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GENETICS OF ROOT CHARACTERISTICS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) UNDER CONTRASTING WATER REGIMES

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SUMMARY

Root characteristics are important while breeding for drought tolerance. Therefore, an experiment was carried out in the large size pots to study the root characteristics under contrasting water regimes. Results indicated repressing effect of drought on root weight and shoot length while root length and root-to-shoot ratio showed higher values under drought stress. Increase in root-to-shoot ratio was observed in almost all genotypes regardless of their drought tolerance while drought tolerant inbred lines only showed increase in root length. Correlation analysis indicated significant positive relationship with root-to-shoot ratio and absence of relationship between root length, shoot length and root weight. There were two types of genetical responses in drought stress regime. The traits, i.e. root length and root to shoot ratio showed higher contribution of additive gene action under drought stress when compared with non-stress regime while traits like shoot length and root weight showed decreased contribution of additive genes under drought. This showed that drought environment was more feasible for the selection of genotypes for traits, i.e. root length and root-to-shoot since it was promoting the expression of additive genes. Breeding procedure such recurrent selection may be used for the improvement of the traits. (AMES-10103 x CM-631) a good specific combiner may be used for the development of molecular markers for root length and root-to-shoot ratio.

Key words: **Drought, gene action, root length, sunflower**

INTRODUCTION

Root characteristics are important while breeding for drought tolerance (Rauf 2008). Response of roots to the drought has been reported earlier in sunflower hybrids and inbred lines (Schneiter 1992, Angadi and Entz 2002, Rauf and Sadaqat 2008). These studies indicated that higher root growth was linked with better drought tolerance. However, breeding sunflower for direct improvement of root characteristics was not taken previously. This may be due to laborious nature of this trait (O'Toole and Bland 1987). However, few breeders turned their efforts for the selection of root characteristics in small pots or at early growth phases (Rauf *et al*. 2008). Such type of breeding strategy has been found unsuitable since full genotypic potential is hard to achieve in pots with very small soil masses. Further more in segregating generation genotype may be lost before reproductive phase.

An alternative approach may be to evaluate the genotype at maturity. However, root evaluation method should be easy and capable of evaluating large number of genotypes. Keeping same in mind, an experiment was

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designed to evaluate genotype for root length at maturity and to determine the type of genetic variability associated with root traits. Hypothesis tested in these experiments was that available and type of genetic variability tended to be modified with contrasting water regimes. Available information would help in the formulation of breeding strategy for the development of genotype with efficient root system under drought.

MATERIALS AND METHODS

Field experiment assures optimum plant vigor, growth and reproduction; however plant performance under field condition should be considered as cumulative effect of some other factors such as weed growth, diseases, inter-varietal competition and soil thermal conductivity (Rauf 2008). On the other hand, controlled experiment provides better control over these factors but do not assure optimum growth, vigor and reproduction due to lower soil masses. Therefore a large pot experiment was designed to provide near field conditions and to overcome the disadvantages of both conditions.

In order to conduct the experiment, large plastic container of 120cm in length and 40 cm in diameter were used. The pots were buried in the soil with 15cm above the ground to avoid water infiltration from surrounding (Plate 1). They were filled with equal volume of fine mixture of field and silt loam soils (50:50). Pot soil had a field capacity of 18%, wilting point 9%, PH 7.9, organic matter 0.88%, available phosphorous 26.1 ppm, and potassium 143 ppm. All pots were irrigated to field capacity before sowing to achieve uniform germination. After germination, pots were equally divided into two regimes, i.e. non-stress and drought regime. Moisture contents with in pots were measured after every 2-3 days. Optimum level of moisture was maintained in the non stressed regime by irrigating with measured quantity of water to keep moisture contents close to field capacity, while stress in the drought regime was provided by skipping irrigations so that plants have 50% water deficit to the total of water applied in the normal regime. Total of 214 liters of water in thirty splits was supplied to non stress regime while 107 liters of water were supplied in fifteen splits to stressed regime in each pot during the whole of crop growth cycle. However, plot in which pots were buried was irrigated at regular interval to avoid heat stress injury with in pot as a result of increase of soil temperature due to moisture stress. Inbred lines and their crosses used are shown in Table 1. There were three pots per genotype in each regime with three replications while each pot consists of four plants. During the experimentation plants were fertilized with urea (40% N) applied to each pot in solution form (10g urea / l of water) at 30, 60,50 and 70 days after sowing.

Plate 1. (a) View of large pot experiment at University of Agriculture, Faisalabad-Pakistan to determine the effect of contrasting water regimes (b) Two differential within pot and field water levels

Root length/root weight/root-to-shoot ratio: All the pots were filled with water to convert the soil into very thin layer of water saturated mud. Using constant pressure of water generated by electric pump, mud was separated from the root. The operation was carried out until all the roots were freed from the soil without exerting manual pressure. Root length was measured

Table 1. Twelve inbredlines and their thirty-six crosses along with their codes.

Code	Inbredlines	Code	Crosses	Code	Crosses	Code	Crosses
1	AMES-10103	1	AMES-10103 x RL-57	13	CM-614 x RL-57	25	ORI-16/B x RL-57
2	PEM-SR-88	2	AMES-10103 x RL-52	14	CM-614 x RL-52	26	ORI-16/B x RL-52
3	CM-614	3	AMES-10103 x CM-815	15	CM-614 x CM-815	27	ORI-16/B x CM-815
4	HA-407	4	AMES-10103 x CM-631	16	CM-614 x CM-631	28	ORI-16/B x CM-631
5	ORI-16/B	5	AMES-10103 x RL-37	17	CM-614 x RL-37	29	ORI-16/B x RL-37
6	HA-350	6	AMES-10103 x CM-619	18	CM-614 x CM-619	30	ORI-16/B x CM-619
7	RL-57	7	PEM-SR-88 x RL-57	19	HA-407 x RL-57	31	HA-350 x RL-57
8	RL-52	8	PEM-SR-88 x RL-52	20	HA-407 x RL-52	32	HA-350 x RL-52
9	CM-815	9	PEM-SR-88 x CM-815	21	HA-407 x CM-815	33	HA-350 x CM-815
10	CM-631	10	PEM-SR-88 x CM-631	22	HA-407 x CM-631	34	HA-350 x CM-631
11	RL-37	11	PEM-SR-88 x RL-37	23	HA-407 x RL-37	35	HA-350 x RL-37
12	CM-619	12	PEM-SR-88 x CM-619	24	HA-407 x CM-619	36	HA-350 x CM-619

with the help of measuring tape. The roots were put in the kraft paper bags and dried to achieve constant weight. Dry root weight was measured on the digital balance while root to shoot ratio was the ratio of root length to the shoot length.

Biomass measurements: For the measurement of root weight, all the plant materials were placed in the glass house that heat up with the help of sunlight and heaters. Maximum temperature in the glass house rose to 70ºC and all the material kept for 15 days in the glass house. The dried biomass was then measured on electronic digital balance to determine root weight.

Biometrical and statistical analysis: Analysis of variance (ANOVA) was carried out in completely randomized design having genotype and moisture levels as factors. All effects were assumed fixed. Variation due to genotypes was further portioned into its subcomponents such as parents and crosses. Similarly, crosses variance was partitioned into females, males, and females × males. Variation due to genotypes × water regimes and crosses × water regimes were also partitioned into subcomponents on the same pattern. The method described by Kempthorne (1957) was adopted for combining ability analyses. Variance due to females and males was considered as general combining ability and that due to females × males, specific combining ability.

RESULTS

Analysis of variance for different root characteristics and shoot length over moisture regimes is given in Table 2. It showed significant variation ($P \leq 0.01$) due to water regimes, genotypes and its components such as parents, crosses and parents versus crosses. These components also showed significant interaction with water regimes ($P \leq 0.01$). Variation due to crosses was further partitioned into its components, i.e. males, females and females × males. These components also showed significant variation for all traits. However, variation due to female was non significant ($P \geq 0.05$) for root length and variation due to males was non-significant ($P \geq 0.05$) for all traits. Females and males component of variation also showed non-significant interaction ($P \geq 0.05$) with water regimes for all traits. Conversely, females × males showed significant interaction with water regimes.

Analysis of variance was also carried out within water regimes (Table 3). Variation due to all components was significant ($P \leq 0.01$) within water regimes. However, interaction due to males were non significant in all regimes and traits. In addition variation due to females showed non-significant differences ($P \geq 0.05$) for root length under non-stress regime.

Table 2. Abstract of the analyses of variance of combining ability in sunflower for root and shoot characteristics, i.e. root length (RL), shoot length (SL), root to shoot ratio (R:S) and root weight (RW) over water levels in pot experiment.

σ^2p, Phenotypic variability; σ^2G, genotypic variability; σ^2G: σ^2p, broad sense heritability

Source of variation	D.F	R.L	S.L	R:S	R.W
Water level (W)	1	2152.23**	98698.78**	5.23**	54444.50**
Genotypes(G)	47	2154.30**	2544.76**	0.25**	5644.67**
Parents(P)	11	3462.50**	3173.93**	0.40**	7776.63**
P vs. C	1	22745.81**	6998.47**	1.17**	2684.92**
Crosses (C)	35	1154.83**	2219.77**	0.18**	5059.19**
Females(F)	5	1596.48^{NS}	7017.36**	0.61**	19829.73**
Male(M)	5	1774.04^{NS}	2632.79^{NS}	0.16^{NS}	750.62^{NS}
Fx M	25	942.65**	1177.65**	0.10**	2966.79**
G x W	47	1733.34**	1366.44**	0.17**	1326.83**
P x W	11	2518.30**	1986.46**	0.26**	393.98**
P vs. C x W	1	581.64**	1241.12**	0.42**	3464.49**
C x W	35	1519.55**	1175.16**	0.14**	1558.93**
F x W	5	2747.95^{NS}	1988.05^{NS}	0.16^{NS}	2679.54^{NS}
M x W	5	712.43^{NS}	426.10^{NS}	0.09^{NS}	177.26^{NS}
F x M x W	25	1435.29**	1162.39**	0.14**	1611.14**
Error	192	7.29	18.60	0.00	31.07
Phenotype		6044.38	6462.16	0.68	12626.52
Genotype		3890.08	3917.40	0.43	6981.85
h^2 (broad sense)		0.64	0.61	0.63	0.55

Mean phenotypic performances for root traits and shoot length: Mean values for root length has been shown in Fig. 1. Generally drought tolerant inbred lines showed higher root length while susceptible lines showed decrease in root length (Fig. 1). Female line AMES-10103 (1) showed the highest root length under drought stress. Inbred line showed an overall increase of 4.36% while crosses showed an increase of 10.22% under drought. Cross combination AMES-10103 × CM-631 (4) showed the highest root length under drought.

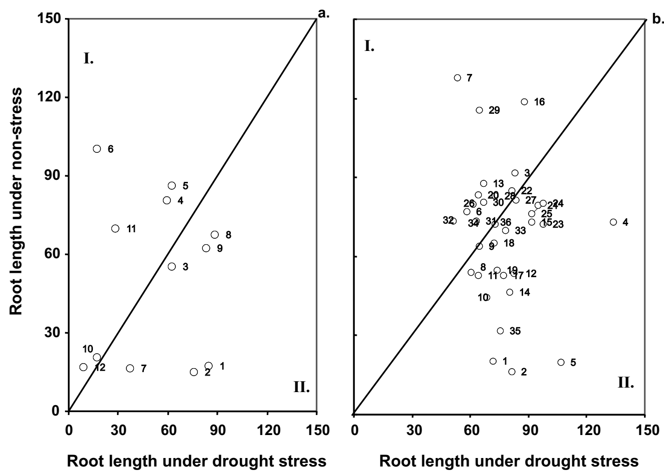

Fig. 1. Effect of contrasting moisture regimes on root length for (a) inbredlines (b) crosses

Inbred lines showed lower shoot length or non-significant ($P \geq 0.05$) increase under drought stress except female line PEM-SR-88 (2) when compared with non-stress regime. The crosses also followed similar pattern. They either showed repressing effect or non-significant increase under drought stress (Fig. 2). Inbred lines and crosses showed a decrease in shoot length of 24% and 28% under drought stress respectively.

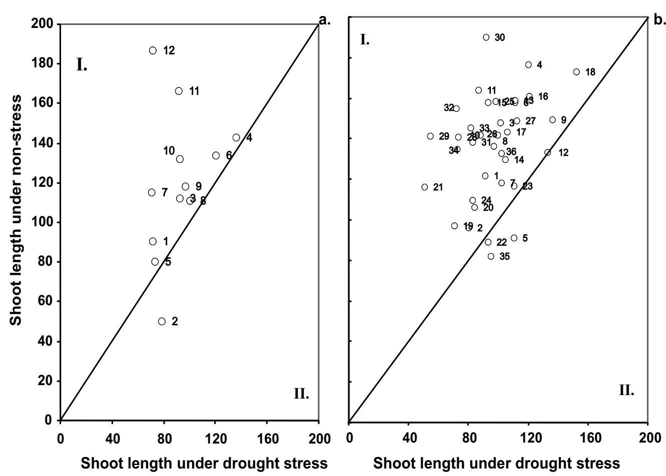

Fig. 2. Effect of contrasting moisture regimes on shoot length for (a) inbredlines (b) crosses

Similar to the shoot length, repressing effects of drought was also evident on root weight (Fig. 3). Inbred lines and crosses showed a decrease of 24% and 40% under drought respectively. However, cross combination ORI-16/B × CM-815 (27) showed significant increase in root weight under drought stress.

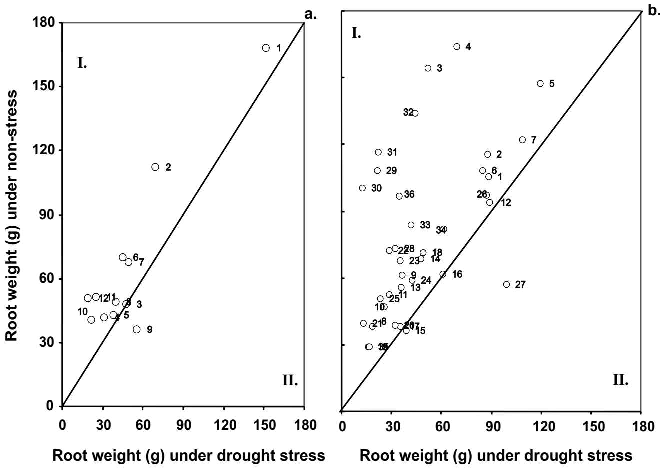

Fig. 3. Effect of contrasting moisture regimes on root weight for (a) inbredlines (b) crosses

Mean values for root to shoot ratio for inbred lines and crosses has been shown in Fig. 4. Generally trend was to show higher root to shoot ratio under drought

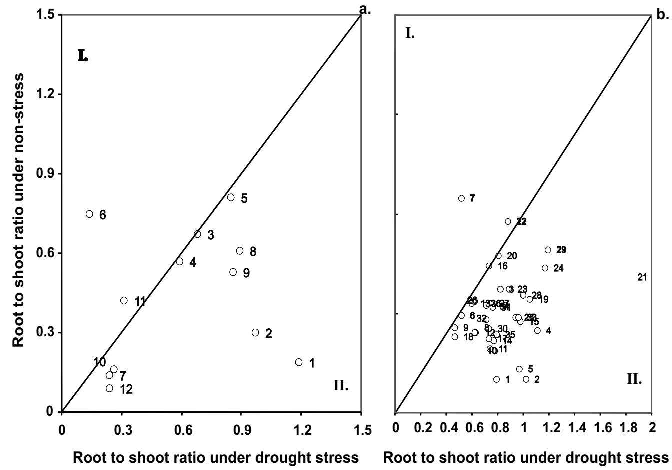

Fig. 4. Effect of contrasting moisture regimes on root to shoot ratio for (a) inbredlines (b) crosses

stress. Among breeding lines only HA-350 (6) showed decrease in root to shoot ratio while female line AMES-10103 (1) showed the highest root to shoot ratio under drought stress. On the other hand only cross combination PEM-SR-88 × RL-57 showed decreased ratio while combination HA-407 × CM-815 showed the highest ratio under drought stress. Increase shown by breeding lines and crosses was 30% and 61% respectively.

Table 3. Analyses of variance of combining ability in sunflower for root and shoot characteristics within water levels, i.e. non stress (NS) and drought stress (DS) in pot experiment.

σ^2p, Phenotypic variability; σ^2G, genotypic variability; σ^2G: σ^2p, broad sense heritability

		Root length		**Shoot length**		**Root shoot ratio**		**Root weight**	
S.O.V	**D.F.**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**
Genotypes	47	2298.07**	1589.58**	2616.49**	1331.31**	0.15**	0.27**	4183.58**	2787.91**
Parents	11	3168.41**	2812.39**	4086.84**	1270.14**	0.27**	0.39**	4461.45**	3709.17**
Parents vs. Crosses	1	8026.43**	15301.02**	7066.99**	730.43**	0.09**	1.49**	6124.60**	24.81**
Crosses	35	1860.87**	813.51**	2027.23**	1367.70**	0.12**	0.20**	4040.80**	2577.32**
Female	5	2705.71^{NS}	1638.72*	5756.49**	3248.92*	0.28*	0.48**	15941.74**	6567.53*
Male	5	1541.18^{NS}	945.29^{NS}	1900.88^{NS}	1158.01^{NS}	0.06^{NS}	0.19^{NS}	667.08^{NS}	260.80^{NS}
Female x Male	25	1755.83**	622.11**	1306.65**	1033.39**	0.10**	0.14**	2335.35**	2242.59**
Error	96	7.89	6.69	15.39	22.51	0.00	0.00	49.55	12.59
Contribution %									
Females		20.77	28.78	40.57	33.94	33.55	34.65	56.36	36.40
Males		11.83	16.60	13.40	12.10	6.94	13.69	2.36	1.45
Female x Male		67.40	54.62	46.04	53.97	59.51	51.66	41.28	62.15

Combining ability variation for root traits and shoot length: Contribution of additive genetic variability (Male + Female) and non-additive genes (Male × Female) was estimated to each trait and are given in Table 3. It showed higher contribution of non-additive genetic variability for all traits and regimes except shoot length and root weight which showed additive gene action under non stress regime. Contribution of non-additive genes decreased for root length and root to shoot ratio under drought stress when compared with non-stress regime. Conversely, contributions due to non-additive genes increased for shoot length and root weight under drought stress.

General combining effects were also estimated which indicated that AMES-10103 was the positive general combiner for all traits under drought stress (Table 4). Furthermore, it was also the best general combiner for root length and root weight. Similarly, CM-614 was the best general combiner for shoot length while HA-407 for root to shoot ratio under drought stress.

Effect of contrasting water regimes was also apparent on specific combining ability effect (Table 5). It changed the direction of SCA values in many cases of all traits. Cross combinations such as AMES-1013 × CM-631 showed the highest SCA for root length, HA-407 × RL-37 for shoot length and ORI-16/B ×RL-37 for root to shoot ratio and PEM-SR-88 × RL-57 for root weight.

Correlations: Correlations between different root traits and shoot length were estimated (Table 6). Root length showed significant positive correlation with root-to-shoot ratio while it showed non-significant relationship with shoot length and root weight. On the other hand, root-to-shoot ratio showed significant negative relationship with root weight and shoot length.

DISCUSSION

Repressing effect of drought was observed on root weight and shoot length while root length and root-to-shoot ratio showed higher values under drought stress. Increase in root-to-shoot ratio was observed in almost all genotypes regardless of their drought tolerance while drought tolerant inbred lines only showed increase in root length. It may be concluded from the result that increase in root length was an adaptive mechanism used by drought tolerant genotypes. Therefore, higher values may be used for the discrimination between drought tolerant and susceptible genotypes. Increase in root length as a

Table 4. Effects of general combining ability for root and shoot characteristics under non-stress (NS) and drought stress (DS) in pot experiment.

	Root length		Shoot length		Root shoot ratio		Root weight	
Parents	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**
AMES-10103	-19.81	12.56	-4.35	5.95	-0.17	0.04	52.57	35.26
PEM-SR-88	-3.50	-11.07	4.36	10.66	-0.03	-0.20	-10.72	2.93
CM-614	4.35	2.69	17.81	18.17	-0.04	-0.12	-24.67	-3.74
HA-407	6.29	8.32	-30.29	-14.44	0.21	0.27	-28.84	-20.47
ORI-16/B	16.54	-3.06	17.38	-8.22	0.05	0.04	1.28	-2.42
HA-350	-3.87	-9.44	-4.91	-12.13	-0.02	-0.03	10.38	-11.55
RL-57	3.88	-6.56	-4.13	-3.78	0.05	-0.06	0.01	0.60
RL-52	-10.66	-10.03	-8.64	-6.88	-0.06	-0.08	2.14	4.66
CM-815	7.00	6.04	8.12	-0.61	0.02	0.14	-7.93	-1.48
CM-631	10.05	7.83	4.53	-1.85	0.08	0.06	3.41	-1.89
RL-37	-12.09	4.31	-13.16	-2.66	-0.06	0.05	-6.01	-5.57
CM-619	1.83	-1.59	13.29	15.78	-0.02	-0.13	8.38	3.69

Table 5. Effects of specific combining ability for root and shoot characteristics under non-stress (NS) and drought stress (DS) in pot experiment.

Crosses	Root length		Shoot length		Root shoot ratio		Root weight	
	NS	DS	NS	DS	NS	DS	NS	DS
AMES-10103 x RL-57	-33.27	-10.97	-6.14	-7.47	-0.23	-0.03	-26.82	4.04
AMES-10103 x RL-52	-22.73	2.50	-27.21	-15.17	-0.12	0.22	-19.22	-0.49
AMES-10103 x CM-815	34.95	-12.41	7.87	-0.60	0.25	-0.20	29.48	-30.21
AMES-10103 x CM-631	13.23	36.64	40.13	19.64	-0.01	0.18	28.05	-12.30
AMES-10103 x RL-37	-17.63	13.50	-27.68	10.44	-0.07	0.05	21.09	41.61
AMES-10103 x CM-619	25.45	-29.27	13.03	-6.83	0.16	-0.22	-32.59	-2.65
PEM-SR-88 x RL-57	57.76	-5.68	-18.25	-1.16	0.54	-0.05	52.74	56.77
PEM-SR-88 x RL-52	-1.30	5.12	4.58	-3.55	-0.03	0.08	-32.93	-37.12
PEM-SR-88 x CM-815	-9.19	-7.02	0.83	29.46	-0.08	-0.30	-0.19	-13.45
PEM-SR-88 x CM-631	-31.61	-4.91	-3.25	-17.08	-0.25	0.09	-25.66	-23.47
PEM-SR-88 x RL-37	-1.20	-5.88	36.78	-17.61	-0.11	0.06	-10.51	-17.13
PEM-SR-88 x CM-619	-14.45	18.36	-20.68	9.95	-0.07	0.12	16.54	34.41
CM-614 x RL-57	9.87	-6.11	9.08	0.33	0.03	-0.06	0.32	-9.36
CM-614 x RL-52	-16.83	11.03	-15.53	-3.23	-0.06	0.14	11.02	-1.29
CM-614 x CM-815	-7.85	6.29	-3.95	-20.83	-0.03	0.12	-11.01	-4.21
CM-614 x CM-631	34.74	0.83	2.47	7.74	0.19	-0.04	2.96	18.33
CM-614 x RL-37	-9.13	-6.64	2.33	-6.12	-0.05	-0.04	-11.03	-4.06
CM-614 x CM-619	-10.81	-5.41	5.61	22.10	-0.08	-0.11	7.75	0.59
HA-407 x RL-57	-24.80	-4.40	-4.60	-7.72	-0.20	0.00	-22.04	-12.56
HA-407 x RL-52	18.27	-10.60	9.07	9.05	0.12	-0.26	-14.38	-0.09
HA-407 x CM-815	-3.52	3.99	2.22	-30.55	-0.06	0.62	-3.47	-13.31
HA-407 x CM-631	-0.77	-11.46	-21.10	13.02	0.16	-0.29	17.73	2.50
HA-407 x RL-37	8.37	8.40	24.10	31.16	-0.04	-0.27	22.67	12.78
HA-407 x CM-619	2.45	14.07	-9.69	-14.95	0.03	0.20	-0.51	10.69
ORI-16/B x RL-57	-13.61	24.78	8.89	13.72	-0.14	0.12	-30.32	-23.45
ORI-16/B x RL-52	4.26	-2.22	-3.10	18.16	0.05	-0.17	13.94	36.46
ORI-16/B x CM-815	-11.73	4.04	-12.74	24.72	-0.04	-0.27	-16.16	54.63
ORI-16/B x CM-631	-13.01	-8.41	-17.27	-13.53	-0.06	0.07	-11.22	-11.62
ORI-16/B x RL-37	41.36	-13.22	0.76	-31.06	0.31	0.27	33.16	-18.65
ORI-16/B x CM-619	-7.26	-4.98	23.47	-12.01	-0.12	-0.02	10.61	-37.37
HA-350 x RL-57	4.06	2.37	11.02	2.30	-0.01	0.01	26.11	-15.45
HA-350 x RL-52	18.33	-5.83	32.19	-5.26	0.04	-0.01	41.57	2.53
HA-350 x CM-815	-2.66	5.09	5.77	-2.19	-0.03	0.02	1.34	6.56
HA-350 x CM-631	-2.58	-12.69	-0.97	-9.78	-0.03	0.00	-11.86	26.58
HA-350 x RL-37	-21.77	3.83	-36.28	13.19	-0.04	-0.06	-55.38	-14.55
HA-350 x CM-619	4.61	7.23	-11.74	1.74	0.07	0.04	-1.79	-5.67

Table 6. Phenotypic correlations among traits for genotypes under stress regime (above diagonal) and under non-stress condition (below diagonal).

Traits	Root length	Shoot length	Root weight	Root to shoot ratio
Root length		0.19^{ns}	0.14^{ns}	0.53^{**}
Shoot length	0.34^{*}		0.38^{*}	-0.65^{**}
Root weight	0.07^{ns}	0.15^{ns}		-0.26^{*}
Root to shoot ratio	0.85^{**}	-0.16^{ns}	-0.05^{ns}	

Where *($P \leq 0.05$)

result of drought stress has also been observed previously (Rauf and Sadaqat 2007, Rauf and Sadaqat 2008). Rauf and Sadaqat, (2008) showed that increase in root length occurred due to higher osmotic adjustment ability of the drought tolerant genotypes. This trait has also shown significant positive relationship with root-to-shoot ratio. Selection for higher root length may also increase root-to-shoot ratio. Furthermore, root length has also showed non-significant relationship with shoot length. This is a good note since it is worthwhile to combine drought tolerance and dwarfness in sunflower. Previously, few reports have characterized variation in sunflower for root length and any greater root length associated with longer growth duration or greater plant height (Fereres *et al*. 1986, Schneiter 1992, Angadi and Entz 2002, Rauf and Sadqat 2008). Angadi and Entz (2002) compared root system characteristics and water extraction patterns of dwarf hybrids with hybrids of standard height. It was observed that most of the dwarf sunflower hybrids showed lower root length, root length density and root distribution, thus showing a positive correlation between plant height and root characteristics studied.

Although there was an increase in the root length but increase in root weight was not observed rather diminishing effects of drought were observed on root weight. This showed that root weight was independent of root length. This was further supported from the absence of correlation between root weight and length. The decrease in root weight may be due to repressing effect of drought on lateral roots, which have decreased overall root weight. Chun *et al*. (2005) also indicated that increased root length occurred at the expense of lateral root number. They further concluded that longer root length may help to explore nutrients deeper in soil profile. This phenomenon is especially important when water supply is limited.

There were two types of genetical responses in drought stress regime. The traits, i.e. root length and root to shoot ratio showed higher contribution of additive gene action under drought stress when compared with non-stress regime while traits like shoot length and root weight showed decreased contribution of additive genes under drought. This showed that drought environment was more feasible for the selection of genotypes for traits, i.e. root length and root-to-shoot, since, it was promoting the expression of additive genes. Breeding procedure such recurrent selection may be used for the improvement of the traits. Promising lines such as AMES-10103, HA-407, CM-631, CM-815, RL-37, positive combiner for root length and root-to-shoot ratio under drought may be utilized for the development of segregating generation.

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