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GENETIC DIVERSITY AND IDENTIFICATION OF TRAIT-SPECIFIC ACCESSIONS FOR DROUGHT STRESS FROM SUNFLOWER GERMPLASM

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Seventy diverse genotypes were evaluated for drought stress for morphological traits i.e. days to flowering, days to maturity, head diameter, number of leaves per plant, stem girth, thousand seed weight, achene yield per plant and oil percentage to find out trait specific genotypes. All genotypes showed highly significant ($P \leq 0.01$) mean square values for all traits. Head diameter expressed highest and significant genotype mean square (5366.93) and days to flowering showed lowest but significant genotype mean square (23.71) in all traits. Achene yield per plant and thousand seed weight having high heritability with increased genetic advance indicated additive gene action and lesser influence of the environment. The characters like days to flowering, days to maturity, head diameter, number of leaves per plant, stem girth and oil percentage showed non-additive gene action. Genotypes ORI 29 and ORI 30 performed better in normal and drought stress conditions that could be used as a drought tolerant material. In Principle Component Analysis (PCA), genotypes RL 110, RL 109, ORI 58, ORI 22, ORI 30 and RL 77 expressed maximum diversity. The bi-plot graph presented that genotype RL 104 had maximum number of leaves per plant, ORI 88 had maximum stem girth and head diameter; however, ORI 25 had high oil percentage value while days to maturity and achene yield per plant were found higher in ORI-66 and ORI-101 under normal and drought stress conditions. The trait-specific accessions will be helpful in effective exploitation of prominent genotypes for drought tolerant breeding programme. Drought tolerant genotypic identification could overcome the shortage of edible oil in country by increasing area and production under arid & semi-arid climatic condition.

Keywords: Sunflower, drought stress, morphological traits, principle component analysis, genetic diversity.

INTRODUCTION

The edible oil is integral component of human diet. Cotton seed, rapeseed, mustard, canola, sunflower, sesame and soybean are the major edible oil producing crops in Pakistan (Imran *et al.,* 2014; Iram *et al.,* 2016). Sunflower ranks second among hybrid crops after maize (Riaz *et al.,* 2017) and ranks 3rd after soybean and groundnut in the world oilseed production (Ramya *et al.,* 2019). Sunflower seeds contain 40 to 45% oil content (Skoric, 1992; Leon *et al.,* 2003; Monotti, 2004), 23% protein (Tahir *et al.,* 2002), 30% carbohydrates, 4% ash (Khalil and Jan, 2002) and its oil contain Palmitic, steric, oleic and linoleic acid (Aftab *et al.,* 2019). High stability, self-fertility, yield productivity and uniform maturity increased sunflower popularity among other oilseed crops (Sujatha *et al.,* 2002; Kaya and Atakisi, 2004).

Pakistan imports edible oil of worth 192.203 billion rupees (U.S. Dollar 1.455 billion) to fulfill the country cooking oil requirement. Local oilseed production is 0.5 million tones i.e.17% of total production while 83% edible oil is imported i.e. 2.421 million tones (Anonymous, 2018-19). The edible oil deficit is aggregating in Pakistan due to the rapid population growth and per capita consumption from 6 kg to 18 kg per annum by lavish eating lifestyle in last twenty years (Fazal *et al.,* 2015). A prodigious amount of foreign exchange has to be spent for the import of edible oil to overcome this deficit every year (Hasan *et al.,* 2015). The imported edible oil contains major share of palm oil that is poor in quality and creating health problems (Mustafa *et al.,* 2018).

Drought causes significant decrease in sunflower seed and oil yield (Mustafa *et al.,* 2015b). The deficit in edible oil production can be reduced by utilizing the area under drought stress condition and by increasing production of oilseed crops (Tan, 2010).

In any breeding programme, genetic diversity plays important role to identify genetic variation among the genotypes (Nafees *et al*., 2019; Riaz *et al.,* 2019). Variability for yield and yield related components assist in improving the productivity of crop plants (Nehru and Manjunath, 2003). Genotypic and phenotypic variability are very important parameters to identify the efficacy of different yield and oil related traits correlation (Resende and Duarte, 2007). Heritability describes how much of the variation in a given trait can be attributed to genetic variation (Cruz, 2004).

Multivariate analysis can be used to analyze the data statistically and to evaluate more than one variable collectively (Mohammadi and Prasanna, 2003; Grahic *et al.,* 2013).

Under changing climatic scenario, the drought tolerant genotypes are the need of time in all over the world to overcome the deficiency of good quality oil requirement and stability of continues production in the country (Ahmad *et al.,* 2009; Mustafa *et al.,* 2015a). To reduce the huge import bill, the area under rainfed climatic condition can be utilized by cultivating drought tolerant genotypes and their hybrids for higher seed production (Tahir *et al*., 2002).

MATERIALS AND METHODS

Total seventy diversifying genotypes were assimilated from different sources (Oilseeds Research Institute, Faisalabad, Plant Genetic Resources Institute, NARC-Islamabad and Department of Plant Breeding & Genetics, UAF). These genotypes were subjected to find out drought tolerant line under field conditions at farm area of Oilseeds Research Institute, Faisalabad during autum-2018.

Genotypes were sown on ridges keeping line to line distance 75cm and plant to plant 23cm in two separate beds using rain shed out conditions under three replications. Replicated complete block design under two factor factorials was used. One bed (T_1: Normal) having the normal irrigation from sowing to maturity while in second bed (T_2: Drought Stress). Water stress was applied at R4 flower stage of sunflower upto maturity by discontinuing its irrigation under rainshed out conditions (Schneiter and Miller, 1981).

Ten plants of each replication was tagged for recording the data of days to flowering, days to maturity, head diameter (cm), number of leaves per plant, stem girth (mm), thousand seed weight (g), achene yield per plant (g) and oil percentage. The days to flowering were counted from planting to 50% flower opening and days to maturity were counted from planting to 50% physiological maturity. Head diameter was measured by measuring tape and number of leaves per plants were counted from base up to the flower at maturity. Stem girth was measured by vernier caliper from base, middle and top of the plant at maturity in millimeters. Thousand seed weight and achene yield per plant was measured by electronic balance in grams. Oil percentage was measured with Gas Chromatography.

Genetic variability among different genotypes analysis of variance was determined according to Steel *et al.* (1997). Genetic heritability was estimated by formula given by Frankham *et al.* (2002). Estimation of genetic advance was done according to Johnson *et al.* (1955). "Minitab 18" and "R Studio" were used for principle component analysis with the software method following Husson *et al.* (2011).

RESULTS

In the present study, all genotypes showed highly significance mean square for all traits values by using combined analysis of variance at both levels of drought (Table 1). In addition, all traits showed highly significance ($P \leq 0.01$) for genotypes and drought interaction (Tahir *et al.,* 2002). Highest genotype mean square (5366.93) was expressed by head diameter while lowest genotype mean square (23.71) was expressed by days to flowering from all significant traits.

***Days to Flowering*:** Highly significant ($P \leq 0.01$) genotypic mean square was found at both drought levels for days to flowering. Grand mean value of normal treatment (T_1) was little higher than drought stress treatment (T_2) (51.23 & 49.91 days). Coefficient of variability was 5.37 at T_1; while, 6.42 at T_2. Genetic variability is lower than phenotypic variability. Broad sense heritability was shown maximum (99.16 & 99.10) at both levels of stress. Genetic advance was shown lower (3.59 & 3.82) under both drought stresses.

***Days to Maturity*:** All genotype of sunflower significantly diversified from each other for all traits. Days to maturity decreased with the onset of the drought stress (Table 2). The days to maturity ranged from 88.50 to 74.50 days under normal condition; while, in drought stress situation its range varied from 86.50 to 70.50 days. Environment variance found less than genotypic and phenotypic variance. High heritability values (99.00 & 98.69) but low genetic advance (6.26 & 6.24) were shown at normal irrigation and drought stress conditions respectively.

***Head Diameter*:** In both (normal and drought stress) condition all genotypes showed highly significant difference for head diameter (cm) (Table 2). Head diameter varied from 20.57 to 5.83 cm under normal situation; while, in drought

Table 1. Mean squares of ANOVA (combined analysis at all level).

SOV	df	DF	DM	HD	NL/P	SG	TSW	AY/P	OP
Genotypes	69	23.71**	67.69**	5366.93**	62.97**	49.63**	245.00**	224.77**	69.84**
Drought	1	181.37**	1133.57**	319.47**	394.40**	352.60**	3.70**	22.85**	606.60**
Genotypes x drought	69	3.59**	10.05**	3.59**	4.88**	9.22**	0.10**	97.33**	7.08**
Error	278	0.04	0.16	3.53	3.32	2.97	4.50	2.75	0.11

*=Significant at 5%, **= Significant at 1%, SOV= source of variance, df=degree of freedom, DF= days to flowering,DM= days to maturity, HD= head diameter, NL/P= number of leaves per plant, SG= stem girth, TSW= thousand seed weight, AY/P= achene yield per plant, OP= oil percentage.

Table 2. Genetic components, heritability, genetic advance and variances estimates for drought stress in sunflower.

	DF		DM		HD		NL/P	
Parameter	**(Normal)**	**(Drought)**	**(Normal)**	**(Drought)**	**(Normal)**	**(Drought)**	**(Normal)**	**(Drought)**
GMS	12.79**	14.49**	38.93**	38.81**	45.12**	36.30**	40.14**	27.72**
EMS	0.03	0.04	0.13	0.17	3.66	2.98	2.27	1.90
GM	51.23	49.91	82.47	79.19	13.59	11.87	24.01	22.07
Max.	55.25	54.25	88.50	86.50	20.57	18.17	37.33	31.33
Min.	44.75	43.75	74.50	70.50	5.83	5.73	17.67	17.00
CV	5.37	6.42	5.44	4.52	14.08	14.54	6.28	6.25
Vg	4.25	4.82	12.93	12.88	13.82	11.11	12.62	8.60
Ve	0.04	0.04	0.13	0.17	3.66	2.98	2.27	1.90
Vp	4.29	4.86	13.06	13.05	17.48	14.09	14.90	10.51
S.D	2.07	2.21	3.61	3.61	4.18	3.75	3.86	3.24
CVg	4.03	4.40	4.36	4.53	27.36	28.08	14.80	13.29
CVp	4.04	4.42	4.38	4.56	30.77	31.62	16.07	14.69
CVe	0.37	0.42	0.44	0.52	14.08	14.54	6.28	6.25
h.b(b.s)	99.16	99.10	99.00	98.69	79.06	78.87	84.73	81.90
G.A	3.59	3.82	6.26	6.24	5.78	5.18	5.72	4.65
G.A%	7.02	7.66	7.59	7.88	42.57	43.64	23.84	21.05

*=Significant at 5%, **= Significant at 1%, GMS= Genotypic Mean square, EMS= Error Mean Square, GM= grand mean, Max.= Maximum, Min.= minimum, CV= coefficient of variability. Vg= Genotypic variance, Ve= Environmental variance, Vp= Phenotypic variance, S.D= Standard deviation, CVg= Genotypic co-efficient of variance, CVp= Phenotypic co-efficient of variance, CVe= Environmental co-efficient of variance, h.b(b.s)= Heritability (Broad sense), G.A= Genetic advance i= 1.76, G.A%= Genetic advance %

Table 3. Genetic components, heritability, genetic advance and variances estimates for drought stress in sunflower.

	SG		TSW		SG		OP	
Parameter	**(Normal)**	**(Drought)**	**(Normal)**	**(Drought)**	**(Normal)**	**(Drought)**	**(Normal)**	**(Drought)**
GMS	27.01**	31.84**	348.76**	32.52**	158.46**	246.50**	42.41**	34.52**
EMS	3.14	2.75	0.08	0.10	2.46	80.18	0.11	14.04
GM	14.67	12.84	29.95	12.88	9.99	9.53	38.96	36.56
Max.	25.67	24.00	47.21	42.53	37.55	31.58	46.22	44.88
Min.	4.76	5.33	12.12	10.54	0.86	0.50	31.29	30.56
CV	12.08	12.91	4.97	6.41	15.68	18.31	4.54	5.87
Vg	7.96	9.70	116.23	10.81	52.00	53.53	14.10	11.47
Ve	3.14	2.75	0.08	0.10	2.46	3.05	0.12	0.10
Vp	11.10	12.45	116.31	10.91	54.46	56.58	14.22	11.57
S.D	3.33	3.53	10.78	3.30	7.38	7.52	3.77	3.40
CVg	19.23	24.25	36.00	25.53	72.18	76.77	9.64	9.26
CVp	22.71	27.48	36.01	25.64	73.87	78.93	9.68	9.31
CVe	12.09	12.92	0.97	2.41	15.70	18.32	0.88	0.87
h.b(b.s)	71.67	77.91	99.93	99.12	95.48	94.61	99.17	99.12
G.A	4.18	4.81	18.86	5.73	12.33	12.45	6.54	5.90
G.A%	28.48	37.46	62.97	44.47	92.44	80.68	16.80	16.14

*=Significant at 5%, **= Significant at 1%, GMS= Genotypic Mean square, EMS= Error Mean Square, GM= grand mean, Max.= Maximum, Min.= minimum, CV= coefficient of variability. Vg= Genotypic variance, Ve= Environmental variance, Vp= Phenotypic variance, S.D= Standard deviation, CVg= Genotypic co-efficient of variance, CVp= Phenotypic co-efficient of variance, CVe= Environmental co-efficient of variance, h.b(b.s)= Heritability (Broad sense), G.A= Genetic advance i= 1.76, G.A%= Genetic advance %

stress its range was 18.17 to 5.73cm. Genotypic and phenotypic variance and their coefficient were higher than environmental variance and its coefficient. High heritability (79.06 & 78.87) and genetic advance (5.78 & 5.18) were found under normal and drought stress conditions, respectively.

***Number of Leaves Per Plant*:** In both stress conditions the number of leaves per plant also highly significant (Table 2). Genotypic variability (12.62 & 8.60) was greater than environmental variability (2.27 & 1.90) at normal irrigation and drought stress conditions respectively; however, it's mean that there was very little influence of environment. This

character also had high heritability (84.73 & 81.90) and low genetic advance (5.72 & 4.65) at T_1 and T_2, respectively.

***Stem Girth*:** Stem girth (mm) had highly significant genotypic mean square at both levels of drought tress (Table 3). Stem girth grand mean values were 14.67 mm in T_1 and 12.84 mm in T_2 due to drought stress. Coefficient of variability was observed higher (12.08 & 12.91) under both levels of stress respectively. A little role of environmental variability (3.14 & 2.75) was found as compared to phenotypic variability (11.10 & 12.45) at normal irrigation and drought stress conditions respectively. The broad sense heritability values were high (71.67 & 77.91) and genetic advance values were lower (4.18 & 4.81) under T_1 and T_2, respectively.

***Thousand Seed Weight*:** Genotypic mean square was highly significant for thousand seed weight (g) (Table 3). There was a huge difference between grand mean weight of T_1 29.95 g and T_2 12.88 g. Genetic variability was also found higher in normal plants (116.23); while, under drought stress plants it was 10.81. Heritability was higher (99.93 & 99.12) than genetic advance % (62.97 & 44.47) at normal irrigation and drought stress conditions, respectively.

***Achene Yield Per Plant*:** Highly significant genotypic mean square values (158.46 & 246.50) for achene yield per plant were found under drought stress conditions respectively (Table 3). Coefficient of variability values were higher under drought stress level (18.31); while, under normal level it was 15.68. Genotypic and phenotypic variability was quite higher than environmental variability in both levels of stress. Higher genetic advance % was found (92.44 & 80.68) under T_1 and T_2, respectively.

***Oil Percentage*:** Highly significant difference was also found for oil percentage (Table 3). Oil percentage was also higher in normal plant (38.96) as compared to drought stressed plants (36.56). High heritability (99.17 & 99.12) and low genetic advance (6.54 & 5.90) were found under both stress levels, respectively.

***Principle Component Analysis*:** Principle component analysis express the genetic diversity among genotypes. The genotypes RL 110, RL 109, ORI 58, RL 96, RL 7, RL 103, A 14, ORI 16, RL 77, RL 82, ORI 61, ORI 35, ORI 30, ORI 29 and OR1 22 showed maximum diversity according to the first and second component (Fig. 1) in normal conditions.

Bi-plot graph also expressed the relationship among all the traits and with genotypes (Fig. 2). The genotypes RL 100, RL 109, RL 110, RL 104 and ORI 36 had maximum number of leaves per plant. Stem girth and head diameter was found high in ORI 88, ORI 86, ORI 37, ORI 27, ORI 47 and ORI 55; while, high oil percentage values was found in ORI 25. Days to flowering, days to maturity, thousand seed weight and achene yield per plant were found higher in ORI-33, ORI-46, ORI-50, ORI-66 and ORI-101.

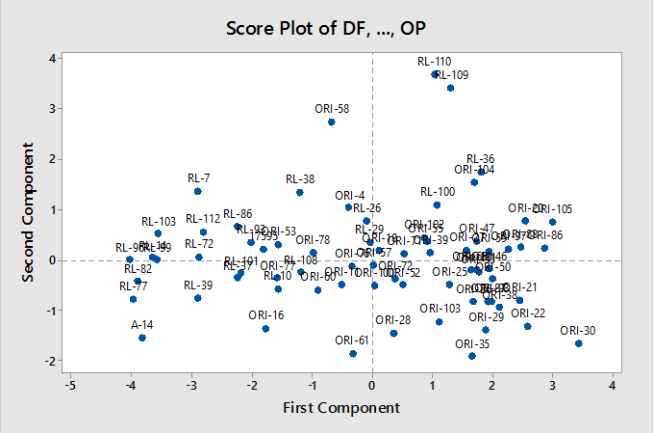

Figure 1. Morphological contributing traits in PC1 and PC2 of 70 genotypes of sunflower under normal condition.

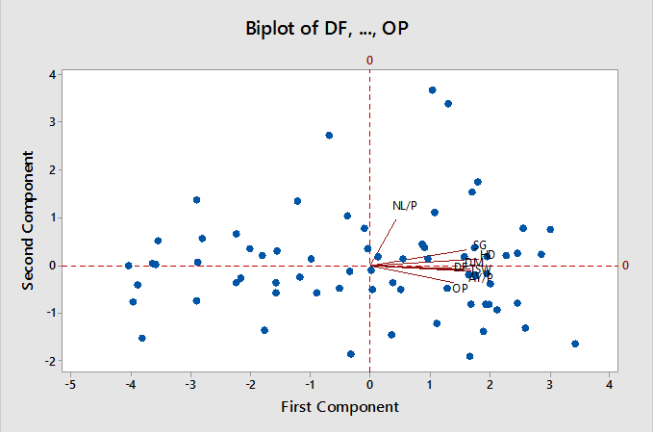

Figure 2. Bi-plot of PC1 and PC2 for all traits (DF= days to flowering, DM= days to maturity, HD= head diameter, NL/P= number of leaves per plant, SG= stem girth, TSW= thousand seed weight, AY/P= achene yield per plant, OP= oil percentage) of sunflower under normal conditions.

Under drought stress conditions, the genotypes ORI 22, ORI 47, ORI 30, ORI 77, ORI 58, RL 14, RL 99, RL 77, RL 110, ORI 20 and RL 109 expressed maximum diversity according to the first and second component of the graph (Fig. 3). Bi-plot graph also expressed the relationship among all the traits (Fig. 4) under drought stress. Achene yield per plant, oil percentage, stem girth, head diameter and days to maturity were projected toward the genotypes ORI 101, ORI 25, ORI 36, ORI 66 and ORI 88. The genotype ORI 39 showed high thousand seed weight. The genotypes ORI 27 and ORI 103 expressed maximum days to flowering. While, ORI 102, ORI 71, ORI 86 and ORI 104genotypes predicted for higher number of leaves per plant (Fig. 4).

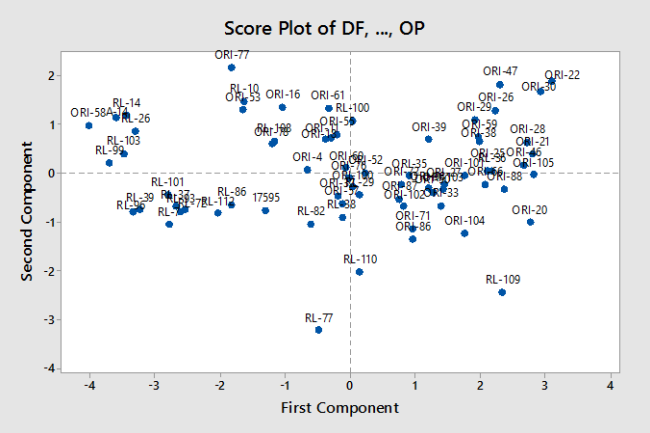

Figure 3. Morphological contributing traits in PC1 and PC2 of 70 genotypes of sunflower under drought stress condition.

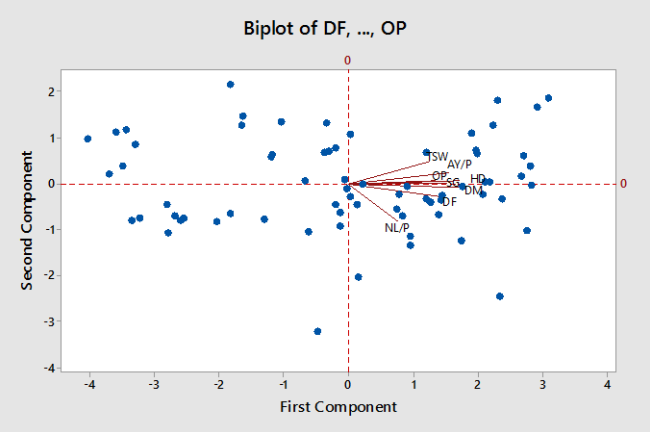

Figure 4. Bi-plot of PC1 and PC2 for all traits (DF= days to flowering, DM= days to maturity, HD= head diameter, NL/P= number of leaves per plant, SG= stem girth, TSW= thousand seed weight, AY/P= achene yield per plant, OP= oil percentage) of sunflower under drought stress conditions.

Stem girth and Head diameter was found high in ORI 88 under both levels of stress. However, genotype ORI 25 had high oil percentage value in drought stress along with normal condition. Days to maturity and achene yield per plant were found higher in ORI-66 and ORI-101 under normal and drought stress conditions (Fig.2 & 4).

DISCUSSION

To increase the yield and oil production in the country, genetic diversity is essential for germplasm strengthening and breeding material improvement in any crop development programme (Dudhe *et al.*, 2019) under drought stress conditions (Shamshad *et al.*, 2014; Tyagi *et al.*, 2014; Jannatdoust *et al.*, 2016; Hussain *et al.*, 2018; Shafqat *et al.*, 2019; Qadir *et al.*, 2019). In this study, highly significant variance was found in analysis of variance and combined analysis of variance for all the characters and their interaction in all seventy genotypes at all levels of drought stress. These results are also in line with the findings of Farooq *et al.* (2018), Divya *et al.* (2019) and Dudhe *et al.* (2019).

Genotypic and phenotypic variance and their coefficient of variability were higher for all characters than environmental variance and its coefficient indicates that environment had a little role on overall results. Phenotypic variance was greatly influenced genetically for all the characters and there was a little role of environmental variance (Rehman *et al.*, 2012: Farooq *et al.*, 2018). Achene yield per plant had high genotypic and phenotypic coefficient of variability indicating that that this character is controlled by additive gene action. Sujatha *et al.* (2002) also endorsed our findings.

Genotypic coefficient of variability did not accurately reflect the actual heritable variation, it requires heritability on the base of genetic advance which could give the trustworthy information to adopt proper selection criteria (Hasan *et al.*, 2020). High heritability along with genetic advance were found for achene yield per plant and thousand seed weight because of additive gene action. Selection procedure has been used for these traits to develop drought tolerant genotypes (Divya *et al.*, 2019). Days to flowering, days to maturity, head diameter, number of leaves per plant, stem girth and oil percentage had high heritability but low genetic advance indicating that these characters are controlled by non-additive genes, thus these characters could be utilized in cross combination programme to exploit the hybrid vigor (Sutar *et al.*, 2010; Rani *et al.*, 2017; Dudhe *et al.*, 2019).

PCA was used to find out the diverse traits and their association with the genotypes within diverse genotypic platform of a crop (Dudhe *et al.*, 2019). It also differentiates morphologically alike phenotypic groups when plotted against principal components. Genetic diversity analysis of genotypes and their division into grouping could be made through bi-plot diagram technique (Tabrizi *et al.*, 2011). The genotypes RL 110, RL 109, ORI 58, ORI 22, ORI 30 and RL 77 expressed maximum diversity according to the first and second component of the graph (Fig. 1 & 3) under both level of stress. The genotype RL 104 had maximum number of leaves per plant under normal and drought stress condition (Fig. 2 & 4). The trait like number of leaves per plant increased the photosynthetic rate, which ultimately increased the crop productivity (Rasool *et al.*, 2013).

***Conclusion*:** Oil and seed yield related traits (days to flowering, days to maturity, head diameter, number of leaves per plant, stem girth, thousand seed weight, achene yield per plant and oil percentage) showed variability, can be used for screening of the germplasm. Environmental variance and its coefficient of variability were quite less than genotypic and

phenotypic variance and its coefficient of variability revealed that role of environment was infinitesimal. Additive gene action was found for achene yield per plant and thousand seed weight used for selection. Days to flowering, days to maturity, head diameter, number of leaves per plant, stem girth and oil percentage had non-additive gene action used for heterosis breeding. In PCA, genotypes RL 110, RL 109, ORI 58, ORI 22, ORI 30 and RL 77 expressed maximum diversity could be used in further hybridization programme of sunflower.

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