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Research Article

Heterosis Expression Analysis and its Impact on Different Agro-Morphological Characters in Sunflower (*H. Annuus* L.) Hybrids

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Abstract | This study was carried out at National Agricultural Research Centre (NARC), Islamabad during 2016-17 with the objective to assess the impact of heterosis on different Agro-morphological traits in sunflower hybrids. Randomized Complete Block Design (RCBD) was used with three replications. The experimental material consisted of nine hybrids, six inbred lines and a commercial check (Hysun-33). The recorded data on days to flowering initiation (DFI), days to flowering completion (DFC), days to maturity (DM), plant height (PHT, cm), stem thickness (ST, mm), head diameter (HD, cm), 100-grain weight (100-GW, gm), oil content percentage (OC %) and seed yield (SY, kg ha^{-1}) revealed statistically significant differences. All the crosses out-performed the standard check in terms of seed yield (kg ha^{-1}) with significant differences in this regard. Positive and significantly high mid-parent heterosis was observed in all the hybrids for all the studied traits except DFI, DFC and DM which showed negligibly low impact of heterosis on their expression. Comparatively higher and positive heterosis was exhibited by all hybrids for traits such as PHT (52.16-84.91 %), ST (2.83-191.85 %), HD (19.98-68.49 %) 100-GW (12.21-64.44 %) and SY (kg ha^{-1}) (125.47-175.21 %) relative to parental average. The highest magnitude of heterosis for seed yield was exhibited by the cross CMS-77 x R-83(1) (175.21 %) relative to mid-parent values. The impact of mid-parent heterosis on the oil content (%) remained negligible. Similarly, all the hybrids showed positive and significant heterosis for PHT (61.36-101.44 %), ST (8.59-134.22 %), HD (1.87-33.20 %), 100-GW (-13.76-32.07 %) and SY (kg ha^{-1}) (32.80-56.01 %) relative to better-parents. The remaining traits studied did not show appreciable effect of heterosis over better-parents. Overall, the heterotic effects were more prominent on the PHT, ST, HD, 100-GW and SY (kg ha^{-1}) as compared to other traits both relative to mid- and better-parent values. The hybrid vigor (%) in the hybrids over the standard check regarding seed yield ranged from 4.84 % in the cross CMS-77 x R-18 to 20.09 % in the cross CMS-77 x R-83(1). The discovery of Cytoplasmic Male Sterility (CMS) and fertility restoration genes gave a new direction to heterosis based sunflower breeding and consequently revolutionized sunflower seed sector. The high impact of heterosis on the yield and other yield contributing traits in the hybrids, as revealed in this study, offers good prospects for the future use of hybrid breeding in sunflower if genetically more diverse germplasm is incorporated in breeding programs.

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Introduction

Sunflower (*Helianthus annuus* L.) is an important edible oil crop and ranks third in terms of its global edible oil production after soybean and rapeseed mustard (Shabir et al., 2010). Edible oil is an important constituent of our daily diet in Pakistan and its demand in the country has increased quite consistently over the years due to exponential increase in our population. The rising demands of edible oil in the country can be met either through increasing the area under oilseed crops or through the use of high yielding hybrids/varieties coupled with the use of highly advanced research based optimum production technology. The option of cultivating oilseeds crops on a larger area has a slim possibility due to diminishing land and water resources. The open option of using high yielding hybrids/varieties with wide adaptability in unison with the scientifically proven/recommended best production technology/management practices can lead to the maximum realization of around 70% (www.parc.gov.pk......oil-seed-program) untapped yield potential of the oilseed crops.

Breeding sunflower hybrids with high oil and seed yield potential has been the major goal in breeding programs around the world (Fick and Miller, 1997; Kaya, 2005). Research work on this crop has shown that there is great potential of growing it under all the soil and climatic conditions in rain-fed as well as irrigated farming system (Shah et al., 2005; Skoric, 2016). Availability of hybrid seed, moderate production requirements plus high seed and oil yield potential have made sunflower an attractive choice for the farmers. Sunflower oil is generally considered a premium quality oil because of its light color, high level of unsaturated oleic acid (29 %) and lenoleicacid (59%) and lack of linolenic acid, bland flavor and high smoke points. As contrasted to soybean, sunflower derives 80% of its value from it oil content (Putnam et al., 1990).

The discovery of the cytoplasmic male sterility system (Leclerq, 1969) and effective male fertility restoration genes (Enns et al., 1970; Kinman, 1970; Leclerq, 1971; Vranceanu and Stoenescu, 1971) in sunflower and the manifestation of high level of hybrid vigor in the economically important traits in the F1 hybrids in the crop and its utilization on commercial scale has led to its acceptance as one of the important vegetable oil crops in the world (Shabir et al., 2010; Encheva et al., 2015). High level heterotic expression in sunflower hybrids over the parents has led to the complete replacement of open pollinated varieties by hybrids and now, in the country, almost 99 % of the area under sunflower is planted to hybrid seed (www.parc.gov.pk......sunflower). Sunflower global acreage and production have increased with the introduction of hybrids due to their way higher yield potential as compared to the open pollinated varieties (OPVs). In Pakistan, almost 100 % of the acreage under sunflower mostly in lower Sindh and Sothern Punjab is planted to imported hybrid seed which is very expensive and its availability in right quantity and at the right time has also been an issue. Locally developed sunflower hybrids would lead to not only bringing down the cost of sunflower production and so making it more attractive for farmers but will also enhance its area and productivity in the country.

Considerable yield increase has been achieved in sunflower through heterotic breeding (Hladni, 2007) and further improvement seems possible if genetically more diverse germplasm is used in the development of sunflower parental inbred lines (Fick, 1983; Seiler, 1992). In the present study, nine F_1 hybrids made from six locally developed highly uniform *CMS* and restorer lines were tested for the expression of heterosis in the important agro-morphological characters. The objective, in this study, was to see the level of heterotic expression of the important traits relative to parents and standard check and to choose the crosses with the combination of agronomic traits having the best heterotic expression over parents and standard check for further testing and evaluation.

Materials and Methods

This research work including the development of parental lines, making crosses and the consequent heterotic impact on the expression of important agro-morphological traits experiment was carried out at Oilseeds Research Program, National Agricultural Research Centre (NARC), Islamabad, Pakistan.

The inbred lines both CMS and restorer lines were developed through selection from heterogeneous parental lines of a local sunflower hybrid PARSUN-3 though manual selfing, crossing and backcrossing for nine generations to gain homozygosity within the parental lines to the highest possible degree. Bagging was used to prevent the material from contamination by unwanted pollens. Selfing, crossing and backcrossing were done manually for development of inbred lines and F_1 hybrids and the practice was repeated every other day until the completion of flowering.

The inbred lines (six) and cross combinations (nine) were evaluated in randomized complete block design (RCBD) and the experiment was replicated three times. Each plot consisted of 5 m long four rows with 75cm and 30cm row to row and plant to plant distances respectively. Recommended production practices (www.parc.gov.pk.....sunflower) were followed right from land preparation through to the harvesting. The middle two rows were used for data collection.

Data on major agro-morphological traits; days to flowering initiation (DFI), days to flowering completion (DFC), days to maturity (DM), plan height (PHT, cm), head diameter (HD, cm), stem thickness (ST, mm), 100-grain weight (100-GW, gm), oil content (%) and seed yield (SY, kg ha^{-1}) were recorded. Flowering initiation and completion data were recorded when respectively 5 and 95 % of the heads opened. Maturity data was recorded when the back of the heads turned yellowish in color. Plant height was measured in cm from the soil surface to the point of attachment of head with the help of a two-meter rod using five randomly selected plants in each plot. Similarly stem thickness was recorded by measuring the stem diameter at 30 cm height above ground level of five randomly selected plants in each entry and for this purpose an electronic vernier caliper was used. Five randomly selected heads in each entry were used for taking data on the head diameter in cm using a 30 cm scale. A representative sample of sun dried 100 seeds from each entry was used for recording data on 100-seed weight in grams. Central two rows of each plot were harvested for recording post-harvest data. Seed yield data were recorded by first determining the moisture factor by taking the fresh and dry weight (after sun drying at 9.5 % moisture) of a seed sample from each entry and then dividing the dry weight by fresh weight of the seed sample to get the moisture factor. The fresh seed weight of central two rows in every plot was multiplied with the moisture factor followed by its conversion to the yield per hectare. Air-dried seed samples of the parental lines and crosses were used for oil content (%) determination using Nuclear-magnetic resonance (Newport Instruments Ltd., 1972) and were done by Oil Quality Lab., NARC, Islamabad.

Data recorded on different traits were subjected to analysis of variance (ANOVA) to find out if (among the genotypes) statistically significant (0.05) differences existed or not. Heterosis effect, expressed as percentage increase (+ve) or decrease (-ve) on the expression of different traits in crosses relative to respective parental average, better parent and standard parent values, was determined using formulae given below (Hayes et al., 1955).

$$\text{Mid-parent heterosis} = F1\text{-}MP/MP \times 100$$
$$\text{Better parent heterosis} = F1\text{-}BP/BP \times 100$$
$$\text{Standard heterosis} = F1\text{-}SC/SC \times 100$$

Where;
F1 stands for the mean performance of *F1* hybrid, *MP* for parental average, *BP* for better parent mean performance and *SC* for mean performance of the standard check.

Results and Discussions

The CMS lines used in the study remained stable while the restorer lines showed 100% male fertility restoration ability. The analysis of variance showed that the differences within and between the parental inbred lines and hybrids were statistically significant for days to flowering initiation (DFI), days to flowering completion (DFC), days to maturity (DM), plant height (PHT), stem thickness (ST), head diameter (HD), 100-grain weight (100-GW), oil content (OC) (%) and seed yield indicating the existence of real genetic differences (Table 1). The DFI, DFC and DM, although significantly different, but were in narrow range because the emphasis during the selection was on the synchronized flowering and maturity in the parents. The mean values of plant height in the female parents ranged from 85 cm in CMS-77 to 103 cm CMS-85 (Table 1). In the male parents, the height range was 100-114.5 cm. Plant height, based on the average of both male and female parents, was around 98.05 cm while the average plant height in the crosses was 166.3 cm showing statistical differences among the parents and crosses in terms of plant height. CMS-64(1) x R-83(1) proved to be the shortest hybrid with height of 155 cm while CMS-85 × R-83(1) turned out to be the tallest cross (172 cm). Hysun-33 used as standard check proved to be the tallest hybrid with mean value of 193 cm. The stem thickness data showed statistically significant differences within as well as between parents and hybrids.

Table 1: *ANOVA showing mean values of days to flowering initiation (DFI), days to flowering completion (DFC), days to maturity (DM), plant height (cm) (PHT), head diameter (cm) (HD), 100-grain weight (g) (100-GW), oil content (OC) (%) and seed yield (kg/ha) (SY) of 15 genotypes including six parents (3 cms & 3 restorer lines), nine crosses and a standard check (Hysun-33).*

Hybrid	DFI	DFC	DM	PHT (cm)	S.T (mm)	HD (cm)	100-GW (g)	OC (%)	SY (kg/ha)
CMS-77	88.33	92.33	117.33	84.97	8.30	12.29	3.28	37.37	1751
CMS-85	86.00	91.33	116.00	102.9	7.18	11.41	3.60	39.59	1792
CMS-64(1)	86.00	90.66	117.33	85.25	5.67	12.09	4.19	38.46	1908
R-83(1)	87.00	93.66	114.66	100.16	10.76	8.44	2.20	35.06	234
R-18	88.33	92.33	115.66	115.35	9.23	7.92	2.91	32.26	340
R-65	87.00	92.66	117.00	99.77	3.32	6.58	2.18	38.90	265
CMS-77 × R-83(1)	88.33	92.66	117.00	171.16	14.46	16.37	3.40	37.48	2732
CMS-85 × R-83(1)	86.00	90.66	115.33	171.68	9.80	14.24	3.63	37.43	2491
CMS-64(1) × R-83(1)	85.33	91.00	115.00	155.02	13.07	12.32	3.61	37.49	2555
CMS-77 × R-18	84.33	89.33	114.66	162.18	9.01	13.03	3.47	38.76	2385
CMS-85 × R-18	85.66	90.66	114.00	166.06	10.35	13.85	4.09	40.07	2472
CMS-64(1) × R-18	85.00	90.33	115.66	167.77	13.28	14.96	4.82	40.11	2534
CMS-77 × R-65	85.66	90.66	116.66	169.85	15.88	14.60	4.03	39.04	2669
CMS-85 × R-65	84.66	90.33	116.00	168.797	13.60	14.60	4.76	39.74	2590
CMS-64(1) × R-65	85.66	90.66	116.00	164.443	13.12	15.73	4.83	40.60	2628
Hysun-33	85.66	91.33	117.00	192.767	8.95	13.18	4.32	38.16	2275
Grand Mean	86.19	91.29	115.96	142.39	10.38	12.64	3.71	38.16	1976
Heritability	0.8	0.52	0.67	0.23	0.8	0.64	0.86	0.77	0.621
Genotype Variance	1.06	0.39	0.61	6.46	4.21	1.01	0.3	1.22	7069.9
Residual Variance	0.79	1.11	0.91	63.58	3.09	1.71	0.14	1.08	12952.1
LSD	1.74	2.14	1.35	13.71	2.73	1.81	0.52	1.61	170.95
CV	1.21	1.35	0.81	5.79	16.05	8.86	8.34	2.56	5.19
Genotype significance	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00

The stem thickness in the female parents ranged from 5.7 to 8.3 mm with a grand mean of 7.1 mm. While the same character in the male parents showed a range of 3.32-10.8 mm with the grand mean of 7.8 mm. The stem thickness in the crosses ranged from 9.02 cm in the cross CMS-77 × R-18 to 15.89 cm in the hybrid CMS-77 × R-65. The stem thickness in the crosses exceeded the stem thickness in their parents except CMS 85 × R-83(1) and CMS-77 × R-18 where the stem thickness was midway between their respective parents. Similar results were obtained for the head diameter data showing statistically significant differences within and between parents and crosses. The head diameter in the cross combinations was larger than their parents. The cross combinations surpassed the standard check in both the stem thickness and head diameter except two crosses, CMS 85 × R-83(1) and CMS-77 × R-18 where the average stem thickness was lower than the standard check. Stem thickness contributes to the strength of the stem, consequently enhancing lodging resistance of the crop while bigger head, being an important yield contributing trait, leads to higher seed yield.

100-grain weight, which is an important yield contributing trait, also has highly significant statistical differences within parental lines, hybrids as well as among them. All the crosses out-performed their parents in 100-grain weight except one crosses (CMS-64(1) × R-83(1)) where 100-grain weight was greater than that in the male parent (restorer) but lesser than the female parent (cms). Oil content (%) also showed highly significant statistical differences. These differences are indicative of genetic differences among the studied genotypes including parents and crosses. The differences in the seed yield/ha were also significant statistically but here as against the other studied traits; three classes emerged on the basis of yield potential.

Table 2: *Impact of heterosis on the expression level of different agro-morphic traits including flowering initiation (DFI), days to flowering completion (DFC), days to maturity (DM), plant height (cm) (PHT), Stem thickness (ST) head diameter (cm) (HD), 100-grain weight (g) (100-GW), oil content (OC) (%) and seed yield (kg/ha) (SY) using mid-parent values (%age).*

Hybrid	DFI	DFC	DM	PHT (cm)	S.T (mm)	HD (cm)	100-GW (g)	OC (%)	S.Y (kg/ha)
CMS-77 × R-83(1)	0.76	-0.36	0.86	84.91	51.72	57.91	24.10	3.49	175.21
CMS-85 × R-83(1)	-0.58	-1.98	0.00	69.08	9.29	43.40	24.96	0.28	145.87
CMS-64(1) × R-83(1)	-1.35	-1.27	-0.86	67.22	59.03	19.98	13.03	1.98	138.46
CMS-77 × R-18	-4.53	-3.25	-1.57	61.92	2.83	28.92	12.21	11.33	128.09
CMS-85 × R-18	-1.72	-1.27	-1.58	52.16	26.12	43.30	25.63	11.54	131.94
CMS-64(1) × R-18	-2.49	-1.28	-0.72	67.27	78.22	49.51	35.87	13.43	125.47
CMS-77 × R-65	-2.28	-1.98	-0.43	83.88	173.28	54.76	47.50	2.37	164.69
CMS-85 × R-65	-2.12	-1.81	-0.43	66.56	159.03	62.23	64.44	1.27	151.79
CMS-64(1) × R-65	-0.96	-1.09	-1.00	77.75	191.85	68.49	51.59	4.97	141.80
Average	-1.70	-1.59	-0.64	70.08	83.48	47.61	33.26	5.63	144.81

Restorer lines have the lowest yield potential which ranged between 200 to 350 kg/ha while the *CMS* parents were better yielders as compared to the male parents. The yield range in the three cms parents was 1700 to 1900 kg/ha. All the hybrids gave statistically higher yield as compared to their parents as well as the standard check (Hysun-33) except one cross (CMS-77 × R-18) where the yield was higher relative to the check but the difference was not statistically significant.

Heterotic effects analysis

Our study revealed either –ve or no heterotic effects on the expression of DFI, DFC and DM in the crosses relative to parental mean and better parents which is preferred in sunflower breeding and has been reported previously by other researchers as well (Phad et al., 2002). The standard heterosis for DFI, DFC and DM were zero or –ve but of negligible magnitude. The expression of important agronomic characters including PHT, ST, HD, 100-GW and SY were positively and statistically significantly influenced by the phenomenon of heterosis both relative to parental averages and better parent values. The manifestation of positive and significantly higher heterosis in the expression of important agronomic traits both relative to parental means and better parent values shows a dominant mode of inheritance and can provide a good basis for breeding superior hybrids (Skoric et al., 2006). The most prominent impact of heterosis was noted in the expression of PHT, ST, HD, SY and 100-grain weight relative to mid-parent and better parent values with an exception of CMS-64(1) × R-83(1) where –ve better-parent heterosis (-13.76) for 100-grain weight was found. Highly variable effects of heterosis on yield and other agronomic traits were also reported previously in sunflower by Kaya (2005).

Heterosis value for PHT in different crosses was +ve and highly significant and ranged from 52.16 to 84.91 % and 61.36 to101.44 % relative to parental means and better parent values (Table 2 and 3). As regards percent heterosis over standard check, all the crosses recorded -ve heterosis for plant height (Table 4) but in this case, it is ok because medium plant height in sunflower hybrids of around 150 cm is more preferable than the taller hybrids like Hysun-33 which reaches up to 200 cm in height or even goes beyond that. Taller hybrids are more vulnerable to falling over in windy/stormy and wet weather during monsoon in the country. Very visible heterotic effects were also observed on ST where its impact ranged from 2.83 % to 191.85 % and 8.59 % to 134.22 % relative to mid-parent and better parent values respectively. CMS-77 × R-18 had negligible heterotic effect (2.83 %) on the stem thickness while the impact of heterosis on the same trait was highest in the cross CMS-64(1) × R-65 (191.85 %) as compared to the parental averages. The level of heterotic effect, as regards the better parent values, was the highest in the cross CMS-64(1) × R-18 (134.22 %) followed by CMS-64(1) × R-65 (131.45 %) and CMS-64(1) × R-83(1) (130.51%) (Table 3). The results show that the crosses involving CMS-64(1) as female parent had over double stem thickness as compared to the female parent (heterobeltiosis). The crosses also showed +ve standard parent heterosis (Table 4).

Table 3: *Expression level of different agro-morphic traits including flowering initiation (DFI), days to flowering completion (DFC), days to maturity (DM), plant height (cm) (PHT), Stem thickness (ST), head diameter (cm) (HD), 100-grain weight (g) (100-GW), oil content (OC) (%) and seed yield (kg/ha) (SY) in nine hybrids as influenced by heterosis using better parent (CMS) (%age).*

Hybrid	DFI	DFC	DM	PHT (cm)	S.T (mm)	HD (cm)	100-GW (g)	OC (%)	S.Y (kg/ha)
CMS-77 × R-83(1)	0.00	0.36	-0.28	101.44	74.23	33.20	3.65	0.29	56.01
CMS-85 × R-83(1)	0.00	-0.73	-0.57	66.82	36.58	24.73	0.65	-5.46	39.01
CMS-64(1) × R-83(1)	-0.78	0.37	-1.99	81.85	130.51	1.87	-13.76	-2.53	33.87
CMS-77 × R-18	-4.53	-3.25	-2.27	90.87	8.59	6.02	5.78	3.72	36.16
CMS-85 × R-18	-0.39	-0.73	-1.72	61.36	44.15	21.37	13.49	1.21	37.95
CMS-64(1) × R-18	-1.16	-0.37	-1.42	96.81	134.22	23.73	15.12	4.28	32.80
CMS-77 × R-65	-3.02	-1.81	-0.57	99.89	91.33	18.85	22.82	4.47	52.40
CMS-85 × R-65	-1.55	-1.09	0.00	64.01	89.46	27.91	32.07	0.39	44.54
CMS-64(1) × R-65	-0.39	0.00	-1.14	92.90	131.45	30.13	15.35	5.56	37.71
Average	-1.16	-0.69	-1.31	75.32	5.44	2.52	10.57	0.47	44.58

Table 4: *Percentage increase (+) or decrease (-) over the standard parent in different agro-morphic traits including flowering initiation (DFI), days to flowering completion (DFC), days to maturity (DM), plant height(PHT) (cm), stem thickness (ST) (mm), head diameter (HD) (cm), 100-grain weight (100-GW) (g), oil content (OC) (%) and seed yield (SY) (kg/ha) of nine crosses.*

Hybrid	DFI	DFC	DM	PHT (cm)	ST (mm)	HD (cm)	100-GW (g)	OC (%)	SY (kg/ha)
CMS-77 × R-83(1)	2.33	2.20	0.00	-11.40	61.68	24.20	-21.06	-1.81	20.09
CMS-85 × R-83(1)	0.00	0.00	-1.71	-10.88	9.61	8.04	-15.97	-1.94	9.49
CMS-64(1) × R-83(1)	-1.16	0.00	-1.71	-19.69	46.03	-6.53	-16.44	-1.78	12.31
CMS-77 × R-18	-2.33	-2.20	-1.71	-16.06	0.78	-1.14	-19.44	1.55	4.84
CMS-85 × R-18	0.00	0.00	-2.56	-13.99	15.64	5.16	-5.32	4.98	8.66
CMS-64(1) × R-18	-1.16	-1.10	-0.85	-12.95	48.38	13.51	11.57	5.08	11.38
CMS-77 × R-65	0.00	0.00	0.00	-11.92	77.54	10.85	-6.48	2.28	17.32
CMS-85 × R-65	-1.16	-1.10	-0.85	-12.44	51.96	10.77	10.19	4.14	13.85
CMS-64(1) × R-65	0.00	0.00	-0.85	-15.03	46.59	19.42	11.81	6.39	15.52

Thicker stems lead to their strength which consequently enhances lodging resistance of the crop under strong winds and flooding. As regards head diameter, an important selection criterion in sunflower breeding and positively yield correlated attribute, the mid-parent and better-parent heterosis values were positive and significant where they ranged from 19.98 to 68.49 % and 6.02 to 33.20 % respectively. The standard parent heterosis in case of HD was also positive for all the crosses except CMS-64(1) × R-83(1) and CMS-77 × R-18 where respectively -6.53 and -1.14 % decrease in the HD over the check was observed (Table 4).

100-grain weight, another important yield contributing factor in sunflower because of its strong and positive correlation with seed yield, revealed significant increase in crosses due to heterotic effects where the highest impact was noted in the crosses CMS-85 × R-65, CMS-64(1) × R-65and CMS-77 × R-65 with 64.44, 51.59 and 47.50 % mid-parent heterosis values respectively. Similarly, CMS-85 × R-65 had highest +ve better parent heterosis for 100-grain weight (32.07 %). However one exception was noted where –ve and significantly different better-parent heterotic effect (-13.76 %) was noted for cross CMS-64(1) × R-83(1) (Table 3). Six of the nine crosses recorded –ve standard parent heterotic impact on 100-grain weight ranging from -21.06 % in CMS-77 × R-83(1) to -5.32% in CMS-85 × R-18 (Table 4). However, three crosses CMS-64(1) × R-18, CMS-85 × R-65 and CMS-64(1) × R-65 showed +ve standard parent heterosis (Table 4).

Oil content (%) proved to be a character with least impact of both mid and better parent heterosis on its expression in the crosses where mid-parent heterosis values ranged from 0.28 % to 13.43 % while better parent heterotic values ranged from -5.46 % to 5.56 %. Similarly, standard parent heterosis was either –ve or +ve but of negligible level which ranged from -1.94 % in CMS-64(1) × R-83(1) to 6.39 % in CMS-64(1) × R-65. It showed that the oil content (%) was not affected by heterosis to large extent as seen in other traits. Similar results were reported by Encheva et al. (2015) regarding no or low impact of heterosis on the expression of different traits in sunflower hybrids relative to parental means or better parent values. The reason may be oil content in sunflower is capped genetically and cannot be increased beyond certain level. However, lack of +ve impact of heteros in the expression of economically important characters can be compensated by selection of parents with higher level of those characters.

Positive and significant improvement in the per unit area seed yield through heterotic effects relative to both parental means and better parent values as well as standard check was noted in all the crosses in this study. Higher seed yield in these crosses augers well for productivity enhancement in sunflower through hybrid breeding using diverse germplasm (Vranceanu, 1998). The highest level of heterosis for yield relative to parental average was recorded by the cross CMS-77 × R-83(1) which was 175.21% followed by the crosses CMS-77 × R-65 and CMS-85 × R-65 with values of 164.69 and 151.79 % respectively (Table 2). The lowest level of hybrid vigor was noted in the cross CMS-64(1) × R-18 which was 125.47 % as compared to the mid-parent yield (Rosa et al., 2002). Similar results were obtained for the above crosses as regards heterotic impact on the yield relative to better parent values (Table 3). All the crosses showed +ve and significantly high impact of heterosis on the average seed yield as compared to the standard check which ranged from 4.84 % in CMS-77 × R-18 to 20.09 % in CMS-77 × R-83(1). Only one cross, CMS-77 × R-18, had although +ve standard parent heterosis value for yield (4.84 %) but the impact was non-significant (Table 1 and 4). Similar results regarding considerable heterosis effects on seed yield were reported by other researchers (Reddy et al., 1985; Singh et al., 1984; Guo-Zhan and Chun-Fang, 1985; Kulkarni and Supriya, 2017).

Conclusions and Recommendations

The statistical differences within and between parental lines and resulting cross combinations indicate real genetic differences and heterotic impact of high magnitude on the important agronomic traits was expected. Highly significant heterosis impact on PHT, ST, HD, 100-GW, seed yield and OC % (upto some extent) both relative to parental mean and better parent was noted. The highest level of heterosis effect was recorded by the seed yield (125.47 to 175.21 %) relative to mid-parent values and (32.80 to 56.01 %) relative to better parent values. Negative or no heterosis observed for DFI, DFC and DM relative to both mid-parent and better parent values show that the impact of heterosis was not the same for all the agronomic traits. The pre-requisite for a successful sunflower hybrid breeding is the availability of information about the impact of heterosis on important agronomic traits in the crosses. Reasonable increase (up to 20 %) in the seed yield in the F_1 hybrids as manifested by +ve and significant heterosis relative to currently grown popular commercial sunflower hybrid used as check (Hysun-33) is very encouraging for us. Genetically diverse parental lines can enhance the chances of +ve and higher heterosis effect on yield and yield contributing traits as is based on the theory, the greater the level of heterozygosity the higher the performance. Characters with no heterosis can be compensated by the selection of parents with high level of expression of those traits like OC (%). Relative to the standard check, the hybrids with high seed yield, medium plant height, thicker stem and bigger head diameter in combination with comparatively higher 100-GW and OC (%) can be considered for further study before their recommendation for release as commercial sunflower hybrids.

Author's Contribution

Ihsan Ullah Khan developed inbred lines, hybrid combinations and wrote the paper; Muhammad Arshad provided technical and financial support for the whole field work; Muhammad Ayub Khan provided the base material and technical help with the paper writing; Muhammad Ashraf analysed the data; Ashiq Saleem helped with the agronomic aspects of the

experiment in the field; Sundas Awan recorded the pre-harvest data; Samra Azam recorded the post-harvest data and Shamim Ul Sibtain Shah helped with field preparation, experimental lay-out and general look after.

References

Encheva, J., G. Georgiev and E. Penchev. 2015. Heterosis effects for agronomically important traits in sunflower (Helianthus annuus L.). Bulg. J. Agric. Sci. 21. (No 2) 2015: 336–341.

Enns, H., D.G. Dorrell, J.A. Hoes and W.O. Chubb. 1970. Sunflower research, a progress report. pp. 162–167. In: Proc. 4th Int. Sunflower Conf., Memphis, Tennessee.

Fick, G.N. 1983. Genetics and breeding of sunflower. J. Am. oil Chem. Soc. Volume 60, (7): pp. 1252–1253. https://doi.org/10.1007/BF02702092

Fick, G.N. and J.F. Miller. 1997. Sunflower breeding. In: A.A. schneiter (ed.) sunflower technology and production. ASA, SCSA and SSSA Monograph. No: 35. Madison, WI, 395-440.

Guo-Zhan, L. and G. Chun-Fang. 1985. Heterosis and its utilization in sunflower. In: Proc. 11th Int. Sunflower Conf. Mar. Del. Plata, Argent. March 10-13, 805.

Hladni, N., D. Škorić, M. Kraljević-Balalić, Z. Sakač and V. Miklič. 2007. Heterosis for agronomically important traits in sunflower (*H. annuus* L.) Helia, 30, Nr. 47: pp. 191-198. https://doi.org/10.2298/HEL0747191H

Hayes, H.K., F.R. Immer and D.C. Smith. 1955. Methods of plant breeding. Mc. grow hill book. Co., Inc., New York.

Kaya, Y. 2005. Hybrid vigor in sunflower (*Helianthus annuus* L.). Helia. 28, Nr. 43, pp. 77-86.

Kinman, M. 1970. New developments in the USDA and state experiment station sunflower breeding programs. Proc. 4th Int. Sunf. Conf. Memphis, Tenn. USA, pp. 181-183.

Kulkarni, V.V. and S.M. Supriya. 2017. Heterosis and combing ability studies for yield and yield component traits in sunflower (*H. annuus*). Int. J. Curr. Microb. App. Sci. 6(9): 3346-57. https://doi.org/10.20546/ijcmas.2017.609.413

Leclereq, P. 1969. Une sterilite male cytoplasmique chez le tournesol. Annales. del'. Amelioration. Des. plantes. 19: 99-106.

Leclercq, P. 1971. La sterilite male cytoplasmique du tournesol. I. Premieres etudes sur la restauration de la fertilite. Ann. Amelior Plant. 21: 45–54.

Phad, D.S., B.M. Joshi, M.K. Ghodke, K.R. Kamble and J.P. Gole. 2002. Heterosis and combining ability analysis of sunflower (Helianthus annuus L.). J. Maharashtra Agric. Univ. 27(1): 115-117.

Putnam, D.H., E.S. Oplinger, D.R. Hicks, B.R. Durgan, D.M. Noetzel, R.A. Meronuck, J.D. Doll and E.E. Schulte. 1990. Sunflower, Altern. Field Crops Mannual. (www.hort.purdue.edu/newcrop/afcm/sunflower.html)

Reddy, P.S., M.V. Reddy, M. Lawrence and N.D.R.K. Sarma. 1985. Heterobeltiosis for seed yield and oil content in sunflower (*H. annuus* L.). India. J. Genet. Plant Breed. 45(1): 166-170.

Rosa, A., H. Leon, G. Martinez and F. Rincon. 2002. Heterosis, combining ability and genetic diversity in commercial hybrids of maize. Agron. Mesoamerica. 11: 113-122.

Seiler, G.J. 1992. Utilization of wild sunflower species for the improvement of cultivated sunflower. Field Crops. Res. 30: 195-230. https://doi.org/10.1016/0378-4290(92)90002-Q

Shabir, H.W., K.S. Hitesh, G. Vikas, M.A. Bhat and N.B. Singh. 2010. Present status and future prospects for heterosis breeding in sunflower (*Helianthus annuus* L.). Asian J. Sci. Tech. Vol. 2: pp. 049-054.

Shah, A.N., H. Shah and N. Akmal. 2005. Sunflower area and production variability in Pakistan: Opportunities and constraints. Helia. 43: 165-178. https://doi.org/10.2298/HEL0543165A

Singh, S.B., K.S. Labana and D.S. Virk. 1984. Heterosis in variety × inbred crosses of sunflower. Crop Improv. 11(1): 33-38.

Skovic, D.S., D. Joccic and Jovanovic. 2006. Achievements of sunflower breeding. Period. Inst. Field Veg. Crops Novi. Sad. 42: 131–173 (Sr).

Škorić, D. 2016. Agricultural and biological sciences: Abiotic and biotic stresses in plants, recent advances and future perspectives. Chapter on sunflower breeding for resistance to abiotic and biotic stresses. Edited by Arun K. Shanker and Chitra Shanker, ISBN, 978-953-51-2250-0.

Vranceanu, A.V. and F. Stoenescu. 1971. Pollen restorer gene from cultivated sunflower (*Helianthus annuus* L.). Euphytica. 20 (4): 536–541.

Vranceanu, A.V. 1998. Sunflower. In: S.S. Banga and

S.K. Banga (ed). Hybrid cultivar development. Narosa publishing house New Delhi, India. pp. 381-400. https://doi.org/10.1007/978-3-662-07822-8_16 www.parc.gov.pk/index.php/en/csi/137-narc/crop-sciences-institue/713-oil-seed-program. www.parc.gov.pk/index.php/en/csi/137-narc/crop-sciences-institue/718-sunflower.