



Full Length Article

Comparative Performance of Sunflower Synthetic Varieties under Drought Stress

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is the world's fourth largest oil-seed crop and its growth is limited by moisture condition. Different varieties of sunflower depict differential response to drought. The objective of this work was to investigate drought tolerance of sunflower genotypes developed at Suranaree University of Technology. Seven sunflower genotypes and one commercial hybrid, Pacific 77, were tested at two water stress levels of -0.6 and -1.2 MPa, using polyethylene glycol 6000 (PEG-6000) as an osmoticum. Dry matter stress index, plant height stress index, root length stress index, relative water content stress index and germination stress index were used to evaluate the plant response to drought. PEG-induced drought stress significantly decreased all these attributes with increase in water stress levels in all sunflower genotypes. Cluster analysis and principal component analysis of all stress indices revealed that synthetic sunflower varieties: S473, S471 and S475 and hybrid Pacific 77 were drought tolerant and could be selected and used for general planting and further genetic improvement programs, whereas inbred lines: 5A, 6A, 9A and 10A were drought sensitive. © 2012 Friends Science Publishers

Key Words: Drought stress; Growth; Stress index; Sunflower; Synthetic variety

INTRODUCTION

Drought is a worldwide problem limiting the survival and growth of plants. It limits plant seed yield and development and severity of the effects depends on the type of plant and its development stage, the extent and duration to which plants are subjected to drought. Generally, the first critical and the most sensitive stage in the cycle of the plants is considered as seed sowing stage. Water deficit not only affects seed germination, but also increase mean germination time in crop plants (Willenborb *et al.*, 2004). Several morphological and physiological characters as affected by drought stress include decrease in leaf area, plant height, root length, head diameter, yield per plant, plant biomass as well as photosynthetic rate (Chartzoulakis *et al.*, 2002; Khalilvand *et al.*, 2007). Kumar *et al.* (2011) reported that relative water content was significantly reduced under water stress condition which was associated with a lower photosynthetic rate. Severe drought stress may result in arresting of photosynthesis, metabolic disturbance and plant death (Reddy *et al.*, 2004).

Sunflower (*Helianthus annuus* L., Asteraceae) is the most important source of edible oil after soybean, rapeseed and peanut, with a worldwide seed production of 33.3 million tons destined almost exclusively to oil extraction, providing 8.5% of total world volume. However, sunflower

production and yield stability is limited by drought stress especially in arid and semi-arid areas. Water stress on sunflower has been reported to reduce plant height, root length, dry matter and grain numbers per plant (Meo, 2000; Nezami *et al.*, 2008; Ahmad *et al.*, 2009). In addition, some evidences have indicated that water stress causes considerable decrease in seed yield, yield components and seed oil content of sunflower (Alahdadi *et al.*, 2011; Stone *et al.*, 2001).

In Thailand, sunflower is a crop of economic significance in vegetable oil production of the country. But limited rainfall or irrigation system that is not well managed during the growing season constraints its seed yield. Therefore, growing of drought tolerance varieties will contribute to more stable sunflower production and could be used as a parent in breeding programs. However, breeding for drought resistance is complicated by the fact that several physiological traits are required for improved plant performance under drought and an efficient screening technique is necessary. Moreover, genotypic differences in sunflower responses to drought may vary with time and severity of the stress.

Many authors reported the use of PEG to induce dehydration for drought screening of genotypes under laboratory conditions (Mohammed *et al.*, 2002; Dutta & Bera, 2008; Ahmad *et al.*, 2009). Several drought tolerance

indices or selection criteria, such as plant height have been proposed as ways to identify genotypes with better stress tolerance. Moreover, Ahmad *et al.* (2009) found dry matter stress index as a reliable indicator of drought tolerance in sunflower.

At Suranaree University of Technology, sunflower breeding project was started in 1997, several inbred lines and synthetic varieties have been developed (Laosuwan, 1997; 2000). However, the performance of these plants in drought stress has not been evaluated. In view of the above facts, the objective of the study was to evaluate drought tolerance of sunflower genotypes to drought stress as created with polyethylene glycol (PEG-6000).

MATERIALS AND METHODS

Plant genotypes: The experiment was carried out at School of Biology, Suranaree University of Technology. Seeds of 4 inbred lines of sunflower (5A, 6A, 9A & 10A) and 3 synthetic varieties (S471, S473 & S475) developed at Suranaree University of Technology (Laosuwan, 2000) and one commercial hybrid, Pacific 77, were used in this study.

Plant growth and water stress treatment: Eight sunflower genotypes were evaluated against drought stress at germination and seedling growth stages for 14 days under laboratory conditions ($25 \pm 3^\circ\text{C}$) in Petri dish bioassays. PEG-6000 was used as a drought stimulator and two water stress levels of -0.6 MPa and -1.2 MPa were developed by dissolving 10 and 20 g of PEG per 100 mL distilled water, respectively. Apart from the stress treatments, one control treatment with no water stress was included. The seeds of each sunflower genotype were split pericarp before sterilization. The seeds were soaked twice with 70% ethanol (two min each time), surface sterilized with 10% sodium hypochlorite solution for 10 min, and finally rinsed four times with distilled water. Ten seeds of each sunflower genotype were placed in each Petri plate containing 2-layers of Whatman filter papers. The experiments were laid out in a 3x8 factorial in a completely randomized design (CRD) with three replications. Five mL of designated treatment solution was applied daily in each Petri plate. Numbers of germinated seeds were counted daily and recorded for 14 days. A seed is considered for germination when both plumule and radicle have emerged to 5 mm.

Determination of morphological and physiological parameters: After 10 days, the numbers of germinated seeds were recorded and the promptness index (PI) and germination stress index (GSI) were calculated using the formula proposed by Bouslama and Schapaugh (1984). From these measurements the plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI), dry matter stress tolerance index (DMSI) and relative water content tolerance index (RWCSI) were calculated as under:

$$\text{PI} = \text{nd}_2 (1.0) + \text{nd}_4 (0.8) + \text{nd}_6 (0.6) + \text{nd}_8 (0.4) + \text{nd}_{10} (0.2)$$

Where; nd_2 , nd_4 , nd_6 , nd_8 , nd_{10} represent the

percentage of germinated seeds at 2, 4, 6, 8 and 10 days after germination, respectively.

$$\text{GSI} = (\text{PISS} / \text{PICS}) \times 100$$

When; PISS is the promptness index of stressed seeds, and PICS is the promptness index of control seeds.

$$\text{PHSI} = (\text{Plant height of stressed plants} / \text{Plant height of control plants}) \times 100$$

$$\text{RLSI} = (\text{Root length of stressed plants} / \text{Root length of control plants}) \times 100$$

$$\text{DMSI} = (\text{Dry matter of stressed plants} / \text{Dry matter of control plants}) \times 100$$

$$\text{RWCSI} = (\text{RWC of stressed plants} / \text{RWC of control plants}) \times 100$$

$$\text{RWC} = [(\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight})] \times 100.$$

Relative water content (RWC) was measured at 14 days after germination, estimated according to Turner (1981) and calculated from the equation given above.

Statistical analysis: The experimental data were analyzed by mean variables and mean values that were taken from measurement of three replicates. Analyses were done using the SPSS program (v. 17). Differences between means were determined by one-way ANOVA and Duncan's multiple range test (DMRT). Principal component analysis (PCA) and clustering analysis were used to determine the relationship among genotypes and stress indices based on morphological and physiological parameters. PCA and cluster analysis were conducted with R program (32-bit) v. 2, and the ranking method (Farshadfar *et al.*, 2012) was used to determine the most desirable drought tolerant genotypes according to all indices.

RESULTS AND DISCUSSION

Analysis of variance and mean comparison: Sunflower plants exposed to PEG-induced drought stress from the germination to seedling growth stage showed a significant reduction in germination percentage and plant growth parameters in all sunflower genotypes (Table I). The results of combined analyses of variances indicated significant differences among genotypes and PEG concentrations for all traits and significant genotype \times PEG concentration for RLSI and RWCSI.

Seed germination and plant growth: Seed germination decreased as osmotic potential became more negative (Fig. 1a). Inhibition of seed germination was greatest under the lowest osmotic potential, -1.2 MPa (20% PEG). The GSI values 10 days after germination ranged from 15.07% to 83.64% for the -0.6 MPa (10% PEG) compared to 0 to 51.81% at -1.2 MPa (data not shown) indicating more pronounced differences among genotypes at the lower osmotic potentials. The highest GSI average over treatments was 67.73% for sunflower variety S473 and the lowest 7.54% for inbred line 5A. At 10% PEG, the highest GSI

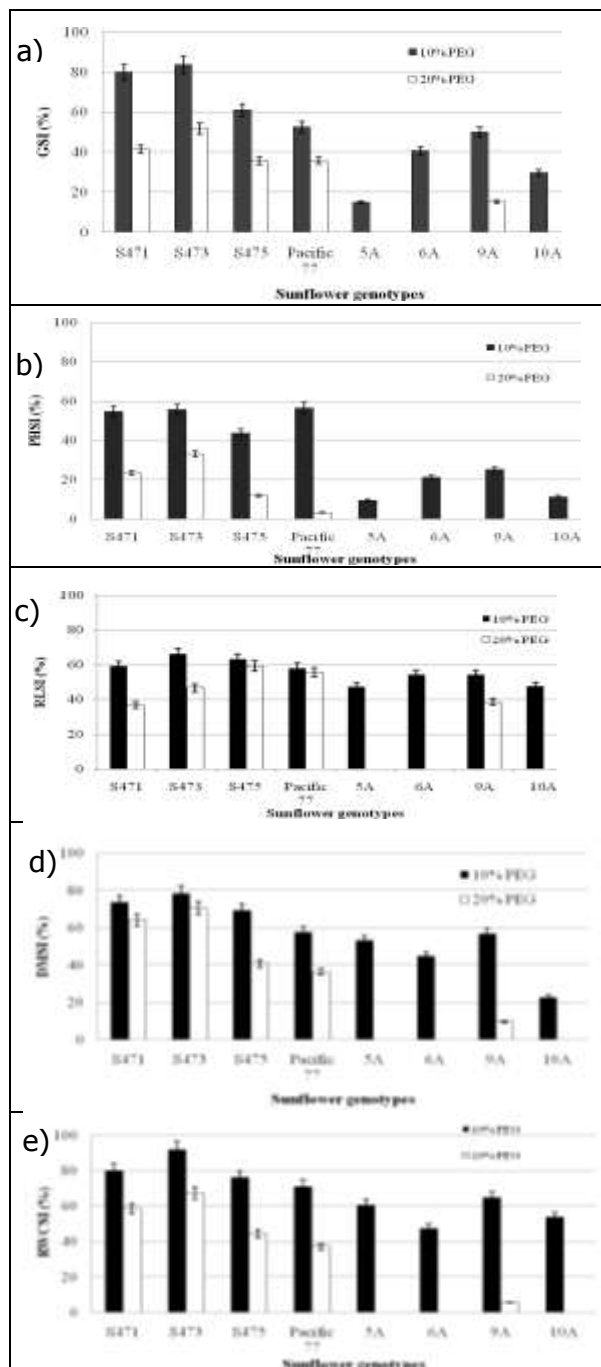
value was recorded in sunflower variety S473 (83.64%), while the lowest (15.07%) was recorded for line 5A. At 20% PEG, the highest (51.81%) was recorded in sunflower variety S473, whereas no germination was observed in lines 5A, 6A and 10A (Fig. 1a). These results were similar to those of Ahmad *et al.* (2009) who reported that PEG-induced water stress at germination and seedling growth stages reduced the GSI in six sunflower hybrids/breeding lines. The GSI was used to account differences in the rate of germination due to osmotic stress (Bouslama & Schapaugh, 1984). High values of the GSI indicated a high rate of germination which was inversely related to moisture stress. According to Ahmad *et al.* (2009), drought stress has an inhibitory effect on sunflower seed germination. Increasing drought stress levels caused delay in seedling emergence as a result of reducing cell division and plant growth metabolism. Similarly, Somers *et al.* (1983) reported that at higher PEG concentrations seedling emergence of four sunflower cultivars was reduced at -11 and -15 bars and completely inhibited at -21 bars. The results of these previous studies are similar with results of our present study.

Analysis of variance on PHSI data in sunflower plants was significantly influenced by genotypes and PEG concentrations, and genotypes \times PEG concentration interaction was not significant (Table I). The PHSI decreased in both 10% and 20% PEG concentrations in all genotypes (Fig. 1b). The highest PHSI average over treatments was 44.40% for sunflower variety S473 and the lowest 4.81% for line 5A. The results of PHSI under drought stress are in agreement with the findings of Ahmad *et al.* (2009) who reported that the PHSI showed significant differences among sunflower genotypes and decreased with the increase in PEG concentration. Water stress on sunflower has been reported to reduce plant height, root length and number of stomata (Pirjol-Sovulescu *et al.*, 1974).

Analysis of variance for the RLSI data in different sunflower genotypes was significantly influenced by genotypes, PEG concentrations and the interaction of genotypes \times PEG concentration (Table I). At 10% PEG, the highest RLSI value was recorded in sunflower variety S473 (66.07%), whereas the lowest (47.23%) was recorded in line 5A. At 20% PEG level, the highest RLSI (59.54%) was recorded in variety S475, followed closely by hybrid Pacific 77, whereas sunflower inbred lines showed lower RLSI values (Fig. 1c). It is interesting to note that line 9A had the highest RLSI value among inbred lines at 10% and 20% PEG. Nejad (2011) reported that major parameters in drought conditions such as root length, number, weight and root volume, decreased in mild water stress (50% of the amount of irrigation treatments). Root length increased in conditions of severe water stress (25% of the amount of irrigation treatments), but reduced root length compared to the control treatments.

Analysis of variance for the data DMSI in different sunflower genotypes revealed significant among genotypes

Fig. 1: Means of (a) germination stress index (GSI), (b) plant height stress index (PHSI), (c) root length stress index (RLSI), (d) dry matter stress index (DMSI) and (e) relative water content stress index (RWCSI) of sunflower plants in response to 10% and 20% PEG concentrations



and PEG concentrations, whereas the interaction genotypes \times PEG concentrations was not significant (Table I). DMSI decreased under drought stress in all genotypes (Fig. 1d). Highest reduction was observed in line 10A.

Table I: Analysis of variances for some growth parameters of sunflower plants exposed to different PEG-6000 concentrations at germination and seedling growth stages

S.V.O	d.f.	Mean square				
		GSI	PHSI	RLSI	DMSI	RWCSI
Genotypes (G)	7	2865.084**	1432.037**	1486.147**	3042.599 **	2701.219**
PEG Concentration (C)	1	10137.434**	7951.343**	8456.175**	12745.435**	20755.074**
G X C	7	133.045	280.303	624.279**	1258.052	367.207 **
Error	32	148.675	54.627	118.723	703.090	8.929
C.V. (%)		27.631	21.480	23.981	28.742	30.080

**= Significant at 0.01 probability level; GSI: germination stress index, PHSI: plant height stress index, RLSI: root length stress index, DMSI: dry matter stress index, RWCSI: relative water content stress index

Table II: Growth characteristics of sunflower plants under water stressed condition at germination and seedling growth stages

Genotypes	GSI		PHSI		RLSI		DMSI		RWCSI	
	10%PEG	20%PEG	10%PEG	20%PEG	10%PEG	20%PEG	10%PEG	20%PEG	10%PEG	20%PEG
S471	79.95ab	41.49a	54.76a	23.52a	59.10ab	36.87a	73.81ab	64.23a	79.95b	58.63b
S473	83.64a	51.81a	55.62a	33.18a	66.07a	46.7a	78.64a	70.59a	91.90a	67.29a
S475	60.9bc	35.72ab	43.72a	12.01b	63.13ab	59.13a	69.36ab	40.91b	76.21b	44.62c
Pacific77	52.71c	35.82ab	56.39a	3.367bc	58.00ab	55.60a	57.82abc	36.39b	71.14c	37.06d
5A	15.07e	0c ^{ng}	9.62b	0c ^{ng}	47.23b	0b ^{ng}	53.33bc	0c ^{ng}	60.63e	0e ^{ng}
6A	40.69cd	0c ^{ng}	21.22b	0c ^{ng}	54.10ab	0b ^{ng}	45.04c	0c ^{ng}	47.44g	0e ^{ng}
9A	49.97cd	15.38bc	25.31b	0c ^{ng}	54.10ab	38.63a	56.89abc	9.78c	64.84d	5.58e
10A	29.72de	0c ^{ng}	11.36b	0c ^{ng}	47.57b	0b ^{ng}	22.73d	0c ^{ng}	53.79f	0e ^{ng}
Mean	51.58	22.53	34.75	9.01	56.16	29.62	57.20	27.74	68.24	26.65

Means followed by the same letters are not significantly different at $p < 0.05$ level of probability using DMRT, ng: no germination, GSI: germination stress index, PHSI: plant height stress index, RLSI: root length stress index, DMSI: dry matter stress index, RWCSI: relative water content stress index

Table III: Ranks (R), ranks mean (\bar{R}), standard deviation of ranks (SDR) and rank sum (RS) on growth characteristics of seedling indicators of drought tolerance

Genotypes	GSI		PHSI				RLSI				DMSI				RWCSI				SDR	RS	Final rank			
	10% PEG	R	20% PEG	R	10% PEG	R	20% PEG	R	10% PEG	R	20% PEG	R	10% PEG	R	20% PEG	R								
	PEG																							
S471	79.95	2	41.49	2	54.76	3	23.52	2	59.10	3	36.87	5	73.81	2	64.23	2	79.95	2	58.63	2	2.5	0.97	3.47	2
S473	83.64	1	51.81	1	55.62	2	33.18	1	66.07	1	46.70	3	78.64	1	70.59	1	91.90	1	67.29	1	1.3	0.68	1.97	1
S475	60.90	3	35.72	4	43.72	4	12.01	3	63.13	2	59.13	1	69.36	3	40.91	3	76.21	3	44.62	3	2.9	0.88	3.78	3
Pacific 77	52.71	4	35.82	3	56.39	1	3.37	4	58.01	4	55.63	2	57.82	4	36.39	4	71.14	4	37.06	4	3.4	1.08	4.47	4
5A	15.07	8	0	6	9.62	8	0	5	47.23	7	0	6	53.33	6	0	6	60.63	6	0	6	6.4	0.97	7.37	8
6A	40.69	6	0	6	21.22	6	0	5	54.10	5	0	6	45.04	7	0	6	47.44	8	0	6	6.1	0.88	6.98	6
9A	49.97	5	15.38	5	25.31	5	0	5	54.10	5	38.63	4	56.89	5	9.78	5	64.84	5	5.58	5	4.9	0.32	5.22	5
10A	29.72	7	0	6	11.36	7	0	5	47.57	6	0	6	22.73	8	0	6	53.79	7	0	6	6.4	0.84	7.24	7

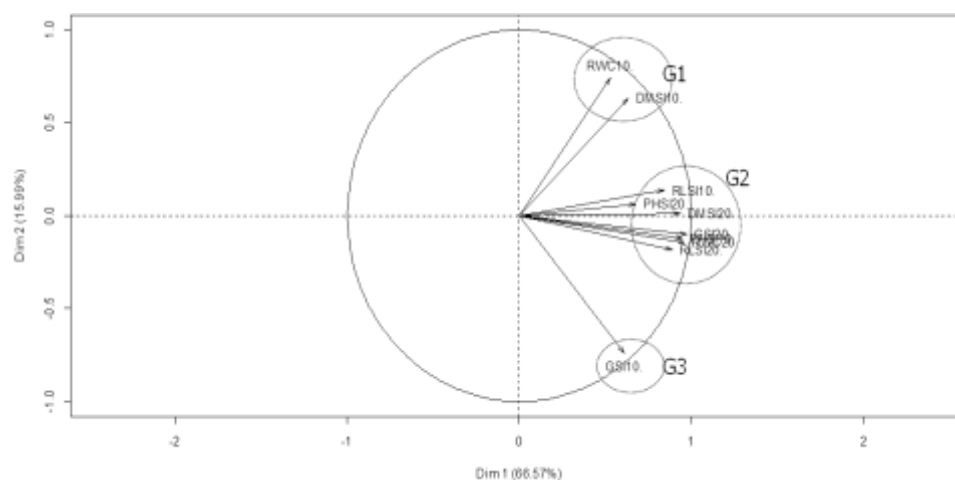
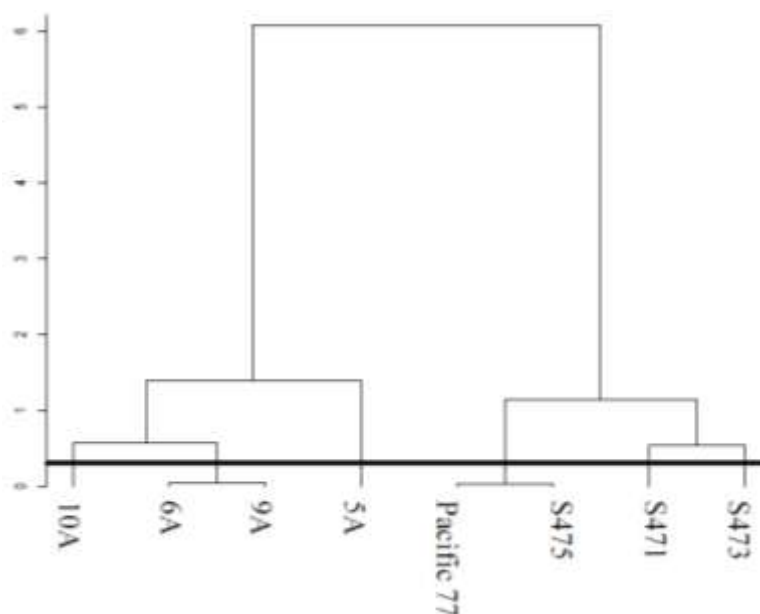
GSI: germination stress index, PHSI: plant height stress index, RLSI: root length stress index, DMSI: dry matter stress index, RWCSI: relative water content stress index

The DMSI values declined when exposed to 20% PEG concentration and variety S473 had the highest DMSI value (70.59%), followed closely by variety S471 (64.23%). Moreover, among four inbred line 9A had the highest DMSI values, 56.89% and 9.78% at 10% and 20% PEG concentrations. Reduced biomass under drought stress was observed in several plant species including sunflower (Vanaja *et al.*, 2011; Ahmad *et al.*, 2009), maize (Vanaja *et al.*, 2011) and soybean (Specht *et al.*, 2001).

Exposure of plants to drought led to noticeable decreases in relative water content. The lowest RWCSI was observed in line 6A at 10% PEG level, and the highest was in variety S473 (91.90%). At 20% PEG level the highest RWCSI was in variety S473 (67.29%) (Fig. 1e). Genotype 9A had the highest RWCSI at both PEG concentrations among inbred lines. Some genotypes of sunflower, e.g. variety S473 maintained relative water content extremely

well at both drought stress levels. This result was agreement with those of Siddique *et al.* (2000) who found that drought stress induced decrease in relative water content in wheat, and the higher leaf water potential and relative water content were associated with a higher photosynthetic rate.

Determination of sunflower genotypes for drought tolerance based on principal component analysis (PCA), clustering and ranking method: To better understand the relationships and similarities among the drought stress indices in sunflower genotypes, PCA based on the rank correlation matrix was used. Eight sunflower genotypes were evaluated for drought tolerance in terms of GSI, PHSI, RLSI, DMSI and RWCSI. The relationships among different indices using PCA are displayed in a biplot of Dim 1 and Dim 2 (Fig. 2). The cosine of the angle between the vectors of two indices approximates the correlation coefficients between them, allowing a whole picture about

Fig. 2: Biplot analysis of morpho-physiological indicators of drought tolerance in sunflower plants**Fig. 3: Dendrogram of cluster analysis of sunflower genotypes based on all morpho-physiological characters standardized to mean 0 and standard deviation =1**

the interrelationships among the morpho-physiological indices. G2 indices were positively correlated (an acute angle), whereas G1 had weak correlation with G3. Similarly clustering was made to categorize drought indices into components for the understanding of the share components that contribute to major variation in the study. Cluster analysis based on all morpho-physiological parameters divided sunflower plants into two groups. The first group included genotypes S471, S473, S475 and pacific 77, whereas the second group included genotypes 5A, 6A, 9A and 10A (Fig. 3).

The ranking method was used to determine overall judgment. All indices, mean rank and standard deviation of rank of all drought tolerance criteria were calculated, rank

sum (RS) and the final rank are shown in Table III. Based on all criteria in terms of morpho-physiological stress indices, the most desirable drought tolerant genotypes were identified. S473 (RS = 1.97), followed by S471 (RS = 3.47), S475 (RS = 3.78) and Pacific 77 (RS = 4.47) were the most drought tolerant genotypes. Genotypes 5A (RS = 7.37), 10A (RS = 7.24), 6A (RS = 6.98) and 9A (RS = 5.22) were the most sensitive to drought, respectively.

The responses of plants to water deficit-induced morpho-physiological changes at germination and seedling stages have been reported by several authors (Busso *et al.*, 1998; Mohammed *et al.*, 2002; Khalilvand *et al.*, 2007; Ahmad *et al.*, 2009; Hossain *et al.*, 2010). Drought tolerance of crop plants is a genetically determined character but

interaction with environment determines the expression of the plant traits. The present study showed clear difference in the contribution of morpho-physiological defense system in the drought tolerance of sunflower genotypes when subjected to drought stress. The exposure of water deficit led to differential GSI, PHSI, DMSI, RLSI and RWCSI response in all genotypes.

PEG caused osmotic stress and affected sunflower early seedling growth. Plant height showed greater reduction than other traits, indicating that this index is sensitive indicator of drought stress at the early seedling stage. Synthetic varieties S471, S473 and S475 performed extremely well among all sunflower genotypes treated with drought stress, and should, therefore be recommended for cultivation in areas where water stress may be common during the germination and early seedling growth stages. These also may serve as excellent germplasm source in breeding program to develop even better water stress tolerant lines. Among inbred lines, 9A was outstanding in its adaptation to drought stress condition that will serve as source material for breeding new hybrids.

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