

## **EFFECT OF MOISTURE STRESS ON COMBINING ABILITY VARIATION FOR BIRD RESISTANCE TRAITS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)**

**SAEED RAUF<sup>1\*</sup>, H.A. SADAQAT<sup>1</sup> AND A. NAVEED<sup>2</sup>**

<sup>1</sup>*Oil seeds Research Laboratory, Department of Plant Breeding & Genetics  
University of Agriculture, Faisalabad-38040, Pakistan*

<sup>2</sup>*Directorate of Research, University of Agriculture, Faisalabad-38040, Pakistan*

### **Abstract**

Experiments on sunflower were carried out under 2 moisture level in the field to study their modifying effect on phenotypic expression and combining ability of bird resistant traits i.e., degree of head shape (DHS), degree of achene compactness (DAC), degree of stem orientation (DSO) and degree of head orientation (DHO). Genotypes indicated substantial shift in their relative morphology for all traits related to bird resistance over moisture regimes. All traits exhibited larger proportion of non additive gene action under both conditions and showed an increase in proportion of non-additive genetic variation under moisture stress condition. Intermating and recurrent selection procedures could be useful breeding procedures for accumulating favorable genes responsible for better bird resistance traits and may produce superior recombinants in segregating progenies under both conditions. Under moisture stress condition, only drought tolerant parents showed peculiar morphology of traits leading to bird attack resistance. Relative sensitivity of these traits to the moisture stress has indicated their potential of acting as morphological marker for selection of drought tolerant genotypes. Among bird resistant traits, degree of head shape showed significant negative relationship with achene yield suggesting the possibility of improving yield through selection of highly concave genotype under drought stress condition.

### **Introduction**

Sunflower is an important oil seed crop of the world. It produces achenes that are rich in oil and protein contents which usually attract large number of birds to feed on it. Over the years, birds have proved to be major pest of sunflower that can cause up to 100% yield losses. In USA, black birds have caused a loss of US \$ 4 to 11 million to the national economy each year by destroying sunflower fields in three states (Linz *et al.*, 1997). Similarly, in Pakistan, large number of birds species (eg., green parrots, brown sparrows and doves) visit sunflower field which not only causes reduction to the yield but also increase the production cost in term of labor deployed for bird watching. Traditional control of birds attack includes use of explosives, puppets, sounds and farm labor to watch these birds. Although, these measures were able to control bird attack to some extent but none of them has been proved to be an absolute control of bird attack. Even in the presence of bird watchers, birds can cause up to 50% yield losses. Therefore a change in the morphology of plants is required to achieve resistance against bird attack. There are number of morphological traits such as concave head shape, droopiness, large head to stem distance that have been proved beneficial for bird attack resistance (Parfitt, 1984; Seiler & Rogers, 1987; Roger, 1988). However, little efforts have been made to breed sunflower for these traits. This may be due to lack of knowledge on the inheritance pattern and effects of environment on these traits.

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Corresponding author: saeedbreeder@hotmail.com, fax+92-041-9200161

All the traits providing bird resistance umbrella are qualitative in nature i.e. head shape can be distinctly classified as convex, concave and flat shape but it has been found that a range exists within a specific class such as less concave to extremely concave which indicate their quantitative nature along with the effect of environment on the expression of these traits. Therefore a quantitative approach must be developed to study these traits. Traits related to bird resistance studied in the quantitative manner would make it possible to apply biometrical approaches such as combining ability analysis for the formulation of breeding strategy for the improvement of these traits.

Keeping above in view, studies were carried to determine inheritance pattern of four traits i.e., degree of head shape, degree of achene compactness, degree of stem orientation and degree of head orientation under contrasting water levels.

### Materials and Methods

Experiments were carried out at the sunflower research area of the department of Plant Breeding & Genetics, University of Agriculture, Faisalabad, Pakistan during the year 2007.

**Development of plant material:** Six female lines (AMES-10103; PEM-SR-88; CM-614; HA-407; ORI-16/B; HA-350) and six male lines (RL-57, RL-52, CM-815, CM-631, RL-37, CM-619) were selected on the basis of drought tolerance (Rauf & Sadaqat, 2007a; Rauf & Sadaqat, 2008) and difference in the morphological traits leading to bird tolerance. Selected material was planted during the crop season 2006 to attempt crosses in line x tester fashion i.e., every female was crossed to each of male resulting in thirty six crosses.

**Growth conditions and experimental lay out:** The soil was a sandy-loam soil with low water retention capacity. The laboratory-measured field capacity and wilting point of the soil averaged 14% and 7% by weight, respectively, pH 7.5, organic matter 0.91%, available phosphorous 27.2 ppm, and potassium 133 ppm. The plots were fertilized with 150 kg N ha<sup>-1</sup>, 50 kg P ha<sup>-1</sup> and 0 kg K ha<sup>-1</sup> was applied. A randomized complete block design with split-plot arrangement was used and each treatment was replicated three times, where levels of water availability were assigned to main plots and genotypes to subplots. Each subplot was 4.8 m wide and 6.0 m long, consisting of eight rows of a single genotype. The inter-row spacing was 60 cm and inter-plant spacing was 30 cm. The near optimum condition was developed by irrigating the plots during the entire growth cycle to maintain the soil water content close to field capacity. In stress condition, the plots were irrigated at the same time and with the same amount of supplemental water as in the non-stress condition during the vegetative phase but supplemental irrigation was completely withheld at the beginning of button stage (R1) so as to achieve moisture stress conditions at anthesis. The soil moisture content was measured every 8-10 days during the whole growth season. Total rainfall during crop growth cycle was only 64.4 mm, of which 41.3 mm fell during the vegetative phase while 23.1 mm during the reproductive phase. Weeds were controlled manually. At maturity, measurements were made eliminating two rows at each side within single plot and within each row, eight competitive plants (plant surrounded by intact plants spaced at specified planting distance, of the same genotype and same row) were selected, thereby eliminating plants at each end. At maturity, data on the following traits were recorded:

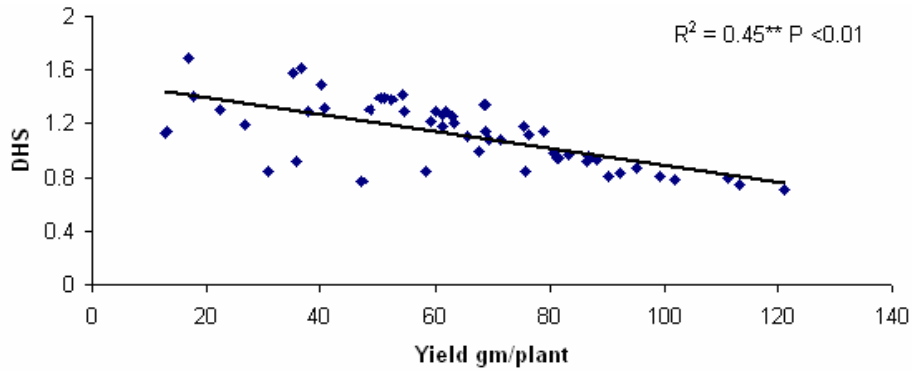


Fig. 1. Effect of degree of head shape (DHS) to the variations in achene yield (gm per plant) under moisture stress condition.



Fig. 2. Head diameter measurements (A), Concave and (B) Convex head shapes.

**i. Degree of head shape:** Degree of head shape was measured in term of ratio of head diameter (measured from front) to the head diameter (measured from back). If the value exceeded form 1, head was considered as convex while if it is equal to 1, head was flat and if it is less than 1, head was found to be concave. These values were also compared with the apparent shape of the head and found almost corresponding. Therefore a value lower than 1 correspond to concave head shape, a desirable trait (Fig. 2).

**ii. Degree of stem orientation:** Sunflower stem achieves maximum girth at its base while tapering towards apex. It was found that sunflower genotypes showing maximum difference of girth from base to head attachment also showed maximum droopiness. Therefore degree of stem orientation was measured as a ratio of stem girth at its base to stem girth at point of head attachment. Sunflower genotypes showing ratio of 0.8 to 1 or higher were found to be erect whereas genotypes having ratio of 0.50 to 0.79 were found to be partially droopy and genotypes having ratio less than 0.5 were droopy. These values were further compared with the apparent stem orientation and found corresponding (Fig. 3).



Fig. 3. (A) Droopy to semi droopy plant canopies and horizontal head orientation under non stress regime, (B). Erect plant canopy and vertical head orientation under drought stress.

**iii. Degree of achene compactness:** Degree of achene compactness was considered as number of achenes per unit area of head. It was calculated by the dividing the number of achenes per head to the total head surface area.

**iv. Degree of head orientation:** Degree of head orientation was calculated as total distance from top of head to the point of its attachment with stem divided by stem height. The value less than 0.1 resulted in vertical orientation of head while a value greater than 0.1 resulted in horizontal orientation of head, a desirable trait. These values were also compared with apparent head orientation and found completely corresponding (Fig. 3).

**Statistical and biometric procedures:** Analysis of variance (ANOVA) was carried out in RCBD with split plot arrangement having genotype and moisture levels as factors. All effects were assumed fixed. Parental breeding lines had been maintained through self-pollination (inbreeding coefficient,  $F=1$ ). Variance due to crosses was partitioned into females, males, and females  $\times$  males. Variation due to crosses  $\times$  water regimes were also partitioned into subcomponents on the same pattern. The method described by Kempthorne (1957) was adopted for combining ability analyses. Relative contribution of female parents, male parents, and females  $\times$  males to total genotypic variation was estimated from their sum of squares. Variance due to females and males was interpreted as general combining ability and that due to females  $\times$  males, specific combining ability.

## Results

**1. Variation among parental breeding lines and commercial hybrids over moisture regimes:** On an average, degree of head shape (DHS) decreased under stress regime, thus parents tended to produce concave head shape under water stress condition while parents produced convex head shape under irrigated condition. However only drought tolerant

parents produced concave head shape under stress condition (Table 1). Average degree of achene compactness (DAC) increased under stress condition when compared with non stress condition. This increase in average DAC was due to tolerant parents which showed increasing tendencies under drought condition. Average degree of stem orientation (DSO) also showed an increase under stress condition. However, this increase was found to be non significant ( $p>0.05$ ). Average values indicated the tendencies for semi droopy stem under both conditions. Average degree of head orientation (DHO) decreased showing vertical head orientation. However, drought tolerant parents showed droopy stem and horizontal head shape under drought stress condition (Table 1).

Among parental lines RL-52 showed maximum concaveness and highest value for DAC while AMES-10103 showed lowest value for DSO, thus showing maximum droopy stem under both conditions (Table 1). This line also showed highest value for DHO, thus showing maximum horizontal head orientation under drought condition. Under optimum water condition, CM-619 showed lowest value for DHS and highest value for DHO while CM-631 showed highest droopiness in this condition.

Average values of all hybrids tended to increase for DHS and DSO under stress condition showing tendencies for convex head shape and semi droopy stem under both condition (Table 2). DAC and DHO decreased under moisture stress condition when average values of hybrids were compared (Table 2). Among hybrids Paradise-21 showed maximum values for bird resistant traits i.e. concave head shape, compact achene, droopy stem and horizontal head orientation under non stress condition while Nova showed its promise under stress condition (Table 2).

Phenotypic variation among parents and commercial hybrids for all bird resistant traits was significant  $p<0.01$  (Table 3). Variation due to parent x moisture (P x W) or commercial hybrids x moisture (Cr. x W) level interaction were also significant for all traits ( $p>0.01$ ), indicating substantial shift in the relative morphology of parental lines or commercial for these traits over moisture regimes.

**2. Combining ability variations:** Significant ( $p<0.05$ ) differences existed among the crosses for all bird resistance traits (Table 3). Similarly, their interactions with moisture levels (C×W) were also significant ( $p<0.05$ ) for all bird resistant traits. Variation due to male parents over environments was non significant ( $p>0.05$ ) for all traits while female parents were significant ( $p<0.05$ ) for DHO. Among interaction components of variation, M×W was significant for DHS and DHO ( $p<0.01$ ) and F×M×W for all the traits except DSO (Table 3). This indicated that moisture regimes had significant modifying effect on general and specific combining ability variations associated with these traits

Combining ability variation for bird resistant traits was also estimated within moisture levels (Table 4). Among crosses, significant differences were observed in each regime for all the traits. However, variation due to females and males was non significant under both regimes except females showed differences for DHS and DHO under non stress regime (Table 4). This may be due to very high magnitude of male x female interaction as significance of both male and female variance was tested by dividing this interaction.

Repressing effect of moisture stress were apparent on general combining ability since its relative contribution to the total variation decreased under moisture stress regime with a corresponding increase in the relative contribution of specific combining ability (Table 4). The relative contribution of general combining ability to the total genotypic variation remained lower than that of specific combining ability in both conditions.

**Table 1. Mean phenotypic expression of parental sunflower for degree of head shape (DHS), degree of achene compactness (DAC), degree of stem orientation (DSO) and degree of head orientation (DHO) under optimum ( $W_1$ ) and stress ( $W_2$ ) for water availability.**

Parents	DHS		DAC		DSO		DHO	
	$W_1$	$W_2$	$W_1$	$W_2$	$W_1$	$W_2$	$W_1$	$W_2$
AMES-10103	1.21	0.79	4.91	10.94	0.60	0.46	0.18	0.21
PEM-SR-88	1.14	0.84	3.56	4.94	0.53	0.47	0.11	0.14
CM-614	1.43	0.92	3.02	4.00	0.50	0.48	0.09	0.12
HA-407	1.25	1.15	4.06	3.20	0.50	0.86	0.14	0.07
ORI-16/B	1.79	1.20	1.73	1.17	0.60	0.77	0.13	0.08
HA-350	0.92	1.13	5.86	4.12	0.90	0.90	0.12	0.05
RL-57	1.11	0.85	7.41	8.35	0.59	0.48	0.11	0.12
RL-52	0.98	0.77	3.85	14.65	0.52	0.41	0.11	0.12
CM-815	1.46	0.84	2.12	2.92	0.46	0.43	0.11	0.11
CM-631	1.37	1.09	3.48	3.29	0.38	0.84	0.19	0.04
RL-37	1.13	1.04	3.74	2.62	0.53	0.62	0.18	0.03
CM-619	0.87	1.29	3.77	2.36	0.47	0.61	0.20	0.04
Average	1.22	0.99	3.96	5.21	0.55	0.62	0.14	0.09
<b>LSD 0.05</b>	<b>0.13</b>		<b>0.98</b>		<b>0.10</b>		<b>0.00</b>	

**Table 2. Mean phenotypic expression of sunflower commercial hybrids for degree of head shape (DHS), degree of compactness (DAC), degree of stem orientation (DSO) and degree of head orientation (DHO) under optimum ( $W_1$ ) and stress ( $W_2$ ) for water availability.**

Parents	DHS		DAC		DSO		DHO	
	$W_1$	$W_2$	$W_1$	$W_2$	$W_1$	$W_2$	$W_1$	$W_2$
Hysun-33	1.00	1.18	5.32	2.5	0.48	0.59	0.09	0.08
Paradise-21	0.90	1.29	7.81	6.1	0.41	0.87	0.13	0.10
Sweet	1.20	1.25	3.42	3.6	0.63	0.79	0.08	0.08
Paraise-24	1.30	1.34	6.39	3.5	0.94	0.57	0.10	0.09
Nova	1.40	1.12	4.17	8.3	0.46	0.54	0.09	0.11
S-278	1.20	1.29	4.96	2.6	.91	0.65	0.09	0.09
DK-4040	1.10	1.16	7.38	4.7	0.43	0.73	0.11	0.11
Average	1.16	1.23	5.64	4.47	0.63	0.68	0.10	0.09
<b>LSD 0.05</b>	<b>0.16</b>		<b>1.01</b>		<b>0.04</b>		<b>0.00</b>	

**3. Status of parental lines for general combining ability (GCA) effects:** Performance of parental lines on the basis of GCA effects was not consistent over moisture levels (Table 5). AMES-10103 was best combiner for all traits under stress condition (Table 5). CM-619 and HA-407 showed highest negative GCA effects for DHS and DSO under optimum moisture level, thus considered best combiners for these traits. A negative or lower value was preferred since lower DHS was associated with concave head shape while DSO with droopy stem. CM-619 also showed highest GCA value for DAC and HA-407 for DHO.

**Table 3. Analysis of variance of combining ability in sunflower for degree of head shape (DHS), degree of achene compactness (DAC), degree of Stem orientation (DSO) and degree of head orientation (DHO) over water levels.**

Source of variation	D.F	DHS	DAC	DSO	DHO
Replication	2	0.00 <sup>ns</sup>	2.14 <sup>ns</sup>	0.00 <sup>ns</sup>	0.00 <sup>ns</sup>
Water level (W)	1	0.38**	69.90**	0.18**	0.00**
Error (a)	2	0.00	1.23	0.00	0.00
Genotypes (G)	54	0.09**	10.91**	0.06**	0.00**
Parents (P)	11	0.20**	29.87**	0.10**	0.01**
Commercial (Cr.)	6	0.09**	8.89**	0.17**	0.00**
P vs. C	1	0.04 <sup>ns</sup>	2.25 <sup>ns</sup>	0.11**	0.04**
C vs. Cr.	1	0.07*	11.99**	0.70**	0.00**
Crosses (C)	35	0.06**	5.56**	0.01**	0.00**
Females (F)	5	0.10 <sup>ns</sup>	2.49 <sup>ns</sup>	0.01 <sup>ns</sup>	0.00**
Male (M)	5	0.02 <sup>ns</sup>	5.42 <sup>ns</sup>	0.01 <sup>ns</sup>	0.00 <sup>ns</sup>
F x M	25	0.05**	6.20**	0.01**	0.00**
G x W	54	0.10**	8.09**	0.02**	0.00**
P x W	11	0.25**	20.60**	0.02**	0.00**
Cr. x W	6	0.05**	8.73**	0.03**	0.00**
P vs. C x W	1	0.05 <sup>ns</sup>	2.01 <sup>ns</sup>	0.05**	0.00**
C vs. Cr. x W	1	0.01 <sup>ns</sup>	42.78**	0.20**	0.00**
C x W	35	0.07**	3.07**	0.01 <sup>ns</sup>	0.00**
F x W	5	0.10 <sup>ns</sup>	2.10 <sup>ns</sup>	0.01 <sup>ns</sup>	0.00 <sup>ns</sup>
M x W	5	0.14**	0.78 <sup>ns</sup>	0.01 <sup>ns</sup>	0.00**
F x M x W	25	0.05**	3.72**	0.01 <sup>ns</sup>	0.00**
Error	216	0.01	1.74	0.01	0.00
$\sigma^2P$		0.28	30.49	0.14	0.01
$\sigma^2G$		0.19	19.58	0.08	0.00
$\sigma^2G:\sigma^2P$		0.69	0.64	0.56	0.53

**4. Estimation of heritability over and with in environment:** Genetic variability and broad sense heritability among genotypes was medium over environments. The broad sense heritability ranged from 53-69% for all traits, indicating involvement of slightly greater genotypic variation than due to environmental variations in the phenotypic expression of bird resistant traits (Table 3). However, heritability estimates were high within regimes except for DHS (Table 4). It varied from 64% to 91% for all traits. Thus, indicating substantial role of genetic variability in the phenotypic expression of bird resistant traits within water level (Table 3). Heritability estimates were higher under optimum condition as compared to non stress except for DSO.

**5. Relationship between achene yield and bird resistant trait:** Relationship among bird resistant traits and achene yield was established under both conditions. However, significant negative relationship between achene yield and degree of head shape was obtained under moisture stress condition (Fig. 1). Thus, indicating concave head shape may be subjected to improvement of achene yield under moisture stress condition.

Table 4. Analysis of variance of combining ability in sunflower for degree of head shape (DHS), degree of achene compactness (DAC), degree of stem orientation (DSO) and degree of head orientation (DHO) with in water level ( $W_1$ ) and ( $W_2$ ).

S.O.V	D.F.	DHS		DAC		DSO		DHO	
		$W_1$	$W_2$	$W_1$	$W_2$	$W_1$	$W_2$	$W_1$	$W_2$
Genotypes	54	0.09**	0.10**	5.21**	13.79**	0.05**	0.04**	0.00**	0.00**
Parents	11	0.21**	0.24**	7.13**	43.35**	0.05**	0.10**	0.01**	0.01**
Commercials	6	0.09**	0.05**	7.97**	9.66**	0.14**	0.07**	0.00**	0.00**
Parents vs. Crosses	1	1.27**	1.65**	150.87**	129.05**	0.29**	0.14**	0.03**	0.03**
Crosses vs. Commercials	1	1.01**	1.47**	248.75**	100.21**	1.03**	0.12**	0.01**	0.01**
Crosses	35	0.05**	0.07**	2.95**	5.67**	0.01**	0.02**	0.00**	0.00**
Female	5	0.11**	0.10 <sup>ns</sup>	0.94 <sup>ns</sup>	3.65 <sup>ns</sup>	0.01 <sup>ns</sup>	0.02 <sup>ns</sup>	0.00**	0.00 <sup>ns</sup>
Male	5	0.06 <sup>ns</sup>	0.09 <sup>ns</sup>	3.32 <sup>ns</sup>	2.89 <sup>ns</sup>	0.01 <sup>ns</sup>	0.01 <sup>ns</sup>	0.00 <sup>ns</sup>	0.00 <sup>ns</sup>
Female x Male	25	0.04**	0.07**	3.28**	6.63**	0.01**	0.02**	0.00**	0.00**
Error	108	0.01	0.02	1.92	1.25	0.00	0.00	0.00	0.00
<b>Contributions</b>									
Females		31.27	18.75	4.54	9.19	11.31	16.63	34.86	28.73
Males		17.96	17.54	16.05	7.28	16.92	4.74	14.03	18.20
Female x Male		50.77	63.71	79.41	83.53	71.77	78.63	51.11	53.07
Heritability ( $H^2$ ) Broad Sense		0.69	0.64	0.86	0.77	0.86	0.91	0.90	0.88



**Table 5. General combining ability effects in sunflower for degree of head shape (DHS), degree of achene compactness (DAC), degree of stem orientation (DSO) and degree of head orientation (DHO) with in water level (W<sub>1</sub>) and (W<sub>2</sub>).**

Parents	DHS		DAC		DSO		DHO	
	W <sub>1</sub>	W <sub>2</sub>	W <sub>1</sub>	W <sub>2</sub>	W <sub>1</sub>	W <sub>2</sub>	W <sub>1</sub>	W <sub>2</sub>
AMES-10103	0.02	-0.07	-0.20	0.73	0.02	-0.06	0.00	0.06
PEM-SR-88	-0.05	-0.04	0.01	-0.06	0.02	0.01	0.00	0.01
CM-614	-0.02	-0.06	-0.14	0.34	0.01	0.05	0.00	0.00
HA-407	-0.05	0.02	0.44	0.11	-0.03	-0.03	0.05	0.00
ORI-16/B	-0.04	0.12	-0.01	-0.71	0.01	-0.03	-0.03	-0.04
HA-350	0.15	0.03	-0.10	-0.26	-0.02	0.03	0.00	0.00
RL-57	0.01	-0.01	-0.19	-0.02	-0.02	0.02	-0.03	0.00
RL-52	0.03	0.03	-0.60	-0.09	-0.01	-0.01	0.00	0.00
CM-815	-0.02	-0.03	-0.13	-0.25	0.04	-0.03	0.00	0.01
CM-631	-0.05	0.10	0.28	-0.44	0.02	0.03	0.00	0.00
RL-37	0.09	0.03	-0.02	0.08	-0.02	0.00	0.00	-0.03
CM-619	-0.07	-0.11	0.66	0.56	-0.01	0.02	0.01	-0.01

## Discussion

Genotypes indicated substantial shift in their morphology for all traits related to bird resistance over contrasting moisture levels. This may be due to significant impact of water availability on dry matter accumulation pattern of genotypes which ultimately affected the plant morphology. Rauf & Sadqat (2007b) also found significant impact of water levels on dry matter accumulation pattern in genotypes differing in osmotic adjustment and found increase in dry root weight on the expense of above ground matter. Rauf & Sadqat (2007c) further found that differential dry matter accumulation pattern was obtained due to differential level of plant growth regulators production under contrasting water level.

The results revealed significance of specific combining ability variations in the inheritance of bird resistance traits. All traits exhibited larger proportion of non additive gene action under both conditions. However, all traits showed an increase in proportion of non-additive genetic variation under moisture stress regime. Intermating and recurrent selection procedures could be useful breeding procedures for accumulating favorable genes responsible for better bird resistance traits and may produce superior recombinants in segregating progenies under both conditions. Breeding lines such as CM-619, HA-407 and CM-631, the good general combiners could serve as base populations for intermating and recurrent selection. Khan *et al.* 2007, has also suggested the use of intermating and recurrent selection procedures for the accumulation of favorable genes for oil and protein contents, under predominance of non additive genes.

It is also important to note here that only drought tolerant parents showed peculiar morphology of traits leading to bird attack resistance such as concave head shape, droopy stem and horizontal head under stress condition. Relative sensitivity of these traits to the moisture stress has indicated their potential of acting as morphological marker for selection of drought tolerant genotypes. Among bird resistant traits, degree of head shape showed significant negative relationship with achene yield suggesting the possibility of improving yield through selection of highly concave genotype under drought stress condition.

### Acknowledgement

This study was a part of Ph.D thesis (Physiogenetics of drought tolerance in sunflower (*Helianthus annuus* L.) and all necessary funds were provided by the Higher Education Commission of Pakistan to PIN No: 042-160183-LS2-181.

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(Received for publication 20 January 2008)