4.17	34.40	33.12
Thatta x ST-2	12.30	9.60	11.40	9.04	-5.74	-13.40	17.34	7.07
SH-3 x ST-2	-1.22	-3.20	-1.45	-4.24	-16.08	-23.60	17.14	13.55
A-2 x ST-2	7.78	3.46	5.99	2.54	4.47	1.11	33.03	21.39

MPH = Mid-Parent Heterosis, BPH = Better Parent Heterosis

Table 3. Heterotic effects of F_1 sunflower hybrids for the number of seeds head^{-1}and seed yield plant^{-1} grown under normal irrigation and water-deficit conditions.

F_1 hybrids	Number of seeds head^{-1}				Seed yield plant^{-1}			
	Normal condition		Water stressed		Normal condition		Water stressed	
	MPH (%)	BPH (%)	MPH (%)	BPH (%)	MPH (%)	BPH (%)	MPH (%)	BPH (%)
FMC-0046 x Turkish	0.57	-12.52	-28.57	-35.78	5.13	-6.82	13.99	3.63
Thatta x Turkish	-18.27	-19.04	-29.82	-34.79	16.30	5.30	16.90	9.98
SH-3 x Turkish	-9.73	-10.28	-0.08	-3.33	12.20	4.55	7.04	3.63
A-2 x Turkish	-29.13	-34.95	-27.95	-28.82	2.38	-2.27	7.33	6.35
FMC-0046 x Mehran	43.84	38.65	2.46	-8.33	25.83	20.73	-4.23	-17.07
Thatta x Mehran	1.50	-13.75	-3.72	-10.99	21.10	18.92	0.91	-9.76
SH-3 x Mehran	-0.10	-15.34	5.41	1.45	8.45	7.03	-7.08	-14.63
A-2 x Mehran	-9.88	-29.09	1.30	0.62	24.68	20.00	3.90	-2.44
FMC-0046 x ST-2	10.52	3.96	19.74	8.64	26.03	17.95	8.57	-5.00
Thatta x ST-2	-4.15	-11.08	15.65	8.49	17.85	12.82	10.60	0.00
SH-3 x ST-2	-3.02	-10.31	12.85	10.29	-2.16	-3.41	12.12	4.18
A-2 x ST-2	-5.63	-19.71	12.60	10.12	-2.96	-4.18	7.89	2.50

MPH = Mid-Parent Heterosis, BPH = Better Parent Heterosis

Morphological traits

Increased head size guarantees a higher quantity of seeds, whether filled or unfilled. The A-2 x Mehran hybrid exhibited the highest MPH (29.84%) and BPH (17.39%) under normal irrigation and MPH (62.00%) and BPH (53.55%) in water-deficit conditions for head diameter. It achieved the biggest head size among all F_1 hybrids (Table 2). Kaleri *et al.* (2023a) also reported that, except for just one hybrid, 14 F_1s demonstrated desired positive relative heterosis in both non-stress and drought-stressed situations. The maximum positive heterosis for head diameter (cm) identified in this study exceeds that reported by Jadhao *et al*. (2023) for various sunflower hybrids examined.

Yield-related traits

Under normal circumstances, the FMC-0046 x Mehran hybrid expressed the highest MPH (43.84%) and BPH (38.65%) for the trait of number of seeds head^{-1}. In the water-stressed situation, the most positive MPH (19.74%) resulted in FMC-0046 x ST-2, with the maximum positive BPH (8.64%) expressed by the SH-3 x ST-2 hybrid (Table 3). For seed yield plant^{-1} in normal irrigation conditions, FMC-0046 x ST-2 and FMC-0046 x Mehran hybrids gave the highest positive MPH (26.03%, 25.83%) and BPH (17.95%, 20.73%), respectively. Under water-deficit conditions, the Thatta x Turkish hybrid fared well with the most positive mid-parent heterosis (16.90%) and heterobeltiosis (9.98%), expressing better yields produced in a water-stressed environment (Table 3). Respective to Memon *et al.* (2015) and Kaleri *et al.* (2023b), this study described practically equivalent findings for the trait of seed yield plant^{-1} for diverse genotypes. SH-3 x ST-2 hybrid demonstrated the greatest MPH (9.78%) for 1000 seed weight in normal irrigation conditions, while FMC-0046 x Mehran hybrid emerged expressing the highest positive BPH (5.51%) in normal irrigation conditions. It also provided the highest positive mid-parent heterosis (16.40%) and better parent heterosis (7.87%) in water-deficit conditions (Table 4).

Oil content (%)

Given that the sunflower crop is famous for producing high-quality edible oil, maximum positive heterosis for oil content is desirable. A-2 x ST-2 and Thatta x Mehran hybrids displayed maximum positive mid-parent heterosis (13.58% and 12.96%) in normal and water-deficit conditions, respectively. Meanwhile, the SH-3 x ST-2 hybrid performed

Table 4. Heterotic effects of F_1 sunflower hybrids for 1000 seed weight and oil content (%) grown under normal irrigation and water-deficit conditions.

F_1 hybrids	1000 seed weight				Oil content (%)			
	Normal condition		Water stressed		Normal condition		Water stressed	
	MPH (%)	BPH (%)	MPH (%)	BPH (%)	MPH (%)	BPH (%)	MPH (%)	BPH (%)
FMC-0046 x Turkish	0.31	-1.05	0.83	0.16	1.93	1.11	2.72	0.00
Thatta x Turkish	2.54	2.21	-1.04	-2.57	6.04	0.57	6.79	-1.41
SH-3 x Turkish	-1.92	-2.55	5.64	4.55	-4.82	-8.19	-4.81	-8.40
A-2 x Turkish	0.67	-0.74	10.60	4.19	8.11	1.92	6.58	0.00
FMC-0046 x Mehran	8.44	5.51	16.40	7.87	-12.86	-13.22	-11.14	-11.65
Thatta x Mehran	-2.96	-7.12	5.42	-0.26	4.18	-0.82	10.84	4.38
SH-3 x Mehran	-0.33	-3.71	4.57	-1.54	2.14	-1.09	-2.10	-3.80
A-2 x Mehran	1.21	-1.49	0.81	-0.28	-0.43	-5.78	-0.14	-4.38
FMC-0046 x ST-2	0.22	-6.50	-4.28	-5.93	0.29	-0.55	0.13	-0.03
Thatta x ST-2	5.03	-3.54	-4.69	-5.16	9.96	5.95	7.95	2.37
SH-3 x ST-2	9.78	1.73	-7.88	-7.91	12.96	10.73	7.92	6.82
A-2 x ST-2	9.14	1.85	0.92	-3.96	13.58	8.77	7.705	3.86

MPH = Mid-Parent Heterosis, BPH = Better Parent Heterosis

well, displaying the greatest positive heterobeltiosis (10.73% and 6.82%) in both normal and water-deficit conditions (Table 4). Similar results came from Kaleri *et al.* (2023b), who also detected considerable heterosis for oil content.

Inbreeding depression in F_2 populations

Earliness traits

The maximum inbreeding depression is a favorable element in judging the performance of F_2 populations, as it leads to decreases in the number of days to maturity, giving a classification as early maturing hybrids. The results obtained from this research demonstrate the performance of the Thatta x Mehran hybrid for expressing maximum positive inbreeding depression (1.87 and 1.70) for days to 90% maturity in both normal and water-deficit conditions (Figure 1). Bakheit and El-Shimy (2014), Memon *et al.* (2015), and Kaleri *et al.* (2023b) found comparable results for inbreeding depression for multiple attributes for earliness.

Morphological traits

Among F_2 populations, in the given stressful environment, the hybrid FMC-0046 x Mehran showed the lowest inbreeding depression (-2.14 and -8.18) and performed well for head diameter in centimeters (Figure 2).

Yield and oil traits

The study discovered that, under water stress, the FMC-0046 x Turkish hybrid had the least amount of inbreeding depression (-41.35) for the trait of the number of seeds head^{-1} (Figure 2). In contrast, under water stress, the A-2 x ST-2 hybrid had the least amount of inbreeding depression (-4.65) for seed yield plant^{-1} (Figure 2). According to assessments of the 1000 seed weight in grams, hybrids Thatta x Turkish and A-2 x Mehran had the lowest inbreeding depressions (-1.85 and -2.50) under normal and water-stressed circumstances, respectively (Figure 3). In both normal and water-stressed environments, SH-3 x Mehran and A-2 x ST-2 hybrids produced the minimum inbreeding depression (-0.82 and 0.86) for oil content (%) (Figure 3). We conclude that from the current data, high to low inbreeding depression emerged for earliness, yield, and oil content, which suggests that selection should be more successful for the establishment of sunflowers' new cultivars from segregating populations. Reports of similar findings of inbreeding depression for sunflower genotypes' earliness,

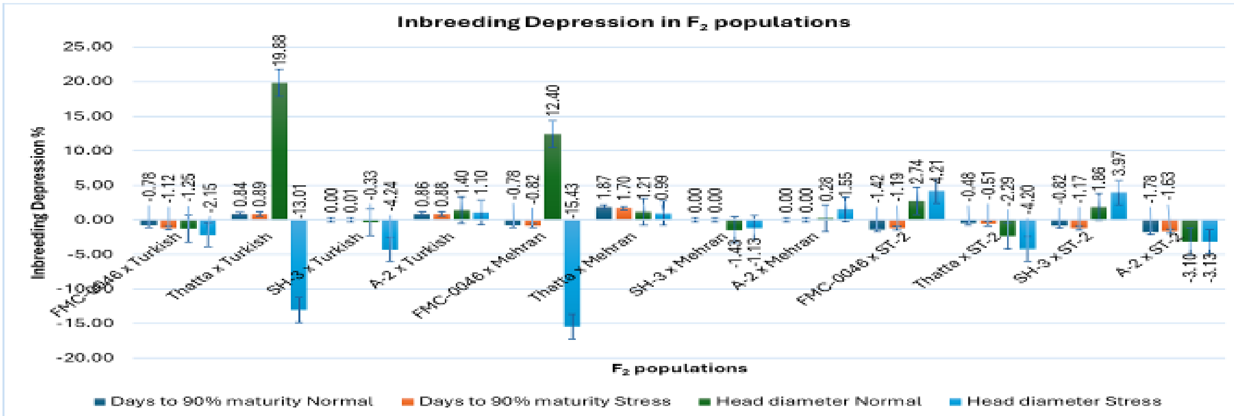

Figure 1. Inbreeding depression in F_2 populations of sunflower for days to 90% maturity and head diameter grown under normal irrigation and water-deficit conditions.

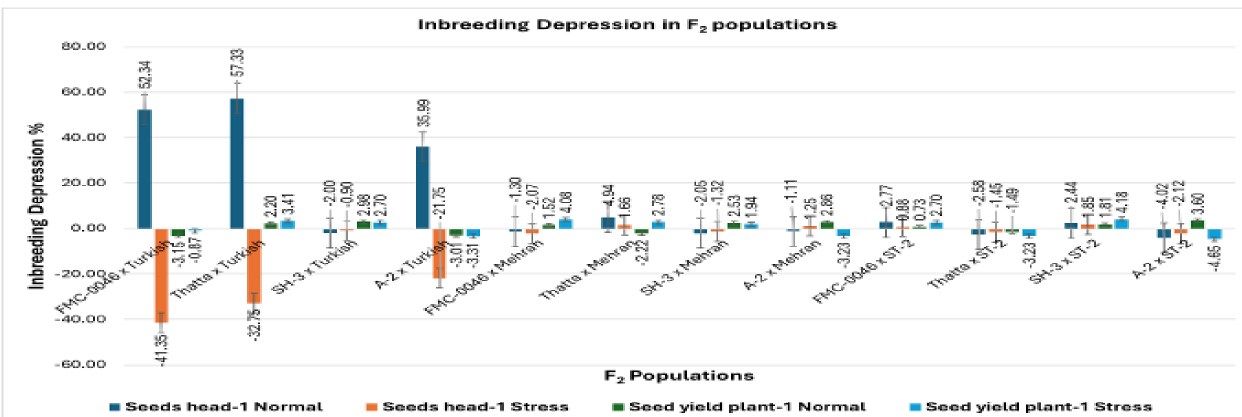

Figure 2. Inbreeding depression in F_2 populations of sunflower for the number of seeds head^{-1} and seed yield plant^{-1} grown under normal irrigation and water-deficit conditions.

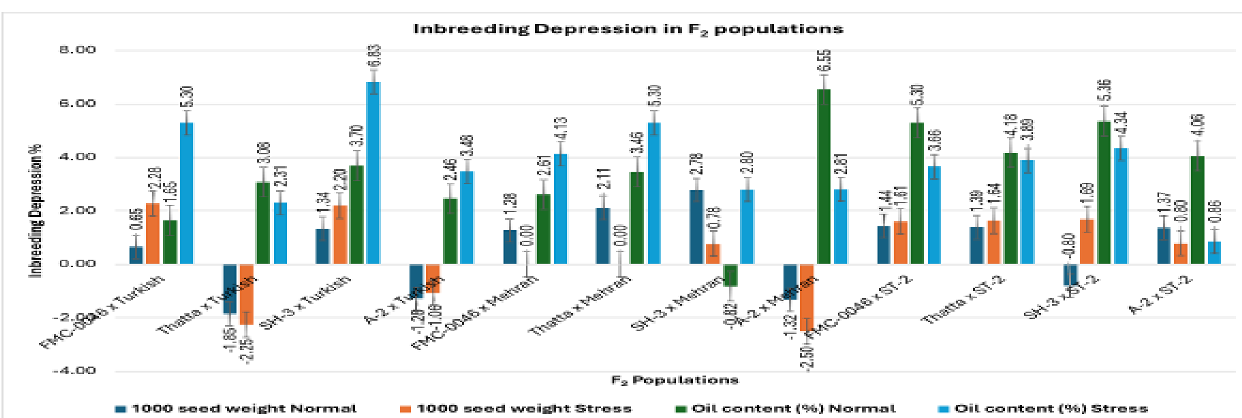

Figure 3. Inbreeding depression in F_2 populations of sunflower for 1000 seed weight (g) and oil content (%) grown under normal irrigation and water-deficit conditions.

yield, and oil content also came from other researchers, including Reddy *et al.* (2006), Bakheit and El-Shimy (2014), Memon *et al.* (2015), and Kaleri *et al.* (2023b).

CONCLUSIONS

Under water-stressed conditions, hybrids FMC-0046 x Turkish, FMC-0046 x Mehran, and Thatta x ST-2 performed well. Conclusively, this study has yielded promising inbreeding depression data, highlighting the highest-performing F_2 populations for producing helpful inbreeding depression for diverse phenotypes under examination. The research determined the Thatta x Mehran hybrid to be an early-maturing hybrid for demonstrating the greatest inbreeding depression for all characteristics.

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