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Exploring Genetic Variation through Line x Tester Analysis of Sunflower (*Helianthus annuus*) Genotypes for Yield and Oil Traits under Adverse Environment

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GENETIC analysis indicated significant differences between treatments, inbred parents, F_1 hybrids and parents versus hybrids for yield and oil traits. The noteworthy variances between parents vs. hybrids indicated the importance of heterosis breeding whereas line × tester revealed the prominence of specific combing ability and involvement of dominant genes. General combining ability of lines and testers indicated that lines, HO.1, Mehran and Thatta and from testers UC-666 and B-2 were good combiners for oil traits were regarded as best general combiners, thus these inbreds may be crossed to produce prolific hybrids. Specific combining ability and heterotic estimates are essential genetic parameters presenting worth of F_1 hybrid expansion. The hybrids like Thatta × UC-666, Mehran × Peshawar-93 and Ho.1 × B-2, on the basis of SCA and heterotic effects were found as potential hybrids for yield and oil parameters in both the environments. These results are suggested that such lines, testers and their F_1 hybrids may be utilized in breeding programme for the development of more recombinants

Keywords: Line x tester analysis, combining ability, oil traits water stress, sunflower genotypes

Introduction

Drought is one of the most significant environmental factors affecting sunflower and other crop yields. Drought stress necessitates an understanding of the nature of phenotypic features that can recover performance under water stress conditions, as well as a comprehension of the complex physiological and genetic systems involved in stress response. In that context, one of the most significant goals of plant breeders is to improve drought tolerance and water productivity in plants suitable for such environments. Considering the morphological, physiological, genetic, and molecular processes that determine drought tolerance can aid in the evolution of drought-tolerant cultivars suitable for cultivation in arid and semi-arid regions (Saremirad et al., 2020). It is also regarded as one of the most severe environmental factors affecting sunflower and other crop yields. As a result, it is critical to identify the subtle physiological and genetic mechanisms that improve sunflower performance under water stress, as well as to consider the type of phenotypic features that enhance performance under stress. One of the primary goals of plant breeders in the near future is to increase drought tolerance in crop plants while also increasing water usage efficiency (Saremirad et al., 2020). By nature and from genetic point of view, sunflower is drought-prone field crop (Tyagi et al., 2018). Droughts are anticipated to become more severe in coming years because of climate change.

To accomplish this goal, it is necessary to document the genetic divergence of current germplasm as well as the genotype combining ability. Heterotic hybrids were developed by mating cytoplasmic male sterile (CMS) inbred females with restorers who had increased general combining ability (GCA) and specific combining

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ability (SCA) effects (Tan et al., 2010; Mena et al., 2013; Ashraf et al., 2024). Larger GCA variation indicates additive gene activity, which represents the line's breeding value, whereas larger SCA variance indicates a greater role for non-additive gene action, which is required for heterosis breeding (Shabbir et al., 2016). Line tester analysis is the most efficient method for analysing a large number of inbreds for GCA and SCA estimates of yield and oil quality attributes. Before beginning a heterosis breeding programme, inbred parents with great combining capacity must be developed. To develop an effective breeding plan, information on the GCA of inbred lines to be used as parents, as well as their unique combining ability, would be incredibly useful (Vikas et al., 2015) and (Saeed et al., 2023). Superiority of hybrid above lines and tester inbreds is an important consideration when developing successful F_1s (Mena et al., 2013). Numerous researchers have observed a high expression of heterotic effects on both yield and oil characteristics (Aslam et al., 2010; Chahal et al., 2019). High heterotic estimations for yield relative to the parental average were obtained (18.3 to 72.38%), as was heterobeltiosis (2.86 to 56.842%) (Ahmed et al., 2021). Several other researches, including Memon et al. (2015), Depar et al. (2017), Khan et al. (2018), and Lakshman et al. (2020), found favourable high parent heterosis for seed yield. The line tester analysis is a valuable method for examining a large number of inbreds and determining their GCA and SCA impacts. This mating design helps. Head diameter, 1000-seed weight, seed output, and oil percentage all displayed greater SCA variability than GCA changes (Andarkhor et al., 2012). Sunflower has already shown substantial heterosis in seed yield and component characteristics (Karasu et al., 2010). Heterosis in sunflower is generally achieved through single-cross hybrids produced by mating cytoplasmic male sterile inbred lines with fertility-restoring males (Hladni et al., 2010). Sunflower has a unique position among water stress tolerant plants, so the present study aims to assess the combining ability and heterotic impacts on yield and oil attributes of sunflower genotypes in adverse circumstances.

Materials and Methods

The ability of CMS lines to combine with restorer testers, as well as their F_1 hybrids were assessed for general and particular combining ability variations and their consequences, F_1s heterosis. The experiment was carried-out in a split plots with two managements (well-watered and water stress from bud formation till seed setting) and four replications at the experimental field of Oil Seeds Research Institute, Tandojam during 2021. Water regimes were regarded as the most important component and in this, they served as a main factor. Irrigation regimes with no water stress (well-watered) received frequent irrigations without any water stress, thus a total of 5 irrigations were applied, whereas water stress treatment received mild to severe stress imposed on 50-day-old plants near to flower bud until seed formation i.e. 80-day-old plants by withholding water for a period of 30 days. The following observations were recorded which are as given below

Seed yield (Kg ha^{-1}): Seed yield per plant (g) was transformed in seed yield Kg ha^{-1}.

Linoleic acid content (%): It is calculated according to procedures adopted by Shamshad et al. (2016).

Oleic acid content (%): It is calculated according to procedures adopted by Shamshad et al. (2016).

Oil content (%): Ten gram grinned sample were heated at 60ºC. The condensed vapor dripped into the Trimble and filled in the siphon tube was poured back into the quick fit round bottom flak. The samples were dried and cooled in desiccators and weighed using metallic electric balance.

Protein content (%): The seed sample were meticulously washed with sample with de-ionized water and dried at 80 ºC. the oven dried sample were grinned very fine on Tema grinder with steel balls for protein analysis .the sample were digested by modified Kjeldahls method in which nitrogen was converted into NH4-N by digest was determined with NaOH 40% and 0.1 NHCL standard.

The acquired data was subjected to analysis of variance using the statistical factorial plot model, as described by Gomez and Gomez (1984). Lines × tester analysis, a method created by Kempthorne (1957) and implemented by Singh and Chaudhry (1984), was used to calculate estimates of combining ability.

Results and Discussions

Seed yield (kg ha^{-1})

Better seed yield production is the absolute objective of efficient sunflower breeding projects. Yet there are many constraints behind the stumpy production of sunflower due to unavailability of quality and high cost of hybrid seed, low yield of open-pollinated varieties and deprived marketing system and poor adaptability of imported hybrid seeds. In present study, out of five, four lines expressed higher positive GCA estimates whilst one parent showed negative effect. Among the female parents, lines Mehran, HO-1 and Thatta exhibited higher GCA effects for seed yield. From three pollinators, only B-2 gave highest GCA estimates in non-stress environments while two others testers such as UC-666 and Peshawar-93 gave detrimental negative GCA impacts and poor mean performance. The uppermost positive GCA for seed yield by tester R50 was achieved by Mohyaji et al. (2014) while Siahbidi et al. (2022) showed high GCA of testers PAC-0306 and PAC-64-A for seed yield and oil content. Saleem-Uddin et al. (2014) perceived that CMS HA-54 and pollinator RHP-71 articulated maximum GCA estimations for seed yield signifying the prevalence of additive gene influence, therefore may help as prospective inbreds for crossing schemes. The maximum GCA estimates was observed in the female line Thatta followed by the PSF-025, Mehran and HO-1 for this trait in stress while tester B-2 showed maximum GCA effects followed by Peshawar-93. More or less similar parents demonstrated upper GCA impacts in both regular and in water drought stress environments; therefore, such parents were identified as good general combiners with additive genes and are suitable for crosses to develop potential hybrids or selection of superior single plants from filial generations. Saleem and Ali (2012) recommended found females L350, L460, L990 and L770 showed astonishing affirmative GCA and evidenced as high combiners for seed yield. Similarly, Imran et al. (2014) observed that inbreds A-34 and A-29 were the best general combiners for obtaining higher seed yield (Table 1).

TABLE 1. GCA effects of lines and testers for various traits of sunflower grown under well watered and water stressedenvironments

Lines	Seed Yield (Kg ha^{-1})		Oleic Acid (%)		Linoleic Acid (%)	
	Well watered	Water stressed	Well watered	Water stressed	Well watered	Water stressed
HO.1	42.25**	11.16	2.53**	2.92**	-5.88**	-4.32**
Mehran	92.12**	59.95**	1.20**	2.03**	6.45**	6.43**
Thatta	24.77**	82.97**	-0.22	-0.38	1.28**	0.01
PSF-025	20.77*	69.01**	-0.47*	-0.55	-0.05	1.35**
SH-3915	-179.49**	-223.01**	-2.89**	-3.30**	-1.77**	-4.49**
S.E. (gi.)	8.10	7.38	0.28	0.92	0.27	0.28
Tester						
UC-666	-19.56**	-27.46**	1.75**	1.07**	-0.16	-0.40*
Peshawar-93	-50.39**	42.46**	-1.40**	-1.03**	-3.98**	-4.70**
B-2	69.97**	70.10**	-0.35*	-0.03	4.17**	5.10**
S.E. (gi.)	6.28	5.71	0.22	0.22	0.21	0.22

**,* = significant at 1 and 5% probability level respectively

Regarding specific combining ability, fifteen cross combinations were evaluated under study, three crosses such as Thatta × UC-666 recorded the highest positive SCA value followed by HO-1 × B-2 and PSF-025. Eight hybrids recorded undesirable SCA effects ranging from -70.81 to -382.17, though appropriate positive SCA effects ranged from 21.89 to 288.16 for seed yield (Table 3). The superior F_1 hybrids were identified as HO-1× B-2, Thatta × UC-666 and Mehran × Peshawar-93 which displayed maximum SCA effects, similarly in non-stress, eight hybrids exhibited negative SCA effects for seed yield under stress. The hybrids which showed higher positive SCA estimates in both the environments are containing favourable dominant genes; hence such hybrids are potential to be exploited for hybrid sunflower expansion. Results further suggested that higher SCA hybrids are equally good for the exploitation of heterosis or single plant selections. Analogous findings were also obtained by Vishwanath and Goud (2006) and Shankar et al. (2007). Rezaeizad and Siahbidi (2015) established high SCA estimates for seed yield shown by hybrids CMS19×R217 and CMS 456/2 × R217, while Tyagi Dhillon (2016) perceived high SCA estimations in hybrids PKU-2A × RHA83R6 and E002-91A × P93R for yield. Saleem-Uddin et al. (2014) evaluated sixteen crosses and noted that 02 crosses demonstrated high SCA for seed yield, while Goksoy and Turan (2004) noted that 4 crosses manifested high SCA estimation for seed yield.

Oleic acid (%)

Sunflower that contains more oleic are highly acceptable against other types owing to its fitness for catering and heat resistance (Smith et al., 2007). Present results revealed that two inbreds lines showed positive GCA effects from five lines while the females HO-1 and Mehran expressed the maximum yet advantageous GCA effects for oleic acid in control irrigation treatment. Similarly, one pollinator UC-666 recorded progressive GCA estimations in regular irrigation. In drought stress, same female lines i.e. HO-1 and Mehran also expressed positive GCA effects (Table 1) and likewise the same tester UC-666 also gave positive GCA effects in stress conditions, showing consistency in performance under both the environments. The four lines viz. L6, L7, L11 and L8 were identified as high combiners for oleic acids in both the seasons (Rizwan et al., 2020). Similar results of parents regarding higher GCA estimates for oleic acid were also reported by Aslam et al. (2010). Crosses Mehran × Peshawar-93, PSF-025 × B-2 and HO-1 × B-2 revealed greater SCA effects in non-stress, whereas, PSF-025 × B-2, Thatta × UC-666 and Mehran × Peshawar-93 manifested highly positive SCA effects in water stress. Outcomes concerning SCA estimations for oleic acid, sixty hybrids of sunflower, crosses L8×T1 and L2×T3 were good combiners in both the seasons (Table 3). Genetic study revealed that additive genes were lower than dominant genes oleic acid because variances owing to GCA were smaller than SCA, thus favored heterosis breeding (Rizwan et al., 2020).

Linoleic acid (%)

This oil component is vital omega-6 fatty acid due to health benefits in depressing blood cholesterol (Orsavova et al., 2015). From present study, it was observed that in non-stress, two female inbreds resembling Mehran and Thatta recorded greater and positive GCA effects for linoleic acid, while from three pollinators, only B-2 showed high GCA estimates in normal irrigation (Table 1). Under drought stress, two parents such as Mehran followed by PSF-025 recorded positive GCA impacts, yet only tester B-2 recorded high GCA estimates for this trait. The females and males parents exhibiting higher GCA effects are recommended as good combiners which are useful parents for hybrids sunflower development. From 15 crosses, the high scoring SCA hybrids were; HO-1 × B-2, Mehran × Peshawar-93 and Thatta × UC-666 in normal irrigation for linoleic acid, nevertheless, 8 hybrids revealed undesirable SCA impacts suggested their being poor enactment for linoleic acid as displayed in Table 3. Under drought stress, nine F_1s manifested deleterious SCA estimations for linoleic acid. The hybrids like HO-1 × Peshawar-93 demonstrated high progressive SCA effects followed by SH-3915 × Peshawar-93 and HO.1 × UC-666, these combinations thus exhibited more tolerant to water stress. Parallel to our findings, Rizwan et al. (2020) carried out experiment on combing ability estimates and reported that the lines L8, L19, L12 L4 and L14 were high general combiners for linoleic oil in both the seasons. Joksimovic et al. (2006) and Manzoor et al. (2016) observed that SCA estimates for linoleic acid were higher in crosses L5 × T2 (7.10) followed by L2 × T2, L13 × T1, L14 × T3 and L5 × T3 in both the seasons. In another study, Skoric et al. (2008) reported analogous results with higher SCA estimations. The trial conducted by Rizwan et al. (2020) demonstrated that dominant genes greater over additive for linoleic acid due to SCA variances being larger than GCA variances. Thus these results favor hybrid expansion.

TABLE 2. SCA effects for various traits in sunflower of sunflower grown under well watered and water Stressed environments

F_1 Hybrids	Seed Yield (Kg ha^{-1})		Oleic Acid (%)		Linoleic Acid (%)	
	Well watered	Water stressed	Well watered	Water stressed	Well watered	Water stressed
HO.1 × UC-666	-112.09**	-99.62**	1.92**	0.10	2.41**	1.57*
Mehran × UC-666	-84.19*8	-73.08**	-2.75**	-2.48**	-4.42**	-9.18**
Thatta × UC-666	288.16**	291.80**	4.67**	5.93**	8.00**	-2.76**
PSF-025 × UC-666	-113.80**	-159.90**	-1.33*	-2.40**	-3.67**	-4.10**
SH-3915 × UC-666	21.89	40.80**	-1.63**	-1.15**	-2.34**	0.74
HO.1 × Peshawar-93	-166.57**	-221.90**	-3.68**	-5.05**	-12.77**	5.87**
Mehran × Peshawar-93	147.89**	238.00**	6.15**	5.87**	9.65**	-4.88**
Thatta × Peshawar-93	53.99**	-73.36**	-0.93	-1.97**	-5.18**	1.54*
PSF-025 × Peshawar-93	-84.22**	25.02**	-4.43**	-4.30**	0.90*	0.20
SH-3915 × Peshawar-93	48.90**	32.24**	3.74**	5.45**	7.37**	5.04**
HO.1 × B-2	278.65**	321.5**	4.77**	4.95**	10.33**	-3.93**
Mehran × B-2	-63.72**	-164.90**	-3.40**	-3.38**	-5.25**	-14.68**
Thatta × B-2	-342.17**	-218.60**	-3.73**	-3.97**	-2.83**	-8.26**
PSF-025 × B-2	198.02**	164.90**	5.77**	6.70**	2.75**	-9.60**
SH-3915 × B-2	-70.81**	-73.01**	-2.56**	-24.85**	-5.03**	-4.76**
S.E. (Si.)	14.04	12.78	0.49	0.50	0.47	0.49

**,* = significant at 1 and 5% probability level respectively

Oil content (%)

Seeds of sunflower comprise a great amount of oil i.e. 40–50% (Riaz et al., 2017). In current study, two from five lines were found to exhibit affirmative GCA in regular irrigation and three manifested deleterious effects for oil content as presented in Table 4. However, the female PSF-025 demonstrated uppermost GCA estimations followed by the parent HO-1 in non-stress, while tester B-2 expressed positive but small GCA effects for oil content. Concerning stress environment, female inbreds like SH-3915 and HO-1 demonstrated positive GCA effects, while the pollinator Peshawar-93 documented high progressive GCA impacts in stressed conditions. The positive SCA effects varied from 0.66 to 3.39 in five crosses out of fifteen F_1 hybrids in non-stress. Ten crosses recorded negative SCA effects (ranging from -0.19 to -2.02) for oil content. Singh et al. (1989) reported the predominance of non-additive genetic effects for seed yield, oil content and linoleic acid content in a line × tester analysis of 30 hybrids. They observed that CMS-234A and inbred line 187-333 were good general combiners for both seed yield and oil content. The combination 86A3 × 68-3 was the best for seed yield and oil quantity. Tyaghi et al. (2018): Mustafa et al. (2023) worked on CMS analogues inbreds and identified inbreds CMS-XA, CMS-ARG-2A, CMS-PRUN-29A, CMS-42A and CMS-234A as the best general combiners with respect to the oil content under the regular and adverse growth conditions. The F_1 hybrids, which exhibited uppermost affirmative SCA estimations, were: Mehran × Peshawar-93, Thatta × UC-666 and PSF-025 × B-2 which reproduced higher SCA impacts (Table 4) in optimal irrigation. In drought environment, the F_1s like Mehran × Peshawar-93 followed Thatta × UC-66 and PSF-025 × B-2 manifested higher SCA impacts for the oil content. Similar to these findings, Ahmed et al. (2022) identified some crosses like A14×Rf14, A28×Rf1, A4×Rf20, A31×Rf1 and A4×Rf11 which demonstrated greater positive SCA impacts for seed oil content.

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Protein content (%)

The seed of sunflower is comprised various organic complexes, addition to oil, it is potential source of proteins. Cultivated cultivars possess 18 to 20% protein. On the basis on parental performance and GCA estimates, three female parents Mehran, SH-3915 and Thatta elucidated maximum advantageous GCA impacts of 1.15, 0.15 and 0.07 in optimal irrigation while B-2 is categorized as topmost tester with uppermost GCA estimation of 0.72 under normal irrigation. Equivalent present results, some male inbreds manifested greater GCA impacts for seed proteins as observed by Nasreen et al. (2014). Two female parents Mehran and Thatta scored maximum GCA positive effects of 0.95 and 0.75 and two testers B-2 and Peshawar-93 scored positive GCA of 1.05 and 0.05 in stress conditions (Table 3). The lines and tester inbreds, which exhibited high positive GCA, are high universal combiners with additive genes; henceforth got the ability to evolve prolific hybrids on crossing. Tan (2010) perceived that CMS lines 4, 46, 58,195 and 704 and testers 0708RF, 084RF, 0951Rf, and 1097Rf displayed desirable GCA impacts. Since some lines and testers show high combining ability, therefore may be involved in hybridization to high protein hybrids. Likewise, some CMS lines 64, 53, H55-2-2-1 and 53 were found as high general combiners for seed proteins (Nasreen et al., 2014).Fifteen cross combinations evaluated, the best hybrid SH-3915 × Peshawar-93 was found with high SCA impacts in both normal and water stress environments followed by HO-1 × UC-666 in normal and PSF-025 × B-2 in drought stress environments (Table 4). Hybrids expressing higher SCA impacts most likely revealed preponderance of non-additive genes contributing to SCA effects, hence such hybrids are suitable candidates to enhance protein content in sunflower. Comparable to current outcomes, Qamar et al. (2018) exposed that hybrids G100×G53, G2×A26 and G7×G12 manifested substantial SCA impacts for protein, whereas Nasreen et al. (2014) who perceived that F1 hybrid CMSNDMTC × RHA-295 presented greater SCA estimations for seed protein also reported similar observations

TABLE 3. GCA effects of lines and testers for various traits of sunflower grown under well watered and water stressed environments

Lines	Oil Content (%)		Protein Content (%)	
	Well watered	Water stressed	Well watered	Water stressed
HO.1	0.97**	0.97**	-0.68**	-1.64**
Mehran	-0.03	-0.45*	1.15**	0.95**
Thatta	-0.95**	-0.03	0.07	0.78*
PSF-025	1.39**	1.47**	-0.68**	-0.05
SH-3915	-1.36**	-1.95**	0.15	-0.05
S.E.(gi.)	0.27	0.25	0.31	0.26
Tester				
UC-666	-0.06	-0.23	-0.58	-1.00*
Peshawar-93	-0.01	0.42*	-0.13	0.05
B-2	0.09	-0.18	0.72	1.05*
S.E.(gi.)	0.21	0.20	0.54	0.45

**,* = significant at 1 and 5% probability level respectively

TABLE 4. SCA effects for various traits of sunflower grown under well watered and water stressed environments

F_1 Hybrids	Oil Content (%)		Protein Content(%)	
	Well watered	Water stressed	Well watered	Water stressed
HO.1 × UC-666	-0.27	-0.27	3.33**	2.34**
Mehran × UC-666	-2.02**	-1.60**	-1.00	-2.00**
Thatta × UC-666	3.40**	3.23**	2.83**	3.17**
PSF-025 × UC-666	-0.94*	-1.27**	-0.92	-0.50
SH-3915 × UC-666	-0.19	-0.10	-4.25**	-3.00**
HO.1 × Peshawar-93	-1.07*	-1.67**	-4.12**	-3.71**
Mehran × Peshawar-93	3.93**	3.75**	2.05**	2.20**
Thatta × Peshawar-93	-1.40**	-1.17**	0.38	-0.13
PSF-025 × Peshawar-93	-1.02*	-1.17**	-2.37**	-2.30**
SH-3915 × Peshawar-93	-0.49	0.25	4.55**	3.95**
HO.1 × B-2	1.33**	1.93**	0.78	1.29**
Mehran × B-2	-1.92**	-2.15**	-1.05*	-0.30
Thatta × B-2	-2.00**	-2.07**	-3.22**	-3.13**
PSF-025 × B-2	1.91**	2.43**	3.28**	3.20**
SH-3915 × B-2	0.66	-0.15	0.20	-1.05*
S.E. (Si.)	0.47	0.44	0.54	0.45

Conclusion

Among the lines, HO.1, Mehran and Thatta and from testers UC-666 and B-2 were good combiners for oil traits were regarded as best general combiners. The hybrids like Thatta × UC-666, Mehran × Peshawar-93 and Ho.1 × B-2, on the basis of SCA and heterotic effects were found as potential hybrids for yield and oil parameters in both the environments.

Consent for publication:

All authors declare their consent for publication.

Author contribution:

The manuscript was edited and revised by all authors.

Conflicts of Interest:

The author declares no conflict of interest.

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