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In vitro AND *In vivo* WATER STRESS IN SUNFLOWER (*Helianthus annuus L.*)

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SUMMARY

In this research, we investigated the response of sunflower cultivars to drought stress under both *in vitro* and *in vivo* conditions. Murashige and Skoog basal medium supplemented with a range of polyethylene glycol (PEG-1000) concentrations was used for *in vitro* drought screening. Results from both *in vitro* and *in vitro* experiments showed that plant growth decreased with increasing PEG concentrations. In addition, there were differences between the cultivars in terms of their response to drought.

The significant correlations between *in vitro* (except number of roots) and *in vivo* characters indicate that an *in vitro* approach could be useful in screening and selecting for drought response prior to field trial. As a result, all *in vitro* characters measured (except number of roots) could give clues for performance of sunflower genotypes against drought *in vivo*.

Key words: drought, plant growth, PEG, screening, tissue culture

INTRODUCTION

Optimal plant growth and successful crop production require suitable soil conditions - adequate water and nutrient supply. Environmental stress is any environmental factor or combination of factors that inhibits plant development and yield (Jones and Qualset, 1984; Erdem *et al.*, 2001). Water stress is one of the most severe limitations of crop growth in semi-arid and arid region of the world as it plays a vital role in plant metabolism at all growth stages. However, depending upon plant species, certain stages, such as germination, seedling or flowering, could be most critical for water stress. Water stress affects protein synthesis, photosynthesis, respiration and nucleic acid synthesis, which may result in yield reduc-

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tion (Dubey, 1994). In addition to genotypic variation, effect of drought on a plant depends on the timing, duration and severity of the stress (Jefferies, 1995).

In Turkey, sunflower (*Helianthus annuus L.*) is an economically important crop as vegetable oil source as well as animal feed. However, limited rainfall during growing season constrains seed yield of sunflower, which is mainly grown without irrigation because of limited resources of available water. Yield reduction amounts to 50% when compared with the seed yield of irrigated crops although sunflower is more tolerant to drought compared with other crops. Therefore, growing of drought tolerant cultivars will contribute to a more stable sunflower production.

Previous investigations showed that there is a variation among sunflower genotypes in terms of their response to drought (Somers *et al.*, 1983; Blanchet *et al.*, 1984; Morizet *et al.*, 1984; Wample and Thornton, 1984; Gomez *et al.*, 1991; Friedt, 1992). *In vitro* screening methods (i.e., at protoplast, cell, root, shoot and plantlet levels) are thought to provide rapid and easy screening tests for response of genotypes to drought stress factors. Moreover, genetic markers have become an important tool in the selection and screening of plants for environmental stresses, for detecting genetic variations between species or within species (Jahromi, 1996).

Investigations have been conducted in order to assess the correlation between *in vitro* and *in vivo* conditions with respect to rapid screening for disease resistance and environmental stress under *in vitro* conditions. According to some published reports, significant correlations between *in vitro* and *in vivo* characters were detected in potato grown under saline conditions (Morpurgo, 1991; Turhan, 1997). Furthermore, Naik and Widholm (1993) found that whole plant level and cultured potato roots *in vitro* showed a correlated relationship. However, there was no correlation at the cell and callus levels, as it might be expected.

The screening of the response of sunflower cultivars or breeding lines to drought stress plays a crucial role in breeding programs. When indiscriminate introduction of many new sunflower cultivars in many developing countries is considered, a rapid and efficient screening procedure gains additional importance for agriculture of a region. However, there are difficulties in field trials for drought screening of genotypes. These difficulties include uncontrolled climatic conditions, insufficient homogeneity of soil, large amount of plant material, time and labor costs and so on.

Therefore, the aims of this study were to determine possible correlation between *in vitro* and *in vivo* plant response to drought and to develop a rapid and easy screening method for providing reliable information on performance of sunflower cultivars against drought prior to field trial.

MATERIALS AND METHODS

Experiments were carried out on three sunflower (*Helianthus annuus* L.) hybrid cultivars (Cargill 207, Turkuaz and Isera) that are widely grown in the

region. The research consisted of two parts, namely *in vitro* and *in vivo*. These two parts are presented separately in this section.

***In vitro* experiment**

Murashige and Skoog (1962) culture medium supplemented with 3% sucrose and pH 5.7 was used as the basal medium for all *in vitro* treatments. Before sterilization, 0.8% Difco Bacto-Agar, a solidifying agent, was added. As an initial experiment, several MS based media with different concentrations of growth regulators were tested in order to obtain the best growth of sunflower cultivars used, as suggested by some researchers for other cultivars (Gurel and Kazan, 1998; Dagustu, 1999). However, the best result for the cultivars used was obtained with MS without growth regulators.

Polyethylene glycol (PEG) with a molecular weight of 1000 was used as a drought simulator *in vitro* (Yeo and Flowers, 1984; Iraki *et al.*, 1989; Dami and Hughes, 1997; Turhan, 1997). Five levels of PEG (0, 3, 6, 9, 12% w/v) were added to the growth regulator free MS medium. 8 ml of molten medium was poured into each 150 × 20 mm glass test tube (borosilicate). After sterilization and cooling down, all test tubes with caps were sealed with 25 mm micropore tape (3M Surgical Division, USA) to reduce evaporation from the medium.

After three-day germination of seeds in darkness at 28°C, about 1 cm long shoot tips were kept in 70% (v/v) ethanol for 5 minutes for surface sterilization and then washed several times with sterile distilled water. The shoot tips were treated with commercial bleach plus one drop of Twin 80 as a wetting agent for 20 min. and again washed several times with sterile distilled water. These explants were placed on the media in the glass test tubes and incubated in a growth room which was illuminated with cool white fluorescent tubes at 25±2°C with a 16 h photoperiod and a photosynthetic photon flux density (PPFD) of 95 μmol $m^{-2}s^{-1}$. After four weeks, several characters were measured and scored. These included shoot length (mm), number of leaves, number of roots, fresh weight of shoot (mg) and dry weight of shoot (mg), root score. For dry weight, shoots were oven dried at 90°C for 48 h, and then immediately weighed on the analytic balance. Root score was based on a visual assessment (i.e., root numbers, degree of branching and thickness), recorded on a 1 to 9 scale where 1 was no root and 9 was a very well established root system.

***In vivo* experiment**

Average monthly temperature during growing period was 14.0, 16.6 and 20.9°C for April, May and June, respectively. In addition, average monthly relative humidity was 84.8, 77.2 and 72.2% for April, May and June, respectively. Prior to the experiment, field capacity of soil in 7 kg pots containing sand, peat compost and field soil in 1:1:1 ratio was determined in order to apply different levels of drought. Field capacity has been defined according to Bilski *et al.* (1988) as that amount of water which is held by soil against gravity. Therefore, Field Capacity (%)=(Amount of Water (g)/Dry Soil Weight (g)) × 100. The amount of water held by the soil was

calculated from the difference between dry and wet soil weight. Drought treatments were determined by using amount of water held by the soil as follows:

1. 100% control in which all pots were kept at field capacity.
2. 75% of amount of water held at field capacity.
3. 50% of amount of water held at field capacity.
4. 25% of amount of water held at field capacity.

Four seeds of each cultivar were sown into 7 kg pots in April, which was sunflower planting time for the region. The experiment was conducted in the open field. During rains, pots were covered with a transparent plastic shed (not touching the plants) in order to minimize shading effect. At the beginning, all pots were kept at field capacity for obtaining germination and emergence of the seeds. Before starting drought treatments, the plants were thinned to one healthy plant per pot. During drought treatment stage, each pot was weighed daily at 9 a.m., and watered until the pot weight reached to pre-determined weight, which was calculated as follows;

$P=P_f - (W_f (100-D)/100)$

- where:

  P=pot weight (g),

  P_f=pot weight at field capacity (g) (in this case, 7 kg),

  W_f=amount of water held by a pot at field capacity (g) (in this case, 500 g),

  D=drought level (%)

For example, control pots at the beginning of the experiment were watered up to 7 kg, whereas in 75% drought treatment, it was 6.875 kg as the soil in a pot holds 500 g water.

After four weeks, plant height (cm), number of leaves per plant, shoot fresh weight (g), root fresh weight (g), shoot dry weight (g) and root dry weight (g) were determined. For root fresh weight, after removing soil from roots in water, they were kept between paper towels for an hour to remove excess water and then weighed. For shoot and root dry weight, they were oven dried at 90°C for 48 h and weighed immediately after drying.

Each *in vitro* treatment replication comprised ten test tubes. In *in vivo* experiment, each treatment comprised four pots. The experimental design in both experiments was a randomized block design. The least significant difference (LSD) test was applied for means separation at 0.05 significance level. The statistical analysis was carried out using the SAS computer package (SAS Institute, 1988).

In order to measure response of cultivars to drought stress, relative tolerance was used instead of absolute tolerance. Relative tolerance was calculated for each genotype and different drought levels using the following formula:

$$\text{Relative tolerance (\%)} = \frac{\text{Absolute value}}{\text{Value at 0.00\% drought level}} \times 100$$

RESULTS AND DISCUSSION

The data taken for four-week old plantlets grown *in vitro* on MS supplemented with different concentrations of PEG were analyzed in terms of the effect of cultivar, PEG and their interaction on all characters measured (data not shown). The highest level of PEG concentration (12%) was not included in statistical analysis, as none of the plants survived at this level.

According to Chaubey and Senadhira (1994), the uptake, accumulation and utilization of mineral elements in plants are genetically controlled, although there is a strong environmental interaction. Our observations on a range of characters in sunflower cultivars showed that there was a genotype dependent-response to drought with respect to drought tolerance. The results showed that the cultivar Turkuaz had a better performance than the other cultivars at increasing levels of drought (Table 1).

Table 1: Effect of Polyethylene Glycol-1000 on all characters measured in three sunflower cultivars grown *in vitro*, after four weeks of treatment

Cultivar	PEG Concentration (%)				
	0	3	6	9	Mean
	Shoot length (cm) (LSD $_{0.05}$: PEG = 1.096, Cultivar = 0.949)				
Turkuaz	14.77 (100)[1]	4.61 (31)	4.14 (28)	3.56 (24)	6.77 a*
Isera	9.67 (100)	4.54 (47)	3.40 (35)	1.96 (20)	4.89 b
C-207	12.03 (100)	3.33 (28)	32.44 (20)	3.04 (25)	5.21 b
Mean	12.16 a	4.16 b	3.33 bc	2.85 c	
	Number of leaves (LSD $_{0.05}$: PEG = 0.684, Cultivar = 0.592)				
Turkuaz	8.00 (100)	4.57 (57)	4.29 (54)	3.71 (46)	5.14 a*
Isera	6.86 (100)	4.57 (67)	4.00 (58)	2.86 (42)	4.57 a
C-207	6.29 (100)	3.57 (57)	2.86 (45)	2.86 (45)	3.89 c
Mean	7.05 a	4.24 b	3.71 bc	3.14 c	
	Number of roots (LSD $_{0.05}$: PEG = 1.126, Cultivar = 0.975)				
Turkuaz	6.43 (100)	5.43 (84)	6.71 (104)	5.14 (80)	5.93 a
Isera	6.29 (100)	5.14 (80)	4.00 (64)	2.57 (41)	4.50 b
C-207	5.43 (100)	4.29 (79)	5.14 (95)	2.71 (50)	4.39 b
Mean	6.05 a	4.95 a	5.29 a	3.48 b	
	Root score (LSD $_{0.05}$: PEG = 0.792, Cultivar = 0.686)				
Turkuaz	9.00 (100)	4.71 (52)	4.14 (46)	3.86 (46)	5.43 a
Isera	8.43 (100)	5.00 (59)	3.86 (46)	2.43 (42)	4.93 ab
C-207	8.71 (100)	3.57 (41)	3.29 (38)	3.29 (45)	4.71 b
Mean	8.71 a	4.43 b	3.76 bc	3.19 c	
	Shoot fresh weight (g) (LSD $_{0.05}$: PEG = 0.054, Cultivar = 0.046)				
Turkuaz	0.61 (100)	0.15 (25)	0.13 (21)	0.12 (20)	0.25 a
Isera	0.58 (100)	0.14 (24)	0.13 (22)	0.08 (14)	0.23 a
C-207	0.44 (100)	0.07 (16)	0.06 (14)	0.08 (18)	0.16 b
Mean	0.54 a	0.12 b	0.11 b	0.09 b	
	Shoot dry weight (mg) (LSD $_{0.05}$: PEG = 4.474, Cultivar = 3.874)				
Turkuaz	42.14 (100)	24.71 (59)	19.14 (45)	18.00 (43)	26.00 a
Isera	39.71 (100)	25.57 (64)	19.14 (48)	14.00 (35)	24.61 a
C-207	34.57 (100)	12.43 (36)	11.43 (33)	9.71 (28)	17.04 b
Mean	38.81 a	20.91 b	16.57 b	13.91 b	

* Means with the same letter are not significantly different at p=0.05.

[1] The values in brackets are relative tolerance (%) of the cultivars at each drought level.

Table 1 also demonstrates that increasing levels of PEG inhibited the growth of all sunflower cultivars *in vitro*, with dramatic reductions in both absolute and relative tolerance values for all the characters.

The results obtained in the *in vivo* experiment (Table 2) were similar to the results from the *in vitro* experiment. The effect of drought on all characters was significant. The means separating each drought level by LSD were shown in Table 2.

Table 2: Effect of drought on all measured characters in three sunflower cultivars grown *in vivo*, after 4 weeks of treatment

Cultivar	Drought level (%)				
	Control (100)	75	50	25	Mean
	Plant height (cm) (LSD $_{0.05}$: PEG = 2.567, Cultivar = 0.2.220)				
Turkuaz	30.25 (100)[1]	26.88 (89)	15.00 (50)	12.88 (43)	21.25 a*
Isera	29.25 (100)	24.50 (84)	13.25 (45)	10.00 (34)	19.25 ab
C-207	24.63 (100)	22.88 (93)	14.13 (57)	9.38 (38)	17.75 b
Mean	28.04 a	24.75 b	14.13 c	10.75 d	
	Number of leaves (LSD $_{0.05}$: PEG = 1.351, Cultivar = 1.170)				
Turkuaz	13.75 (100)	12.50 (91)	5.00 (36)	4.50 (33)	8.94 a
Isera	14.00 (100)	11.25 (80)	5.75 (41)	4.00 (29)	8.75 a
C-207	11.75 (100)	10.75 (91)	5.00 (43)	3.50 (30)	7.75 b
Mean	13.17 a	11.50 b	5.25 c	4.00 c	
	Shoot fresh weight (g) (LSD $_{0.05}$: PEG = 5.441, Cultivar = 4.712)				
Turkuaz	34.52 (100)	30.35 (88)	1.69 (5)	1.46 (4)	17.01
Isera	37.77 (100)	18.98 (50)	1.94 (5)	1.41 (4)	15.03
C-207	37.04 (100)	21.85 (59)	4.86 (13)	0.60 (2)	16.09
Mean	36.44 a	23.73 b	2.83 c	1.15 c	
	Root fresh weight (g) (LSD $_{0.05}$: PEG = 7.188, Cultivar = 6.225)				
Turkuaz	86.48 (100)	57.94 (67)	4.56 (5)	3.41 (4)	38.09 a
Isera	73.00 (100)	32.32 (44)	6.36 (9)	3.63 (5)	28.83 b
C-207	72.98 (100)	52.08 (71)	7.48 (10)	1.80 (3)	33.58 ab
Mean	77.48 a	47.45 b	6.13 c	2.95 c	
	Shoot dry weight (g) (LSD $_{0.05}$: PEG = 1.240, Cultivar = 1.076)				
Turkuaz	13.34 (100)	9.07 (68)	0.81 (6)	0.64 (5)	5.96
Isera	12.44 (100)	5.98 (48)	1.28 (10)	0.75 (6)	5.11
C-207	13.00 (100)	9.33 (72)	1.49 (11)	0.35 (3)	6.04
Mean	12.93 a	8.13 b	1.19 c	0.58 c	
	Root dry weight (g) (LSD $_{0.05}$: PEG = 0.456, Cultivar = 0.395)				
Turkuaz	3.31 (100)	2.54 (77)	0.38 (11)	0.18 (5)	1.56
Isera	3.68 (100)	1.96 (53)	0.33 (9)	0.25 (7)	1.56
C-207	3.93 (100)	2.27 (58)	0.38 (10)	0.09 (2)	1.67
Mean	3.64 a	2.26 b	0.31 c	0.18 c	

* Means with the same letter are not significantly different at p=0.05.

[1] The values in brackets are relative tolerance (%) of the cultivars in each drought level.

According to relative tolerance, the extent of reduction was 2-43% at the highest level of drought (25%). There were also significant variations among means of cultivars in terms of their response to drought for plant height, number of leaves and root fresh weight. Considered overall in terms of relative tolerance, Isera was more

tolerant than the other cultivars at the highest drought level (25%) although it showed a lower performance at 75% drought level.

The analyses of the characters measured indicated clearly that drought has a highly significant and generally detrimental effect on sunflower cultivars grown under both *in vitro* and *in vivo* conditions. The *in vitro* experiment showed that PEG could be successfully used as drought simulator, as previously declared by other researchers (Yeo and Flowers, 1984; Iraki *et al.*, 1989; Turhan, 1997). PEG exposure causes osmotic stress, reduction of turgor pressure, limitation of nutrient uptake and inhibition of photosynthetic CO_2 uptake (Pugnarie *et al.*, 1994). Therefore, PEG started to affect the sunflower cultivars as its level increased. In general, the cultivars were drastically affected at the higher levels, and at 12% PEG level none of the plants could survive. In addition, these experimental results showed that drought sensitivity was not uniform across cultivars. In overall, the cultivar Turkuaz was the most tolerant for all the characters measured *in vitro* according to relative tolerance.

When plants were exposed to drought under *in vivo* conditions, plant growth was also dramatically declined. According to relative tolerance, shoot length and shoot fresh weight were the most affected traits under *in vitro* conditions and shoot and root weight were the most affected characters under *in vivo* conditions.

Correlations between *in vitro* and *in vivo* characters (except between number of roots *in vitro* and shoot and root weight related characters) were found to be significant and positive (Table 3).

Table 3: Comparison of the effect of drought on sunflower cultivars grown under *in vitro* and *in vivo* conditions

		In vivo characters					
		PH	NL	FSW	FRW	DSW	DRW
In vitro characters	SL	0.75 **	0.61*	0.81**	0.85***	0.83***	0.83**
	NL	0.83***	0.65*	0.83***	0.86***	0.84***	0.84***
	NR	0.66*	0.59*	0.53	0.53	0.51	0.52
	RSC	0.81**	0.67*	0.85***	0.86***	0.86***	0.87***
	FSW	0.73**	0.60*	0.79**	0.81**	0.80**	0.80**
	DSW	0.81**	0.62*	0.82**	0.83***	0.81**	0.83***

PH: Plant height, NL: Number of leaves, FSW: Fresh shoot weight, FRW: Fresh root weight, DSW: Dry shoot weight, DRW: Dry root weight, SL: Shoot length, NR: Number of roots, RSC: Root score. *, ** and ***: Significant at 0.05, 0.01 and 0.001 level, respectively.

On the other hand, root score, an *in vitro* character, was highly and significantly correlated with all *in vivo* characters. Morpurgo (1991) and Turhan (1999) also found a high correlation between *in vitro* root growth and *in vivo* tuber yield of potato genotypes exposed to NaCl. This may be an indication that roots are actively involved in moderate drought stress by controlling water uptake from the soil.

In conclusion, these significant correlations indicate that an *in vitro* approach could be useful in screening and selection of sunflower genotypes for responses to

drought prior to field trial. Except for the number of roots, all *in vitro* characters measured could provide clues for pot or field performance of sunflower genotypes grown under drought conditions.

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ESTRÉS HÍDRICO EN GIRASOL (*Helianthus annuus L.*) EN LAS CONDICIONES *in vitro* e *in vivo*

RESUMEN

En este trabajo fue investigada la reacción de las variedades de girasol en el estrés provocado por sequía, en las condiciones *in vitro* e *in vivo*. Para la selección de plantas en las condiciones *in vitro*, fue utilizado el suelo Murashige y Skoog, al cual se anadían diferentes concentraciones de glicol polietileno (PEG-1000). En ambos tipos de condiciones, el crecimiento de las plantas iba disminuyéndose con el incremento de concentración de PEG. Aparte de ello, también fueron notadas las diferencias entre las variedades, en cuanto a la reacción a sequía.

Unas correlaciones significantes, establecidas entre las características investigadas en las condiciones *in vitro* (salvo el número de raíces) e *in vivo*, indican que el acercamiento *in vitro* podría ser de utilidad en la elección y selección de plantas para la reacción a sequía, antes de los ensayos de campo. Como consecuencia de ello, todas las características medidas en las condiciones *in vitro* (salvo el número de raíces) pueden servir de indicadores de rendimiento de los genotipos de girasol en las condiciones de sequía *in vivo*.

STRESS D'EAU DU TOURNESOL (*Helianthus annus* L.) DANS LES CONDITIONS *In vitro* ET *In vivo*

RÉSUMÉ

Dans cette étude la réaction des sortes de tournesol au stress provoqué par la sécheresse dans les conditions *in vitro* et *in vivo* était analysée. Les bases de plantes Murashige et Skoog étaient utilisées dans les conditions *in vitro* avec le supplément de concentrations différentes de polyéthylène glycol (PEG-1000). Dans les deux types de conditions le développement de plantes se diminuait avec l'augmentation de concentration de PEG. En outre, certaines différences concernant la réaction envers la sécheresse sont remarquées.

Les importantes corrélations constatées entre les traits examinés dans les conditions *in vitro* (excepté le nombre de racines) et *in vivo* prouvent que l'approche *in vitro* pourrait être utile pendant la procédure de sélection de plantes concernant la réaction envers la sécheresse avant le processus expérimental de champ. En conséquence, tous les traits testés dans les conditions *in vitro* (excepté le nombre de racines) pourraient être utilisés comme indicateurs de performance de génotypes de tournesol dans les conditions de sécheresse *in vivo*.