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HETEROSIS FOR THE IMPROVEMENT OF OIL QUALITY IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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Abstract

To improve the oil quality of the available sunflower genotypes by exploiting heterosis-breeding program. Four Cytoplasmic Male Sterile lines TS-17, TS-18, TS-228, TS-335 and four Restorer lines 291RGI, R-25, TR-9, TR-6023 sunflower parents and their sixteen F_1 hybrids were evaluated in randomized complete block design with three replicates at Agricultural Research Institute, Tarnab, Peshawar. Highly significant genetic differences ($p<0.01$) were observed among parents and F_1 hybrids for oleic acid (C18: 1), linoleic acid (C18:2) and behenic acid (C20:0).

Mid and high parent heterosis estimates of F_1 hybrids ranged from -100 to 157.31% and -100 to 113.59% for C20, –29.84 to 52.02% and –31.23 to 50.49% for C18:1, -20.12 to 16.19% and-20.66 to 9.69% for C18: 2 and –100 to 201.08% and –100 to 100% for C20:0 respectively.

TS-335 x 291RGI has highest negative mid and high parent heterotic effects for TS-18 x R-25 has maximum positive mid and high parent heterosis for C18:1, TS-18 x TR-6023 has maximum positive mid and high parent heterotic effects for C18:2 and highest negative mid parent heterosis was observed for C20:0 by TS-17 x TR-9, TS-18 x 291RGI.

It is concluded that the mid and high parent heterotic effects improve oil quality of the parent of these eight hybrids and are suggested for use in sunflower breeding program.

Introduction

Study of inbreeding and heterosis in sunflower has been taking place for over 80 years now. Practical application of the phenomenon of heterosis in this species began after the discovery of a suitable source of cytoplasmic male sterility in 1969 and that of restorer genes. Sunflower hybrids are now planted in all parts of the world where sunflower is grown commercially as an oil crop worldwide (Anon., 1995). Utilization of heterosis has allowed sunflower to become one of the major oilseed in many countries. Present day sunflower cultivars contain more than 40% oil and 18-20% protein. Sunflower oil is of good quality as it contains high proportion of linoleic acid which is a polyunsaturated fatty acid. It is also a good source of calcium, phosphorus, nicotinic acid and vitamin E. There are a number of advantages of growing sunflower for oil compared with other cultivated species. No special machinery is needed to produce this crop. It can be grown as catch crop in many situations. Being drought resistant it is well suited for rain fed as well as irrigated areas.

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Quality of sunflower oil is analysed on the basis of the ratio of oleic/linoleic acid. The most frequent fatty acid composition in sunflower oil is: 55-65% of linoleic acid, 20-30% of oleic acid and the remaining including other fatty acid, primary palmitic and stearic (Joksimovic *et al*., 2006). There exists a negative correlation between the contents of oleic and linoleic acid, that their contents are genetically controlled (Fick & Mill, 1997) and have strong linkage which needs to be broken. Many authors have reported significant manifestation of heterosis for oil quality and seed yield as well (Khalil *et al*., 2000 & Flagella *et al*., 2002 & Khan *et al*., 2008. However the quality of oil is different in the various sunflower genotypes (Joksimovic *et al*., 2001).

Pakistan grows about 161 thousand hectares of the sunflower with annual production of 106 thousands tones with an average yield of 1726.7 kg ha^{-1} (Anon., 2004). Edible oil is Pakistan's largest single food import with consumer demand steadily increasing at 7.7% a year (Anon., 1995). Total domestic requirement of edible oil is 2.0 million tons of which about 29% comes from local production and the remaining 71% has to be imported every year. The aims of the studies were to improve the oil quality in the available sunflower genotypes by exploiting heterosis-breeding program.

Materials and Methods

Sunflower seed: Seed of eight different genotype of the sunflower were received from Federal Seed Certification and Registration Department, Swat. The experiment was conducted on a sandy clay loam soil at Agricultural Research Institute, Tarnab and Peshawar during autumn. Environmental temperature and physio- chemical properties of the soil were noted.

Experimental design: Eight Sunflower parents i.e., four Cytoplasmic male sterile (CMS) lines TS-17, TS-18, TS-228 and TS-335 (Tamable Sterile) and four Restorer lines 291RGI, R-25, TR-9 and TR-6023 (Tarnab Restorer) were selected for the desirable character including head size, plant height, early maturity, high yield and oil content. These parents were breed to produce F_1 hybrids. Sixteen F_1 hybrids were obtained, which were TS-17 × 291RGI, TS-17 × R-25, TS-17 × TR-9, TS-17 × TR-6023, TS-18 × 291RGI, TS-18 × R-25, TS-18 × TR-9, TS-18 × TR-6023, TS-228 × 291RGI, TS-228 × R-25, TS-228 × TR-9, TS-228 × TR-6023, TS-335 × 291RGI, TS-335 × R-25, TS-335 × TR-9, TS-335 × TR-6023. Parents and F_1 hybrids are arranged in Randomized Complete Block Design (RCBD) with three replicates. Each hybrid and parental line was planted in five-meter long rows with plant-to-plant distance of 0.3m and row-to-row distance of 0.75m in each plot. A basal fertilizer doze of 120 kg $hectare^{-1}$ Nitrogen (Urea) and 60 kg $hectare^{-1}$ of Phosphorus (Diammonium Phosphate) were applied. Full doze of DAP and half doze of Nitrogen was applied at the time of sowing. While the remaining half doze of nitrogen was applied just before head initiation.

Analysis of the oil content: The oil content was determined by the method described by AOAC (Anon., 1990) and Gas Liquid Chromatography (Shimadzu, GC-9A) was carried out for determining the relative composition of the different fatty acid in oil by the method described by Martin *et al*., (1979) with slight modification. Analysis of the fatty acid profile of each genotype seed was analyzed in Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Peshawar.

Statistical analysis: The data were analyzed by Least Significance Difference (LSD) Test using MSTAT-C software for mean separation. Mid parent and High parent

heterosis were computed for each trait using the following formula (Sharma & Singh 1978 & Ahmed *et al*., 2005).

a. Mid-Parent Heterosis (%) = $(F_1 – MP/MP) \times 100$

b. High-Parent Heterosis (%)= $(F_1 – HP/HP) \times 100$

The significance of F_1 hybrids vs Mid-Parents and High-Parents means was determined via t-test as follow: -

a. t-test for mid parent = $(F_1 – MP) / \sqrt{3/8 \times \delta^2_E}$

b. t-test for high parent = $(F_1 – HP) / \sqrt{3/8 \times \delta^2_E}$

wherein,
F_1 = mean of hybrid
MP = mean of two parents for a trait in a cross (parent 1 + parent 2)/2
HP = mean of high parent for a trait in a cross.
δ^2_E = mean squares of the pooled error (MSE)

Results and Discussion

The hybrid combinations which exhibited significant differences from the mean values of the parents and the average value of the parents were analyzed for heterosis of Oleic acid (C18:1), Linoleic acid (C18:2) and Behenic acid (C20:0).

Oleic Acid (C18:1): Differences among parents and hybrids for oleic acid (C18:1) were highly significant $p<0.01$ (Table 1). Among the parents C18:1 percentage ranged from 10.26 to 13.00% indicating a net difference of 2.74%. Such variations among different hybrid have also been observed by Ahmed *et al*., (1999). Maximum C18:1 percentage was observed for TR-6023 (13%), which was significantly higher than all parents followed by R-25 (12.6%) and TS-18 (12.11%). C18:1 percentage among the F_1 hybrids ranged from 8.10 to 18.60% representing a net difference of 10.5%. Minimum C18:1 percentage was observed for TS-228 x TR-9 (8.10%), which was significantly lower than all F_1 hybrids followed by TS-335 x R-25 (8.50%), TS-335 x 291RGI (9.32%), TS-18 x 291RGI (10.66%), TS-228 x 291RGI (10.03%), while maximum C18:1 percentage was observed for TS-18 x R-25 (18.60%), which was significantly higher than all F_1 hybrids followed by TS-17 x TR-6023 (16.90%), TS-17 x 291RGI (16.10%), TS-17 x TR-9 (14.80%) and TS-17 x R-25 (14.70%).

Analysis of variance for oleic acid shows that sufficient genetic variability existed among the sunflower genotypes in this study supported those obtained by Skoric *et al*., 1978. Heterotic and heterobeltiotic effects of oleic acid were highly significant for all F_1 hybrids noted by Khalil *et al*., (2000) and Joksimovic *et al*., (2006) as similar to our study. Heterosis effect of oleic acid was positive for nine F_1 hybrids and ranged from –1.64 to 52.02%. Maximum positive heterotic effect was produced by TS-18 x R-25 (52.02%). Heterobeltiotic magnitude of oleic acid was positive for eight F_1 hybrids and ranged from 1.54 to 50.49%. Maximum positive magnitude of heterobeltiosis was expressed by TS-18 x R-25 (50.49%). The F_1 hybrid TS-18 x R-25 expressed positive values indicating that it has outperformed mid and high parent in increasing the C18: 1 percentage. Shekar *et al*., (1998) crossed 6 CMS & 5 tester and found best cross combination for oleic acid and seed yield. A slightly similar relationship was established in our study.

Table 1. Mean balues, hetertosis (MPH%) and heterobeltiosis (HPH%) for loeic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$) and behenic acid ($C_{20:0}$) in sunflower genotypes during autum.

Genotype	C18:1(#)	MPH (%)	HPH (%)	C18:2 (#)	MPH (%)	HPH (%)	C20:0 (#)	MPH (%)	HPH (%)
Parents									
TS-17	10.26 S	-	-	77.90 E	-	-	0.80 G	-	-
TS-18	12.11 L	-	-	74.32 I	-	-	0.70 H	-	-
TS-228	10.86 Q	-	-	78.80 C	-	-	0.23 M	-	-
TS-335	11.87 N	-	-	74.10 J	-	-	0.27 L	-	-
291RGI	12.00 M	-	-	73.02 K	-	-	1.10 C	-	-
R-25	12.36 G	-	-	75.12 H	-	-	0.97 E	-	-
TR-9	11.02 P	-	-	75.81 G	-	-	0.30 K	-	-
TR-6023	13.00 H	-	-	66.00 O	-	-	0.70 H	-	-
Mean	**11.69**	-	-	**74.39**	-	-	**0.63**	-	-
Hybrids									
TS-17×291RGI	16.10 C	44.7**	34.17**	68.35 N	-9.4**	-12.27**	0.70 H	-26.3	-36.36
TS-17×R-25	14.70 E	29.97**	18.93**	69.40 M	-9.30**	-10.92**	0.00 O	-100.00	-100.00
TS -17×TR-9	14.80 D	39.10**	34.30**	74.50 I	-3.07**	-4.38**	0.00 O	-100.00	-100.00
TS-17×TR-6023	16.90 B	45.31**	30.00**	78.30 D	8.82**	0.50**	0.90 F	20.00	12.50
TS-18×291RGI	10.66 R	-11.57**	-11.97**	78.67 C	6.79**	5.85**	0.00 O	-100.00	-100.00
TS-18×R-25	18.60 A	52.02**	50.49**	63.70 Q	-14.75**	-15.20**	1.40 A	67.66	44.33
TS-18×TR-9	12.83 I	10.94**	5.95**	75.84 G	1.03**	0.04	0.49 I	-2.00	-30.00
TS-18×TR-6023	11.20 O	-10.79**	-13.85**	81.52 A	16.19**	9.69**	0.80 G	14.29	14.29
TS-228×291RGI	10.03 T	-12.25**	-16.42**	79.09 B	4.19**	0.37**	0.20 N	-69.92	-81.82
TS-228×R-25	11.80 N	1.64**	-4.53**	63.60 QR	-17.36**	-19.29**	1.20 B	100.00	23.71
TS-228×TR-9	8.10 W	-25.96**	-26.50**	81.35 A	5.23**	3.24**	0.00 O	-100.00	-100.00
TS-228×TR-6023	13.20 G	10.65**	1.54**	64.50 P	-10.91**	-18.15**	1.40 A	201.08	100.00
TS-335×291RGI	9.32 U	-21.91**	-22.33**	76.42 F	3.89**	3.13**	0.31 K	-54.74	-71.82
TS-335×R-25	8.50 V	-29.84**	-31.23**	59.60 S	-20.12**	-20.66**	1.00 D	61.29	3.09
TS-335×TR-9	13.40 F	17.08**	12.89**	72.20 L	-3.68**	-4.76**	0.40 J	40.35	33.33
TS-335×TR-6023	12.20 K	-1.89**	-6.15**	63.40 R	-9.49**	-14.44**	1.20 B	147.42	71.43
Mean	**12.65**			**71.90**			**0.63**		
LSD%	**0.084**			**0.210**			**0.015**		

*, ** Heterotic and Hetrobeltiotic effects significant at 5 and 1% probability levels respectively.

Means in a column sharing same letter(s) are not significantly different at 5% probability level.

MPH = Mid-Parent Heterosis, **HPH** = High-Parent Heterosis

Linoleic acid (C18:2): Variation for linoleic acid (C18:2) was highly significant $p<0.01$ among parents and their F_1 hybrids (Table 1). Parents for C18:2 ranged from 66 to 78.80% indicating net difference of 12.8% slightly similar variations in linoleic content have also been observed by Ahmed *et al*., (2001). Minimum C18:2 percentage was observed in case of TR-6023 (66%), which was significantly lower than all parents followed by 291RGI (73.02%) and TS-335 (74.10%) and TS-18 (74.32%), while maximum C18:2 percentage was observed for TS-228 (78.80%), which was significantly higher than all parents followed by TS-17 (77.90%), TR-9 (75.81%) and R-25 (75.12%). F_1 hybrids for C18:2 ranged from 59.60 to 81.52% representing a net difference of 21.9%. Maximum C18:2 percentage was observed for TS-18 x TR-6023 (81.52%), which was significantly higher than all F_1 hybrids except TS-228 x TR-9 (81.35%) followed by TS-228 x 291RGI (79.09%), TS-18 x 291RGI (78.67%) and TS-17 x TR-6023 (78.30%) as presented in Table 1.

The positive effects of the heterosis for C18:2 in cross between cultivated and wild sunflower. Fernandez & Knowles (1987) and Joksimovic *et al*., 2006) found that the gene for high C18:2 content were dominant in respect to the gene high C18:2 as similar to our study. Sunflower genotypes for linoleic acid have sufficient genetic variability. Heterotic magnitude was significant for all F_1 hybrids while heterobeltiotic effect was also significant except TS-18 x TR-9. Heterosis was negative for nine hybrids and ranged from –3.07 to 16.19%. Khalil *et al.*, (2000) evaluated ten sunflowers for oil content and fatty acid composition. They found variation in the content of C18:2 among the hybrids.

Maximum positive high parent heterosis of linoleic acid was observed for TS-18 x TR-6023 (16.19%). Heterobeltiotic effect of linoleic acid was negative for nine F_1 hybrids and ranged from –20.66 to 9.69%.

Behenic acid (C20:0): Sufficient genetic variability ($p<0.01$) existed among the sunflower genotypes for C20:0 as evident from Table 1. Mean values of behenic acid for parents ranged from 0.23 to 1.10% for behenic acid percentage indicating a net difference of 0.87%. Minimum percentage of C20:0 was observed for TS-228 (0.23%), which was significantly lower than all parents followed by TS-335 (0.27%), TR-9 (0.30%) and TS-18 and TR-6023 (0.70%). Mean values of the (C20:0**)** for F_1 hybrids ranged from 0 to 1.4% indicating a net difference of 1.4%. Maximum values of (C20:0**)** percentage was observed for TS-18 x R-25 and TS-228 x TR-6023 (1.40%), which was significantly higher than all F_1 hybrids followed by TS-228 x R-25 and TS-335 x TR-6023 (1.20%) as presented in Table 1.

Heterosis was positive for eight F_1 hybrids and ranged from –100 to 201.08%. Maximum negative heterosis was expressed by TS-17 x TR-9, TS-17 x R-25, TS-18 x 291RGI and TS-228 x TR-9 (-100%). Heterobeltiotic effect was positive for 8 F_1 hybrids and ranged from -100 to 100%. Maximum negative heterobeltiotic effect was expressed by TS-17 x R-25, TS-17 x TR-9, TS-228 x TR-9 and TS-18 x 291RGI (-100%), indicating that these F_1 hybrids have outperformed mid and better parent values in reducing C20:0 percentage.

Based on mean performance and mid and high parent heterotic effects for morpho-physiological traits, parent of these 8 hybrids are suggested for use in sunflower breeding program.

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