Breeding Progress for morphology and genetic pattern in *Helianthus annuus* L.

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Abstract: Two field experiments were conducted during 2010-2011 and 2011-2012 to evaluate the achene yield of single cross (F_1) sunflower hybrids. Seven lines and three testers were crossed in Line × tester fashion and cross combination (A-18 × G-79) was found the most promising early mature hybrid for plant height, head diameter, 100 achene weight and achene yield/plant. It was found that plant height and 100 achene weight had positive and significant SCA effects for cross combination A-18 × G-79. The additive variances (σ^2A) was more for plant height, head diameter, 100 achene weight and achene yield per plant as compared to dominance variances (σ^2D) and high heritability was found in these traits. Heterosis and heterobiltosis of plant height, 100-acehne weight and achene yield/plant were found the most significant ($p^* < 0.01$) for cross combination A-18 × G-79. The present study results demonstrated that A-18 × G-79 hybrid has potential to obtain high yielding genotypes in arid/semiarid regions such as Pakistan.
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Introduction

Sunflower (*Helianthus annuus* L.) is an important oilseed crop, belongs to the genus *Helianthus* and family 'Asteraceae'. It is native to Southern part of USA and Mexico. Sunflower, in Pakistan is cultivated on 443.2 thousand hectares, whereas, seed production is 643 thousand tons and oil production is 244 thousand tons (Khan *et al*. 2008). Among the non-traditional oilseed crops, sunflower has shown the great potential under our agro-ecological environment. It is most important oilseed crop and is widely grown for edible oil in different countries of the world. Sunflower is a short duration crop (90-110 days) and can be grown profitably twice a year under irrigated as well as rainfed conditions. Sunflower, being a highly cross pollinated crop, is ideally suited for exploitation of heterosis. Heterosis breeding in sunflower evolved successfully ever since the discovery of first cytoplasmic male sterility (CMS) source (Leclercq, 1969) and fertility restoration (Kinman, 1970) that gave the required impetus to commercial hybrid seed production. Since then many hybrids have been released for commercial cultivation both by public and private sectors and available variability in the parental material have been used in breeding program very efficiently. The selection of parents/inbreds in heterosis breeding programme (Habib *et al*. 2006) with good specific combining ability is very important in producing superior hybrids. For a plant breeder heritability is also helpful to predict the function and genetic causes of yield attributing traits. More over it helps to estimate genetic advance and selection could be done in multi-locational trails. The traits having higher the heritability estimates could be included in the breeding program (Nasreen *et al.,* 2011). The estimation of general combining ability (GCA) and specific combining ability (SCA) helps in identifying the potential parents/inbreds in the production of superior hybrids for seed yield (Ali et al., 2014abc; Bibi et al., 2012; Masood et al., 2014ac; Ahsan et al., 2011,2013) . The information resulting from this study may help future breeding program to develop varieties and hybrids of sunflower with high achene yield. The present studies had the following objectives: 1) Understanding genetic behavior of sunflower lines for various plant characters 2) Estimation of the general and specific combining abilities of sunflower lines 3) Selection of potential hybrids for general cultivation.

Materials and methods

Experimental layout

The experiment was conducted in the research field area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad-Pakistan. Ten parental lines were sown in crossing block in randomized complete block design during March 2009-2010. These lines were crossed in line × tester fashion to get 21 single cross hybrids. The seeds of 21 crosses and their parents were planted in the field during February 2010-2011 and 2011-2012 consecutively over a period of two years, following a randomized complete block design with three replications. The seeds were sown in 10m long rows keeping plant × plant and row × row distance 25cm and 75cm respectively. In both growing seasons all the agronomic and cultural practices were performed uniformly from sowing till harvesting. Moreover crop phenology, monthly average temperature (minimum and maximum) and rainfall are presented in (Table 1 and Fig. 1).

Genetic Material

The genetic material consisted of ten parental lines namely A-40, G-79, A-7, A-27, A-18, A-19, A-174, A-5, A-95 and A-85 of sunflower (*Helianthus annuus L.*) obtained from the Oilseeds Research Group, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad Pakistan. Out of these parental lines three were kept as male lines and seven as female lines respectively. These were grown in the crossing block during spring 2010-2011 and 2011-2012 crosses namely A-27×A-40, A-27×G-79, A-27×A-7, A-18×A-40, A-18×G-79, A-18×A-7, A-19×A-40, A-19×G-79, A-19×A-7, A-174×A-40, A-174×G-79, A-174×A-7, A-5×A-40, A-5×G-79, A-5×A-7, A-95×A-40, A-95×G-79, A-95×A-7, A-85×A-40, A-85×G-79, A-85×A-7 were made in line × tester fashion.

Breeding procedure

The flower head is protected with suitable cover before anthesis initiation in any of the florets and the cover was retained till fertilization was over in all florets. Artificial self pollination with pollen collected from the same flower or another flower of the same plant using a soft brush will enhance seed settings. Hand emasculation was done at 4 am by removing the anthers of disc floret in 3 to 4 whorls with the help of forceps and remaining florets in the head were removed. About 9-10 am the pollens are collected from the desired male parent and dusted on the emasculated head. This process was continued for 3 to 4 days. Pollens from male parent were collected in the Petri dish and pollination is done by applying the collected pollens with the help of camel hair brush on female parent. After pollination, care must be taken to avoid contamination by cleaning the hands with alcohol.

Data recording

Ten representative plants were randomly selected from each line and data were recorded on 6 plant traits viz. days to 50% flowering (numbers of days were recorded as days taken from sowing date to a stage when 50% flowers were appeared), days to maturity (numbers of days were counted when 50% plants showed maturity and recorded as number of days to maturity), plant height (plant height was measured from ground level to the base of head in centimeter (cm) with the help of measuring tape), head diameter (head diameter was measured with the help of measuring tape in centimeter squares (cm^2) and data were recorded), 100-achene weight (100 achenes were counted from heads and weighed in grams (g) with the help of electronic balance) and achene yield per plant (heads were harvested and threshed separately. Achenes per head were sun dried and weighed in grams (g) with electronic balance). After compiling the data was analyzed for the analysis of variance technique (Steel *et al.,* 1997) to study the significance level between parents and their F_1 hybrids and then subjected to the analysis of general and specific combining ability (Kempthorne 1957) and heterosis (Falconer and Mackey 1996).

Results and discussion

The accessions had significant differences among all studied traits revealed by analysis of variance P* < 0.01. Mean values (Fig. 2 a, b, c, d, e and f) for days to flowering ranged from 73 to 101, days to maturity ranged from 113 to 125, plant height from 135.47 to 203.53, head diameter from 13.1 to 31.1, 100-achene weight from 4.2 to 6.2 and achene yield per plant from 34.2 to 56.7. Table 2 showed that Line A-18 and tester G-79 were the best general combiner (GCA) thus had the positive and significant GCA effects for days to maturity, plant height, head diameter, 100-achene weight and achene yield per plant. Dedio (1993); Ghaffari *et al.* (2011); Andarkhor (2012); Ahsan et al., (2013) and Ali et al., (2014d) reported that plant height and 100 achene weight were the most contributing traits while examining GCA of seven lines and three testers. The further studies (Habib *et al*., 2007 and Aslam *et al*., 2010) also demonstrated that plant height, head diameter were the most attributing traits for achene yield/plant in sunflower. 100-achene weight also contributes to increase achene yield/plant in sunflower if fresh biomass of plant has significant effect to 100-achene weight and head diameter (Ahmad *et al*., 2005 and Devi *et al*., 2005). Similarly Devindra and Singh (2003) reported that 50 % days to flowering and days to maturity were negatively contributing traits to achene yield/plant and these results are in complete concordance with the present study as shown in (Table 2). GCA (Ansaril *et al*., 1999 and Ashoka *et al*., 2000) in parental lines while SCA in hybrids (Bajaj *et al*., 1997) is an efficient statistical tool to examine the effect of any independent trait on the dependant structure (Deniz *et al.,* 2015; Imran et al., 2014; Qasrani et al., 2014). The cross combination A18 x G79 had maximum SCA for plant height, 100-achene weight, head diameter and achene yield/plant thus found to be the most promising hybrid in the present study (Table 3).

Similar results were found by Devindra and Singh (2003) and Ahmad *et al*., (2005) when they calculated the SCA effects and found plant height and stem diameter a positive and significant contributor to achene yield/plant in 51 single cross hybrids. Previous literature also shows (Devi *et al*., 2005 and Aslam *et al*., 2010) that to obtain high achene yield hybrids in sunflower the early maturing genotypes are the possible solution. In context to this Andarkhor (2012) reported that to achieve high achene yield hybrids 50 % days to flowering and days to maturity are the factors which had negative SCA effects. The present study results (Table 3) also shows that 50 % days to flowering and days to maturity are significant and negative contributing traits to achene yield per plant for the most promising hybrid (A18 x G79). Additive and dominance variances were also estimated for all traits and the gene action was determined by calculating the degree of dominance. Table 4 results showed that the plant height, head diameter, 100 achene weight and achene yield/plant were controlled by additive type of gene action while 50 % days to flowering and days to maturity were controlled by dominance type of gene action (Table 4). Ansaril *et al*., (1999) and Ashoka *et al*., (2000) reported that the traits which had positive SCA effects these are also heritable and under the control of additive type of gene action. Similar results were found by Bajaj *et al*., (1997) who found the more additive variance as compared to dominance variance for plant height and 100 achene weight (Dedio 1993; Jinbao *et al.,* 2014; Imran et al., 2014). Mid and better parent heterosis among hybrids were observed for all traits studied. The combination A-18 × G-79 had the best performance for plant height, head diameter, 100 achenes weight and achene yield per plant and had positive and significant heterosis over mid and better parent (Table 5a and 5b). Days to flowering and days to maturity had negative and significant heterosis over both mid and better parent (Table 5a,b) for cross combination A-18 × G-79. Similar results were obtained by Bajaj *et al*., (1997) and Andarkhor (2012) when they found the positive and significant heterosis for fresh plant biomass, stem diameter and 100-acehene weight. The broad sense heritability (Table 6) results show that the selection of high achene yield genotypes may be effective on the basis of achene yield per plant and its attributing traits and these findings afe in complete concordance with the previous literature (Ashoka *et al*., 2000; Devi *et al*., 2005 and Seneviratne 2004). Present study results demonstrated that the hybrid (A-18 x G-79) had the potential to improve achene yield of sunflower and could be used for further breeding program by a plant breeder in arid/semi-arid regions.

Table 1: Crop Phenology

	Stem colour	Leaf colour	Stem Pubescence	Leaf pubescence	Leaf Serration	Head droopiness	Head shape	Achene colour
(A-18)	Light	Green	Little	Little	Little	Droopy	Flat	Grey
(G-79)	light	Green	Much	Intermediate	Intermediate	Droopy	Flat	Grey
(A-18 x G-79)	Slightly light	Green	Much	Little	Little	Droopy	Flat	Grey

Table 2: Estimation of General Combining Ability effects of sunflower lines and testers.

Lines	DTF	DTM	PH	HD	100AW	AYP
A-27	3.5*	-1.4**	17.16**	-0.39	0.14	-2.75
A-18	0.4	1.33**	27.98**	7.16**	1.08**	9.01**
A-19	-1.71*	0.33	-17.3**	2.3**	-0.30	5.13**
A-174	-0.38	0.44	-13.5*	-0.86*	-0.04	-1.17
A-5	2.62**	1.67**	-11.4**	-0.53*	-0.29	0.39
A-95	-1.38	-1.0**	3.80**	2.23**	-0.07	-3.72
A-85	-3.1**	-0.67*	-6.66	-5.2**	-0.52**	-0.35*
S.E.	0.42	0.24	3.69	0.26	0.13	1.95
Testers						
A-40	1.71*	-0.44	5.53*	-1.05*	-0.01	-1.25
G-79	-1.10	-0.11	5.25*	1.17*	0.10*	1.96*
A-7	-0.62	0.56	-10.77*	-0.12	-0.09	0.71
S.E.	0.27	0.15	2.41	0.17	0.08	1.28

Table 3: Specific Combining ability effects of the best single cross sunflower crosses.

Crosses	DTF	DTM	PH	HD	100AW	AYP
A-27 × G-79	-10.7**	-5.5**	10.0**	1.34	0.21	2.14*
A-18 × A-40	-4.49**	-1.00	-9.06**	-3.7**	-0.64	-4.1**
A-18 × G-79	-2.32*	-1.33**	11.2**	5.70**	0.91*	6.30*
A-18 × A-7	2.17*	-2.33*	-1.16	2.37*	0.38	1.75
A-19 × G-79	3.43**	2.33*	3.90**	-2.41*	-0.27	2.34*
A-19 × A-7	-0.95	-1.67	1.22**	1.63	0.08	2.34**
A-174 × A-40	-4.71**	-2.44*	-6.57**	-1.31	0.10	2.79*
A-5 × A-40	1.29	2.33*	6.14**	3.89**	0.48	2.85**
S.E. (SCA)	0.72	0.41	6.39	0.64	0.23	3.39

Table 4: Estimates of variances due to GCA, SCA, additive variance, dominance variance, GCA: SCA ratio and degree of dominance (HD= head diameter, PH= plant height, DTF= days to flowering, DTM= days to maturity, 100AW= 100-achene weight, AYP= Achene yield per plant, Cov=covariance, HS= half sib, FS= full sib)

Genetic components	Cov. H.S. (L)	Cov. H.S. (T)	Cov. H.S. (av.)	Cov. F.S.	σ^2GCA	F=1, σ^2A	σ^2 SCA	F=1, σ^2D	σ^2SCA / σ^2GCA	$(\sigma^2D/\sigma^2A)^2$
HD	11.2	-0.39	4.16	24.4	4.16	66.6	11.28	45.1	2.71	0.45
PH	246	67.84	96.9	563	96.9	1550	93.75	375	0.96	0.05
100AW	0.195	-0.02	0.071	0.359	0.071	1.13	0.17	0.69	2.43	0.37
DTF	-7.9	-3.52	0.803	24.3	0.803	12.8	39.97	159	49.77	12.43
DTM	-1.6	-1.05	0.16	5.01	0.16	2.58	9.06	36.2	56.62	197.3
AYP	21.66	-0.08	8.06	36.52	8.06	128.9	9.66	38.6	1.19	0.08

Table 5(a): Heterosis effects in the sunflower hybrids for yield related traits (HD= head diameter, PH= plant height, DTF= days to flowering, DTM= days to maturity, MP= mid parent, BP= Better parent).

Crosses	DTF		DTM		PH		HD	
	MP	BP	MP	BP	MP	BP	MP	BP
A-27 × G-79	1.6	2.8	-2.4**	-0.9	19.9**	22.4**	29**	44.8**
A-18 × A-40	14.5**	14.5**	8.2**	8.4**	29.8**	40.4**	-29.3	36.8**
A-18 × G-79	-18.6**	-20.3**	-10.9*	-12.6**	37.4**	54.8**	85.7**	84.2**
A-18 × A-7	14.9**	20.8**	5.7**	8.1**	-0.09	34.0**	56.6**	34.6**
A-19 × A-40	8.5**	11.6**	1.6	6.9**	-7.09**	-3.7*	27.1**	26.0**
A-19 × G-79	-13.9**	-15.5**	-3.1*	-7.0**	-1.13	6.6*	4.6*	1.2

Table 5(b): Hetrosis effects for sunflower hybrids for achene weight and achene yield per plant (MP= mid parent, BP= Better parent, 100AW= 100-achene weight, AYP= Achene yield per plant).

Crosses	100AW		AYP	
	MP	BP	MP	BP
A-27 × G-79	21**	16.3**	8.3**	4.6*
A-18 × A-40	6.2**	-2.1	11.2**	10.4**
A-18 × G-79	24.2**	16.9**	32.6**	32.4**
A-18 × A-7	13.3**	11.0**	19.2**	13.7**
A-19 × G-79	-16.2	-23.0	15.9**	9.3**

Table 6: Heritability estimates for sunflower yield related traits.

Traits	Heritability
DTF	0.9
DTM	0.9
HD	0.5
PH	0.7
100AW	0.6
AYP	0.9

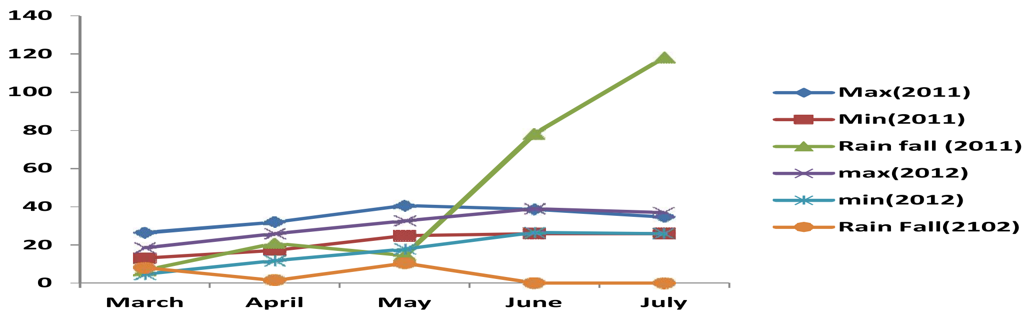

Fig. 1: Maximum and minimum data of temperature and rainfall during crop growing seasons 2011 and 2012

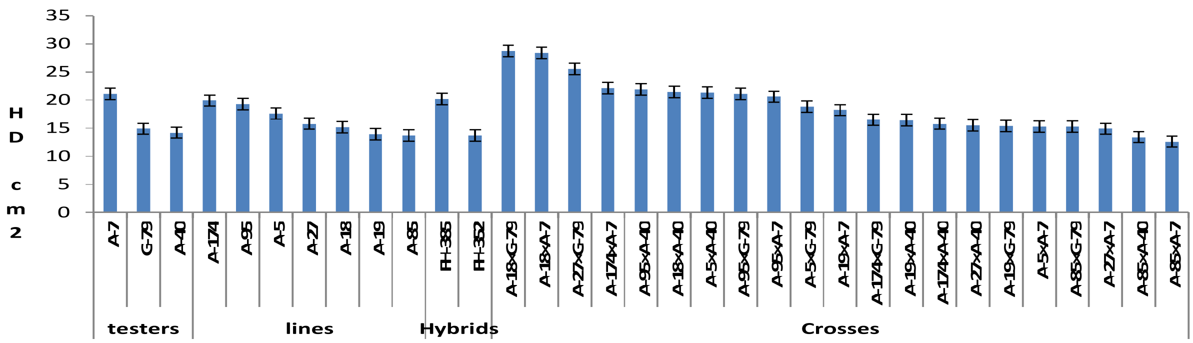

Fig. 2 a: Mean performance of sunflower lines, testers, commercial hybrid (hybrids) and single cross hybrids (F_1) for head diameter

CD_1 for Testers = 2.6 CD_1 for Lines = 4.8 CD_1 for Hybrids = 3.1
CD_1 for Crosses = 1.4
CD_2 for Testers = 4.4 CD_2 for Lines = 6.7 CD_2 for Hybrids = 2.1
CD_2 for Crosses = 1.9

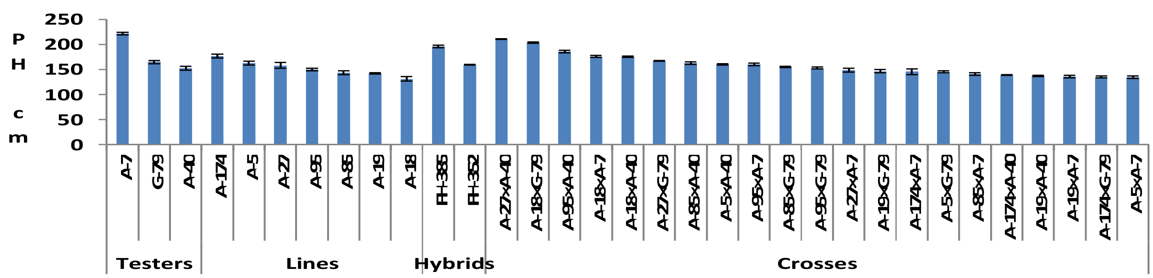

Fig. 2 b: Mean performance of sunflower lines, testers, commercial hybrid (hybrids) and single cross hybrids (F_1) for plant height diameter

CD_1 for Testers = 17.9 CD_1 for Lines = 14.0 CD_1 for Hybrids = 10.1
CD_1 for Crosses = 7.8
CD_2 for Testers = 29.6 CD_2 for Lines = 19.6 CD_2 for Hybrids = 13.2
CD_2 for Crosses = 10.5

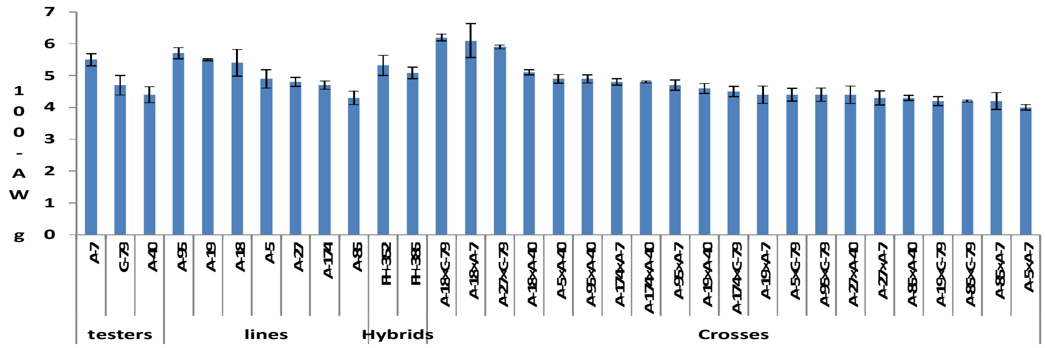

Fig. 2 c: Mean performance of sunflower lines, testers, commercial hybrid (hybrids) and single cross hybrids (F_1) for plant achene weight

CD$_1$ for Testers = 0.7 CD$_1$ for Lines = 0.4 CD$_1$ for Hybrids = 0.2
CD$_1$ for Crosses = 0.6
CD$_2$ for Testers = 1.2 CD$_2$ for Lines = 1.3 CD$_2$ for Hybrids = 0.5
CD$_2$ for Crosses = 0.8

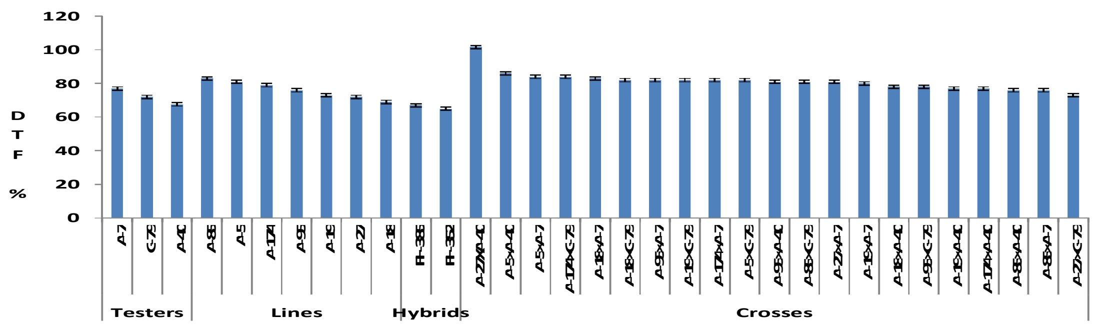

Fig. 2 d: Mean performance of sunflower lines, testers, commercial hybrid (hybrids) and single cross hybrids (F_1) for 50 % days to flowering

CD$_1$ for Testers = 3.0 CD$_1$ for Lines = 1.3 CD$_1$ for Hybrids = 1.2
CD$_1$ for Crosses = 3.1
CD$_2$ for Testers = 2.0 CD$_2$ for Lines = 1.9 CD$_2$ for Hybrids = 1.4
CD$_2$ for Crosses = 4.2

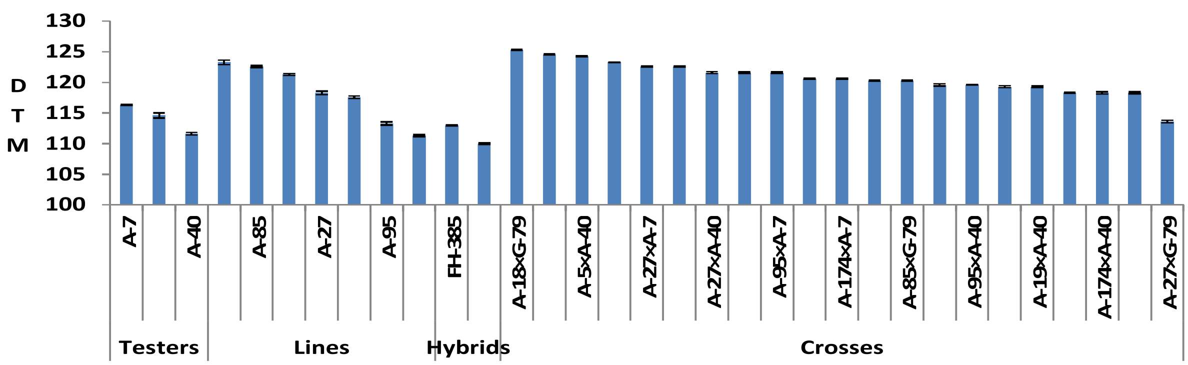

Fig. 2 e: Mean performance of sunflower lines, testers, commercial hybrid (hybrids) and single cross hybrids (F_1) for 50 % days to maturity

CD$_1$ for Testers = 1.5 CD$_1$ for Lines = 1.4 CD$_1$ for Hybrids = 1.8
CD$_1$ for Crosses = 1.1
CD$_2$ for Testers = 2.5 CD$_2$ for Lines = 2.01 CD$_2$ for Hybrids = 1.6
CD$_2$ for Crosses = 1.5

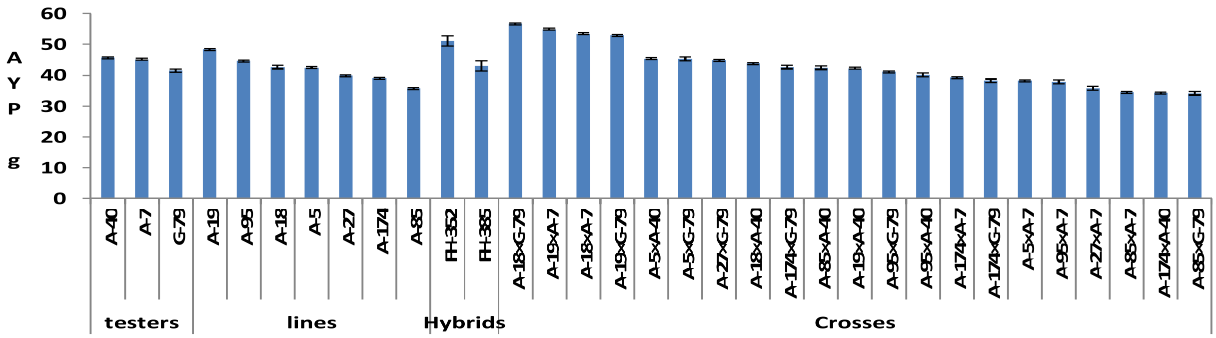

Fig. 2 f: Mean performance of sunflower lines, testers, commercial hybrid (hybrids) and single cross hybrids (F_1) for achene yield per plant

CD_1 for Testers = 2.4 CD_1 for Lines = 9.3 CD_1 for Hybrids = 6.9
CD_1 for Crosses = 7.5

CD_2 for Testers = 24.7 CD_2 for Lines = 13.1 CD_2 for Hybrids = 7.5
CD_2 for Crosses = 10.1

Conclusion

Plant height, 100-achene weight, head diameter and achene yield per plant showed positive and significant GCA effect while 50 % days to flowering and days to maturity had negative GCA effect in parental accessions: the line (A-18) and tester (G-79) and subsequent results were found for cross combination cross combinations A-18 × G-79. Furthermore the additive variance and heritability values of above mentioned traits were also high which depicts that these traits could be included by a plant breeder in hybrid developing program to increase achene yield/plant. However, multi environment trails are needed to exploit present study genome for desired traits. The present study demonstrates that these hybrids and their potential parental lines and their cross combination (A-18 × G-79) had the great importance to increase achene yield per plant in sunflower for arid/semi-arid regions like Pakistan.

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