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MORPHO-PHYSIOLOGICAL SCREENING OF SUNFLOWER INBRED LINES UNDER DROUGHT STRESS CONDITION

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ABSTRACT

Efficiency of morphological and physiological characteristics was studied for screening of sunflower (*Helianthus annuus* L.) inbred lines for drought tolerance in flowering stage using principle component analysis. Sixteen sunflower inbred lines were evaluated under rainout shelter for two years. According to the results stress tolerance index and stress susceptibility index each had more efficiency for identifying of drought tolerant and sensitive lines respectively. Principle component analysis was emerged as a powerful method for identifying of drought tolerant and sensitive lines which separated BGK 329 and RGK 21 as the most drought tolerant and sensitive lines respectively in the reverse direction of its biplot. The tolerant line was differentiated principally by higher plant height, stem and head diameter, seed weight, seed number, root and shoot dry weight, root length, leaf area, osmotic adjustment, Fv/Fm, SPAD value and seed yield and lower leaf temperature and specific leaf area compared to the sensitive line. Plant height, stem and head diameter were identified as morphological and leaf area, leaf temperature, Fv/Fm and SPAD value as efficient, none destructive physiological indicators for screening of sunflower genotypes under drought stress condition.

Key words: Biplot, Correlation, Drought stress, Flowering, Principle component analysis

INTRODUCTION

Water availability is the most important key factor that determines yield potential of plants. About one quarter of world's arable areas is under drought stress (Singh, 2000). Sunflower following oil palm, soybean, and rapeseed constitute over 87% of global production of vegetable oils (Murphy, 2010). Productivity of sunflower is greatly affected by drought, however it is considered moderately tolerant to drought stress (Tahir *et al*., 2002). It is well known that sunflower yield decreases under drought stress (Erdem *et al*., 2006) but this is dependant to level of water deficit and cultivar (Rodriguez *et al*., 2002). Drought stress during the growth period has deleterious effects on yield and oil content of sunflower (Razi and Assad, 1999) but greatest yield losses occurs when water shortage occurs at flowering stage (Rauf, 2008).

The effect of drought stress on morphological and physiological characteristics of sunflower is well documented. Reduction of head size, stem diameter, plant height, seed weight, seed yield, root to shoot ratio, leaf water potential (LWP), relative water content (RWC) of leaves, leaf area index and total chlorophyll content were reported previously (Sharp and Boyer, 1986; Razi and Assad, 1998; Petcu *et al*., 2001; Hossain *et al*., 2010; Vanaja *et al*., 2011). Maury *et al*. (2000) reported increasing of osmotic adjustment and Germ *et al*. (2005) indicated differential response of sunflower cultivars for chlorophyll fluorescence (Fv/Fm). Plant breeders have used different criteria for screening of sunflower genotypes for drought tolerance. The most important of them are stress susceptibility index (SSI) (Fischer and Maurer, 1978), mean productivity (MP) and tolerance index (TOL) (Rosielle and Hamblin, 1981), geometric mean productivity (GMP) and stress tolerance index (STI) (Fernandez, 1992). There is no information about using of morphological and physiological traits together for screening of sunflower genotypes under drought stress. The objective of this study was to evaluate efficiency of morphological and physiological attributes for screening of sunflower inbred lines under drought stress and to identify the most drought tolerant and sensitive lines.

MATERIALS AND METHODS

This study was carried out at the research station of Faculty of Agriculture, University of Tabriz, Iran (latitude of 38°, 5' and longitude of 46°, 17' and altitude of 1360 m above mean sea level) during 2009 and 2010. The climate of the region was cold and semidry with average rainfall amount of 184 mm and the area temperature of 12 °C. Sixteen sunflower inbred lines coming from Khoy, Iran Agricultural Research Station were planted in normal and drought stress conditions under rainout shelter using a Randomized Block Design with 9 replications. Drought stress was imposed by water withholding during flowering stage (R4 to R6) (Schneiter and Miller, 1981). Three seeds of each line planted in a pot, 20 cm diameter and 1m length and thinned to one seedling after emergence.

Seed yield and its components and some of plant characteristics were measured after harvest while RWC, LWP, Fv/Fm, leaf area, leaf temperature, SPAD reading and proline content were measured at the end of flowering stage (R6). The upper most fully expanded leaves were used for measurement of RWC using RWC =100 × (fresh weight – dry weight) / (turgid weight – dry weight). Turgid weight was determined after 24 h rehydration at 4°C in a dark room with the leaf discs placed in a container with distilled water and dry weight determined after oven drying for 24 h at 80°C. Leaf water potential was determined using a pressure chamber. Proline content was quantified according to the method of Bates *et al*. (1973). Leaf chlorophyll concentration was assessed using a SPAD-502 chlorophyll meter (SPAD-502, Minolta). The measurements being taken at upper, middle and lower part of the leaf and average of them was considered as SPAD reading. Drought tolerance indices and principle component analysis (PCA) were used to identify of the tolerant and sensitive lines. Data were analyzed using SPSS and Stat Graphics.

RESULTS AND DISCUSSION

Among the 16 sunflower inbred lines, BGK 329 had significantly higher seed yield compared to the other lines in both normal and drought stressed conditions (Table 1). This line discriminated as the drought tolerant line with the highest STI, MP and GMP. In other hand RGK 21 with 61% reduction of seed yield was affected more than any other lines by drought stress. Sensitivity of this line was reflected by its high values of SSI and TOL.

Table 1. Average seed yield and drought tolerance indices of sunflower inbred lines.

Inbred lines	HYn[a] (g head $^{-1}$)	HYs[b] (g head $^{-1}$)	STI[c]	SSI[d]	TOL[e]	MP[f]	GM[g]
BGK-345	8.072	4.651	0.429	1.069	3.421	6.36	6.13
BGK-221	9.714	5.238	0.582	1.251	4.476	7.48	7.13
BGK-355	7.074	4.423	0.358	1.018	2.651	5.75	5.59
BGK-329	12.045	8.003	1.102	0.911	4.041	10.02	9.82
BGK-109	7.958	5.323	0.484	0.899	2.635	6.64	6.51
BGK-195	9.357	5.239	0.561	1.195	4.118	7.30	7.00
BGK-343	9.596	5.523	0.606	1.153	4.074	7.56	7.28
BGK-309	8.370	5.153	0.493	1.044	3.217	6.76	6.57
BGK-259	9.181	5.362	0.563	1.130	3.819	7.27	7.02
BGK-147	8.928	6.813	0.696	0.643	2.115	7.87	7.80
RGK-46	10.358	6.611	0.783	0.983	3.747	8.48	8.27
RGK-56	9.321	5.571	0.594	1.093	3.750	7.45	7.21
RGK-26	10.147	7.074	0.821	0.822	3.073	8.61	8.47
RGK-23	8.988	5.104	0.525	1.174	3.884	7.05	6.77
RGK-21	10.636	4.162	0.506	1.653	6.474	7.40	6.65
RGK-3	9.876	6.069	0.685	1.047	3.807	7.97	7.74

[a] Head yield in normal condition; [b] Head yield in stress condition; [c] Stress tolerance index; [d] Stress susceptibility index; [e] Tolerance; [f] Mean productivity; [g] Geometric mean productivity; LSD for comparison of seed yield means is 0.53 at probability level of 5%.

Darvishzadeh *et al*. (2010) suggested STI, MP and GMP as the suitable indices for screening of sunflower genotypes under drought stress condition. In accordance with Clarke *et al.* (1992) SSI failed in identifying of the tolerant lines. It is concluded that none of these indices cannot be used as a criterion for identify of sensitive or tolerant lines solely. In this study STI and SSI each had more efficiency for identifying of the tolerant and sensitive lines respectively.

Three dimensional graphs using seed yield in normal and drought stressed conditions and STI and SSI as drought tolerance/sensitivity indices were used to partitioning lines based on Fernandez (1992) groups. These graphs confirmed BGK 329 as the most drought tolerant line, followed by RGK 46, RGK 26 and RGK 3 which composed all together group A lines (Fig. 1). RGK 21, BGK 221 and BGK 343 were located in B region and represented the sensitive lines. There was only one line, BGK 147 in C region with lowest SSI and TOL showing drought tolerance characteristics but had low STI. Most of the lines which aggregated in D region were not really sensitive lines because of lower seed yield potential in well watered condition.

Principle component analysis which facilitates selection of genotypes especially when there are many lines to be selected and many traits to be involved was used to determine whether there was any structure associated with agronomic performance and drought tolerance indices or not.

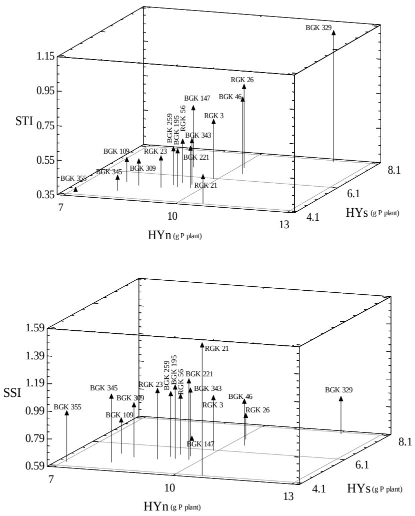

Figure 1. Three dimensional plot of head yield in normal (HYn) and drought stressed condition (HYs) with STI (up) and SSI (down).

Three principle components had eigen values more than 1 and together accounted for 79.3% of the variability of original data under drought stressed condition. Plant height, seed yield, stem diameter, root dry weight and STI had the highest weight in the first component while root to shoot ratio, seed number and RWC were important in the second component (Table 2).

The first PCA separated 2 lines in the extreme horizontal ends of the biplot, BGK 329 expressed as the tolerant line in the right and RGK 21 as the sensitive line in the left side of the biplot (Fig. 2). The tolerant line was differentiated with the high values of a cluster of traits like that plant height, Fv/Fm, leaf area, STI and shoot dry weight. These traits had high correlation with each other because of close adjacent of their vectors and lines in the same direction of the respective vectors had the higher value for these traits (de la Vega *et al.*, 2001), so BGK 329 as well as RGK 46 and RGK 26 at the same direction of STI vector were drought tolerant. The lines RGK 21 and BGK 355 in the reverse direction of STI were differentiated with high values of leaf temperature, specific leaf area (SLA), LWP and SSI and were drought sensitive lines. Comparison of lines under drought stress revealed significant difference between BGK 329 and RGK 46 as the tolerant lines and RGK 15 and BGK 355 as the sensitive lines regarding all the measured traits (Table 3). These two groups of lines were separated and located in the reverse side of PCA biplot (Fig. 2) which represents efficiency of PCA as a powerful tool for identifying of drought sensitive and tolerant lines.

Table 2. Eigen value of equations in principal component analysis (PCA).

Abbreviation	Traits	PC-1	PC-2	PC-3
PH	Plant Height	0.260	0.087	-0.071
SD	Stem Diameter	0.245	-0.139	-0.046
HD	Head diameter	0.240	-0.095	-0.073
SW	1000 Seed Weigh	0.240	-0.173	-0.191
SN	Seed Number	0.127	0.453	0.337
SDW	Shoot Dry Weight	0.240	0.105	0.157
RDW	Root Dry Weight	0.243	-0.226	0.081
RS	Root to Shoot ratio	0.099	-0.455	-0.094
RL	Root Length	0.225	-0.210	0.215
LA	Leaf Area	0.237	0.026	-0.100
SLA	Specific Leaf Area	-0.188	-0.106	0.305
RWC	Relative Water Content	0.215	-0.261	-0.023
LWP	Leaf Water Potential	-0.196	0.158	0.184
Fv/Fm	Chlorophyll Fluorescence	0.218	0.036	-0.055
LT	Leaf Temperature	-0.218	-0.071	0.240
SPAD	Leaf chlorophyll content	0.195	-0.057	0.002
OA	Osmotic Adjustment	0.223	0.202	0.007
PC	Proline Content	0.174	0.204	0.027
HYn	Head Yield normal	0.170	-0.133	0.552
HYs	Head Yield stress	0.252	0.201	0.104
STI	Stress Tolerance index	0.242	0.087	0.299
SSI	Stress Susceptibility index	-0.153	-0.391	0.383

PC-1, 2 and 3 refers to principle component 1, 2 and 3 respectively

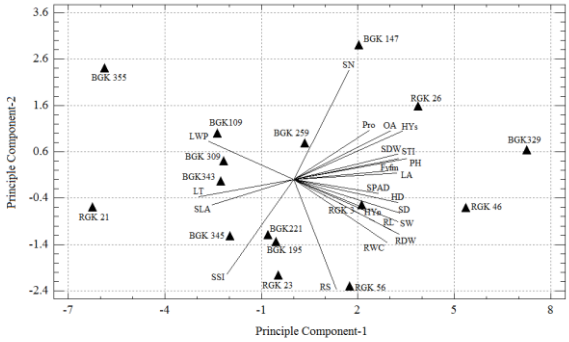

Figure 2. Biplot of principle components analysis for morphological and physiological traits of sunflower inbred lines under drought stress condition. Triangles show position of inbred lines. Each beeline is a vector of morphological or physiological trait. Abbreviations are presented in table 2.

Although the above mentioned traits are important drought stress related traits but there is need to simple and easy measurable characteristics which could help to improving efficiency of selection, a major challenge in plant breeding programs. In accordance with PCA, plant height and head and stem diameter having significant positive correlation with HYs and STI (Table 4) were stabilized as simple morphological markers for screening of sunflower genotypes under drought stress condition. Razi and Assad (1998) also stated the important role of these traits under drought stress. Significant correlation of plant height with seed yield is reported by Dagustu (2002) in normal condition too, so plant height is an important determinant of seed yield in both normal and drought stress condition. Sadras *et al.* (1993) indicated the critical role of stem reservoirs in seed filling of sunflower under drought condition. Physiological characteristics like that leaf area, leaf temperature, Fv/Fm, SPAD, proline content, osmotic adjustment, RWC, LWP and shoot dry weight had especially high significant correlations with each other and with HYs (Table 4). Among them leaf area, leaf temperature, Fv/Fm and SPAD value are suitable physiological traits for screening of sunflower genotypes in selection programs because of simplicity in measuring.

Table 3. Comparison of sunflower inbred lines for morpho-physiological traits under drought stress

Root length (mm)	**Root/Shoot**	**Root dry weight (g)**	**Shoot dry weight (g)**	**Seed No./Head**	**1000 seed weight (g)**	**Head diameter (mm)**	**Stem diameter (mm)**	**Plant height (cm)**	**Lines**
211	0.389	7.101	18.279	225.97	20.94	112.56	15.21	117.8	BGK-345
230	0.338	8.078	24.165	252.47	20.84	121.07	15.5	120.8	BGK-221
191	0.278	5.131	18.782	257.62	17.11	89.85	12.52	108.4	BGK-355
242	0.352	9.34	26.731	312.73	25.75	130.23	18.11	136.6	BGK-329
219	0.306	6.248	20.44	256.59	21.56	102.52	13.24	116.3	BGK-109
219	0.324	7.173	21.699	226.29	23.09	108.38	15.62	119.9	BGK-195
217	0.378	7.333	19.068	292.44	19.07	103.19	13.78	113.1	BGK-343
207	0.343	6.341	18.52	256.52	20.27	115.9	13.78	122.9	BGK-309
221	0.313	7.134	22.99	255.36	21.5	125.27	14.89	125.3	BGK-259
217	0.292	7.294	25.187	302.2	22.58	117.55	16.16	130.3	BGK-147
229	0.373	9.473	25.462	271.62	24.95	132.7	17.93	134.7	RGK-46
224	0.404	8.919	22.356	233.39	24.39	119.54	17.03	126.3	RGK-56
224	0.314	7.875	25.581	292.24	24.27	127.11	14.97	132.1	RGK-26
223	0.343	7.174	20.832	205.97	25.22	119.83	14.31	115.2	RGK-23
209	0.299	5.696	19.058	250.31	17.08	93.07	12.23	105.2	RGK-21
231	0.364	8.794	24.436	266.52	23.1	113.17	15.42	127.3	RGK-3
14.21	0.03	0.74	2.07	27.53	1.63	7.28	1.17	7.16	LSD 5%

Table 3. Continue

Proline content (mg/g fresh weight)	**Osmotic adjustment (MPa)**	**SPAD**	**Leaf Temperature (C°)**	**Fv/Fm**	**Leaf water potential (MPa)**	**Relative water content (%)**	**Specific leaf area**	**Leaf area (cm^2)**	**Lines**
12.322	0.21	27.89	22.34	0.676	2.25	61.02	58.98	1475	BGK-345
12.164	0.21	25.04	22.7	0.692	2.23	62.56	70.98	1521	BGK-221
12.418	0.20	22.53	21.97	0.676	2.5	59.76	69.07	1333	BGK-355
16.389	0.40	29.57	20.43	0.751	2.16	66.8	59.64	2447	BGK-329
9.392	0.19	29.27	21.56	0.689	2.43	59.49	64.92	1550	BGK-109
10.883	0.26	27.4	21.49	0.681	2.15	65.3	69.38	1858	BGK-195
13.140	0.23	27.62	21.84	0.672	2.29	60.07	75.17	1223	BGK-343
14.350	0.16	25.45	21.67	0.667	2.16	59.52	70.28	1940	BGK-309
17.157	0.41	27.67	21.62	0.685	2.13	60.29	66.9	1454	BGK-259
12.428	0.44	26.61	21.14	0.694	2.07	59.59	64.37	2164	BGK-147
15.423	0.38	30.01	20.77	0.747	2.17	66.78	61.4	2434	RGK-46
11.088	0.32	27.3	21.2	0.676	2.08	64.98	62.79	2060	RGK-56
16.485	0.37	29.69	20.86	0.693	2.09	65.47	58.6	1994	RGK-26
11.897	0.21	26.58	20.71	0.696	2.06	64.31	70.81	1617	RGK-23
9.176	0.15	25.43	23.13	0.642	2.45	58.54	74.89	1225	RGK-21
13.171	0.37	27.06	21.1	0.682	2.08	63.85	66.54	2193	RGK-3
1.60	0.06	1.66	0.31	0.02	0.09	2.4	4.96	71.67	LSD 5%

Table 4. Correlations of some morphological and physiological traits in sunflower lines under drought stress

	PH [a]	SD	HD	SDW	RL	LA	RWC	LWP	Fv/Fm	LT	SPAD	OA	PC	STI
SD	.861**													
HD	.879**	.810**												
SDW	.855**	.765**	.750**											
RL	.708**	.748**	.751**	.784**										
LA	.893**	.820**	.696**	.755**	.613*									
RWC	.655**	.771**	.675**	.663**	.715**	.700**								
LWP	-.725**	-.667**	-.786**	-.587*	-.596*	-.648**	-.577*							
Fv/Fm	.717**	.758**	.716**	.676**	.628**	.678**	.685**	-.334						
LT	-.727**	-.611*	-.630**	-.608*	-.539*	-.765**	-.678**	.646**	-.705**					
SPAD	.634**	.567*	.608*	.506*	.679**	.503*	.539*	-.379	.569*	-.570*				
OA	.829**	.716**	.667**	.844**	.564*	.680**	.476	-.638**	.559*	-.654**	.504*			
PC	.682**	.448	.701**	.525*	.354	.428	.375	-.463	.559*	-.504*	.377	.636**		
STI	.804**	.731**	.686**	.841**	.792**	.745**	.668**	-.487	.698**	-.646**	.638**	.714**	.588*	
HYs	.888**	.739**	.714**	.861**	.716**	.810**	.611*	-.558*	.723**	-.767**	.655**	.799**	.618*	.942**

[a] Abbreviations are presented in table 2 , * and ** refer to level of significance, P<0.05 and P<0.01 respectively.

It seems that tolerant lines with lower leaf temperature (Table 3) can control excess energy of light which can dissipated as heat or re emitted as chlorophyll fluorescence. Reciprocal relationship of leaf temperature and chlorophyll fluorescence was revealed by PCA biplot (Fig. 2), where the respective vectors were expressed in reverse directions. Lower values of Fv/Fm ratio in RGK 21, indicated an injury to electron transfer system in photo system II, causing an imbalance between generation and utilization of electrons, resulting changes of quantum yield efficiency (Reddy *et al*., 2004). Reduction of Fv/Fm after severe water stress was reported by Germ *et al.* (2005) in sunflower leaves exposed to limited water supply. Although drought stress blemish to chlorophyll content of sunflower leaves (Petcu *et al*., 2001), however tolerant lines with higher SPAD value (Table 3) endured this injury better than sensitive lines.

Proline content had a significant positive correlation with plant height, osmotic adjustment and HYs (table 4). Proline accumulation is an adaptive behavior of plants when they are subjected to different environmental stresses (Oncel *et al*., 2000). These correlations indicated that proline content can affect osmotic potential under drought stress which is in accordance with Morgan (1984). Rauf and Sadaqat (2008) suggested RWC as a physiological marker of osmotic adjustment. In accordance with them, RWC had a significant positive correlation with seed yield. There was a significant positive correlation among RWC, root length and HYs, indicated that deeper roots provide more water for plant, the ability which was observed in BGK 329 (Fig. 2 and Table 3) and explains higher seed yield of this line under drought stress. Higher LWP inhibits the photosynthesis capacity of sunflower (Tezara *et al*., 2002) which was a characteristics of sensitive line i.e. RGK 21. Measuring of LWP and RWC has its complexity but PCA represented leaf temperature as a good indicator of them. The sensitive lines, BGK 355 and RGK 21 were diverged from other lines with higher value of SLA. Leaves with high SLA are thinner and have lower chlorophyll content and photosynthetic capacity (Songsri *et al*., 2009). This explains the low productivity of the sensitive lines, RGK 21 and BGK 355 under drought stress.

CONCLUSIONS

Among 16 sunflower inbred lines BGK 329 and RGK 21 were expressed as the most drought tolerant and sensitive lines respectively. The two indices, STI and SSI each had more efficiency in identifying of drought tolerant and sensitive lines respectively. Principle component analysis separated drought tolerant and sensitive lines effectively. Plant height, stem and head diameter were stabilized as efficient morphological and leaf area, leaf temperature, Fv/Fm and SPAD value as physiological indicators for screening of sunflower genotypes under drought stress condition.

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