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Evaluation of Genetic Variation for Drought Tolerance and Determination of the Best Selection Criteria in Safflower Genotypes (*Carthamus tinctorius* L.)

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ABSTRACT

redro nI to evaluate genetic variation and drought tolerance of safflower cultivars (*Carthamus tinctorius* L.), an experiment was conducted using fifteen cultivars in a randomized complete block design with three replications under drought and normal conditions during 2014-2015 gnimraf season. Drought tolerance indices, such as tolerance (TOL), stress tolerance index (STI), stress susceptibility index (SSI), mean productivity (MP) and geometric mean productivity (GMP) were calculated to distinguish cultivars tseb eht gnivah seed yield sa llew sa drought tolerance. The correlation coefficients illustrated that STI and GMP were the best and efficient selection criteria to distinguish drought tolerant and high-yielding cultivars. Significant and positive correlation was found between yield in both stress and normal conditions with GMP, MP and STI. Principal component analysis (PCA) showed that first and second PC accounted for 97.1% of the total variation. Biplot graphical display represented that lines 2, 11, 14 and 15 were highly adapted to the both normal stress conditions, and classified them in high-yielding and drought tolerant groups, while genotypes numbered as 10, 12 and 13 were potential and stable under normal. condition. Based on data analysis, cultivars numbered as 1, 5, 6 and 9 had lowest yield under both moisture regimes, lines 3, 4, 7 and 8 showed high-yielding under stress regimes. Cluster analysis ordered the genotypes into six groups with 5, 3, 2, 2, 2 and 1 genotypes, respectively. In conclusion, present investigation revealed that drought conditions induced reduction of yield of some cultivars, while others were tolerant to drought stress. Hence, breeders can select drought tolerant safflower lines based on the GMP and STI indices.

***Key words*:** Safflower, Drought tolerance indices, Genetic improvement, Biplot, Cluster analysis.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is one of the oil seed crops grown in Iran. It is one of the plants, which have a high conformity to various conditions such as resistance to drought, and it is appropriated to be grown in arid and semi-arid areas. Due to the growing request for edible oils, improvement of oilseed crop is very important (Safavi *et al.*, 2013; Rameshknia *et* *al.*, 2013). In normal condition plants are subjected to various stresses factors with harmful influence on growth and crop production (Roudbari *et al.*, 2012). Drought as an environmental stress is the limitation that induces a highly negative effect on yield (Khalili *et al.*, 2014). Drought tolerant is an important characteristic for increasing and enhancing crop efficiency in dry regions (Guo *et al.*, 2009).

Recognition of the important yield component is very efficient in genetic programs of these traits via indirect selection (Golparvar, 2011). The identified genes from wild plant species provide a mean for sustaining genetic improvement in plant cultivated in dry regions. The cultivated lines tolerated less drought stress than wild plants and fluctuating water stress levels caused meaningful more declines in the seed yield of cropping genotypes as compared with wild genotypes (Majidi *et al.*, 2011). The inheritance of agronomic traits was studied in safflower under drought stress condition. In order to improve seed yield and seed yield of safflower under drought regimes, obtained outcomes could be suitable for designing of breeding programs (Mirzahashemi *et al.*, 2014). Detection of the drought tolerance genes in barley (*Hordeum Vulgare* L.) will facilitate the molecular mechanisms conception of drought tolerance, and also facilitate the genetic breeding of barley via marker-assisted selection or transformation of genes. These results showed that new understanding into further comprehension of drought tolerance procedures in barley plants could be provided (Guo *et al.*, 2009). Various plants reacte to drought stress differently. Drought condition induced varied molecular and physiological responses such as changes in gene expression in plants (Savitri *et al.*, 2013). Shiranirad *et al.* (2011) announced that drought is a common obstacle seriously influencing rapeseed (*Brassica napus* L.) production, mostly in arid region in the world. They observed that MP, GMP and YI parameters were the best for screening high seed yield genotypes under stress conditions. Farshadfar *et al.* (2013) cleared that grain yield of bread wheat in normal and stress regimes were significantly and negatively correlated with SSI. Their findings indicated that some indices such as RDI, ATI, SNPI and DI can be used as the most favorable indicators for identifying drought tolerant genotypes. Cluster analysis of mentioned investigation classified the cultivars into three groups including; tolerant, susceptible and semi tolerant or semi-sensitive to drought regimes. In order to assess of drought tolerance indices under different environmental conditions for screening of Turkish oat (*Avena sativa* L.), fourteen landraces and cultivars were used. The experiments were applied both under rain-fed and irrigated regimes for three cropping seasons. Correlation coefficient matrix showed that the drought parameters were significantly inter-correlated with each other and can be classified into four groups. Their results demonstrated that the STI, GMP, MP, YI and HM indices under dry and irrigated conditions can be suggested to screen drought tolerant cultivars with high-yielding potential (Akcura *et al.*, 2011).

MATERIALS AND METHODS

A filed trial was carrdei out during 2014-2015 at the Isfahan (Khorasgan) Branch, Islamic Azad University, research station (50˚ 44′ N, 32˚ 40′ W and altitude of 1517 m above mean sea level). The study location is characterized by arid climate with an annual average rainfall of 120 mm, and the annual mean maximum and minimum temperatures of 25 °C and 1 °C, respectively. Soil type of the study site was silty loam and soil pH was 7.7 to 8. Generally, there is no precipitation during safflower growth cycle in this region.

Fifteen spring safflower cultivars including U.S.10, Kuseh landrace, Nebraska-10, Gila, S149, Bushehr landrace, Shiraz landrace, Arak-2811, Kerman landrace, Isfahan landrace, C111, Lordegan landrace, S3110, A.C.Sterling and Semnan landrace were planted at first of March 2012. The plots comprising of three rows were 3 m long and 0.5 m apart. Interplants distance within rows was 5 cm, hence, seedling density was 400/000 plants ha^{-1}. The experiment was irrigated at planting and flowering stages. Irrigation regimes were started at emerging of seedling. Two irrigation programs were considered in this study: IR1, irrigation after 75 mm cumulative evaporation from class A evaporation pan (CE) during the whole growth cycle as optimum irrigation treatment. IR2, irrigation after 150 mm cumulative evaporation from class A evaporation pan (CE) during the whole growth cycle as stress treatment. Various drought tolerance indices were evaluated (Table 1).

Table 1. Drought tolerance indices to calculate the reaction of safflower cultivars to stress

Code	Drought tolerance indices	Equation*	References
1	Stress Susceptibility Index (SSI)	$SSI = \frac{1-(Y_s / Y_p)}{1-(\bar{Y}_s / \bar{Y}_p)}$	Fischer and Maurer, 1978
2	Geometric Mean Productivity (GMP)	$GMP = \sqrt{Y_s \times Y_p}$	Fernández *et al.*, 1992
3	Stress Tolerance Index (STI)	$STI = \frac{Y_s \times Y_p}{\bar{Y}_p^2}$	Fernández *et al.*, 1992
4	Mean Productivity (MP)	$MP = \frac{Y_s + Y_p}{2}$	Rosielle and Hambling, 1981
5	Tolerance Index (TI)	$TOL = Y_p - Y_s$	Rosielle and Hambling, 1981

* Y_s and Y_p are seed yield in stress and normal conditions, respectively.

The research was conducted in two independent randomized complete block design (as stress and non-stress conditions) with three replications in each experiment. Analysis of variance and Duncan's multiple range test for mean comparisons were applied using proc GLM procedure of SAS software (version 9.2, SAS institute Inc., NC, USA). Correlation analysis, principal component analysis (PCA) and biplot graphical display were done by using STATGRAPHICS PLUS software. Cluster analysis based on Ward's method was carried out by $SAS_{9.2}$ software.

RESULTS AND DISCUSSION

Yields ranged from 3534 kg ha^{-1} (cultivar S149) to 1366 kg ha^{-1} (cultivar Arak-2811) in non stress treatment (Yp) (Table 2). The values of yield under stress conditions (Y) varied from 1326 to 1966 kg ha^{-1} and the Semnan and Lordegan landraces had lower seed yields. Gila, U.S.10 and Nebraska-10 showed higher yields (1966, 1946 and 1934 kg ha^{-1}, respectively) in non-stress condition. Mean yields under stress conditions were 2253 and 1657 kg ha^{-1}, respectively, revealing a reduction of 27% compared to normal irrigation conditions (data not shown).

Table 2. Average values of drought tolerance indices in safflower genotypes

Code	Genotype	Yp	Ys	TOL	MP	SSI	GMP	STI
1	Esfahan landrace	1714	1472	242	1593	0.52	1588.39	0.49
2	Kuseh landrace	2392	1740	652	2066	1.01	2040.11	0.81
3	Arak-2811	1366	1686	-320	1526	-0.87	1517.58	0.45
4	Nebraska-10	1838	1934	-96	1886	-0.19	1885.38	0.69
5	Semnan landrace	1566	1326	240	1446	0.57	1441.01	0.4
6	Lordegan landrace	1494	1326	168	1410	0.42	1407.49	0.39
7	Bushehr landrace	1496	1846	-350	1671	-0.87	1661.81	0.54
8	Shiraz landrace	2146	1680	466	1913	0.81	1898.75	0.7
9	Kerman landrace	2234	1360	874	1797	1.46	1743.05	0.59
10	A.C.Sterling	2600	1446	1154	2023	1.66	1938.96	0.74
11	S3110	3146	1820	1326	2483	1.57	2392.84	1.12
12	C111	3080	1466	1614	2273	1.96	2124.91	0.88
13	S149	3534	1754	1780	2644	1.88	2489.7	1.22
14	U.S.10	2794	1946	848	2370	1.13	2331.76	1.07
15	Gila	2406	1966	440	2186	0.68	2174.9	0.93

The data indicated that drought stress could significantly reduce yield. The genotypes S149 and S3110 showed reporp seed yield under both moisture regimes (Table 2). The values of MP varied from 1410 kg ha^{-1} (Lordegan landrace) to 2644 kg ha^{-1} (line S149) and the genotypes S149, S3110, U.S.10, C111, Gila, Kuseh landrace, A.C.Sterling and Esfahan landrace were the most productive (1955 kg ha^{-1}). Based on GMP, yields varied from 1407.5 kg ha^{-1} (Lordegan landrace) to 2490 kg ha^{-1} (line S149), proposing that the genotypes 13, 11, 14, 15, 12, 2 and 10 were the most productive. TOL index varied from -350 to 1780 kg ha^{-1}. Lower or negative TOL indices show tolerance to moisture stress. Hence, Esfahan, Semnan and Lordegan landraces, Nebraska10, A.C.Sterling, S3110, C111 and S149 were more tolerant (297 kg ha^{-1}). STI ranged from 0.39 (Lordegan landrace) to 1.22 (S149). The value of stress susceptibility index (SSI) varied from -0.87 (lines Arak and Bushehr landraces) to

1.96 (line C111). To detected the most desirable drought tolerance measures, correlation coefficient between yields under non-stress and stress conditions, and other quantitative indices of drought tolerance were estimated (Table 3). The outcomes indicated that the indices GMP, MP, STI and SSI were very similar for selection as Yp. This was supported by the high correlations among Yp and SSI (r= 0.84), TOL (r= 0.94), MP (r=0.95), GMP (r= 0.92) and STI (r=0.93). Correlation analysis demonstrated that the indices GMP and STI were similar for selection as Ys. Correlations between yields under stress regime and GMP (r= 0.58) and STI (r= 0.57) confirmed this conclusion. The indices SSI, TOL and MP illustrated the lowest correlations with Ys (Table 3). Results of Safavi *et al.* (2013), investigations indicated that significant positive correlations were observed between grain yield in the drought regime (Ys) with stress tolerance index (STI), harmonic mean (HAR) and geometric mean productivity (GMP) and therefore, these indices were suitable criteria for screening stress tolerant cultivars. Majidi *et al.* (2011) believed that GMP, STI and HM are superior criteria for identifying high yield genotypes under drought and normal regimes. The present results verified significant and positive correlation amongst Yp and Ys with GMP and STI; so these indices may be better predictors of Yp and Ys than MP, SSI and TOL indices. Our findings are in coincident with study of Rameshknia *et al.* (2013) who believed STI and GMP indices were the best parameters for identification and screening of genotypes under normal and stress regimes in breeding programs. Safavi *et al.* (2013) also stated that tolerant index (TOL) and mean productivity (MP) can be regarded as desirable indices for detecting drought tolerant genotypes. Khalili *et al.* (2014) announced STI, MP, GMP and YI indices were the most appropriate criteria in safflower breeding plans and they revealed that these indices were used for screening high-yielding cultivars under both normal and stress conditions. In assessment of genetic properties of drought tolerance indices for durum wheat, the parameters such as; MP, GMP and STI had high positive genetic correlations with each other as well as with grain yield under stress regime (Ys) and normal condition (Yp). Hence, through these indices it is possible to select high-yielding cultivars in either conditions (Hussain Ali, 2015). SSI values changed from -0.69 – 1.54, which were significantly and positively correlated with yield under non-stress and TOL index and negatively correlated with Ys. MP is the mean production under both moisture regimes, and was highly correlated with Yp and TOL indices.

Table 3. Pearson’s correlation coefficients among drought tolerance indices

index	Yp	Ys	SSI	TOL	MP	GMP	STI
Yp	1						
Ys	0.249^{ns}	1					
SSI	0.849^{**}	-0.231^{ns}	1				
TOL	0.94^{**}	-0.095^{ns}	0.955^{**}	1			
MP	0956^{**}	0.521^{*}	0.678^{*}	0.799^{**}	1		
GMP	0.927^{**}	0.587^{*}	0.634^{*}	0.746^{**}	0.994^{**}	1	
STI	0.93^{**}	0.574^{*}	0.635^{*}	0.754^{**}	0.993^{**}	0.996^{**}	1

For abbreviations, see Table1.
*, ** significant at 0.05 and 0.01 probability levels; ns: not significant.

Results of Rameshknia *et al.* (2013) assessment illustrated that STI, GMP and MP indices could screen tolerant and sensitive genotypes under both environmental conditions, and mentioned that these indices could be used for selection of tolerant cultivars of spring safflower. TOL varied from -350- 1780 kg ha^{-1}. A positive correlation between TOL and Yp (yield under non-stress conditions) and a negative correlation between TOL and yield under water stress (Ys) offered that selection based on TOL indices resulted in reduced yield under optimum irrigation regime. Hussain ali (2015) revealed that the genetic correlation of TOL and SSI indices with yield under stress conditions were high and negative, while correlation coefficient between TOL index and Yp was high and positive. Their findings cleared that selection can be based on TOL index to improve drought tolerance in durum wheat. Our correlations coefficient matrix illustrated that both GMP and STI indices were correlated with yield under both conditions. Moreover, a suitable index must be significantly correlated with yield in any of the two moisture regimes and show a low coefficient of variation. Therefore, these indices can be used to determine drought resistance cultivars with high yield in both moisture regimes. Selection based on a combination of indices may be more useful for improving drought resistance of safflower, but correlation coefficients are helpful for determining the degree of overall linear association between any two attributes (Safavi *et al.*, 2013). Hence, a better approach than a correlation analysis such as biplot analysis is required to identify supreme cultivars for both moisture regimes. For further assessment of the relation among drought tolerance indices, principle component analysis was applied. Accordingly, tow PC, components accounted for 97.1% of the total variation (Table 4). The results of the principle component analysis of safflower cultivars indicated that the first PC accounted for 77.3% of the total variation, while the second PC justified 19.81% of the remaining variation (Table 4). Also reported that the first component with more than 68% of total variation is able to separate high-yielding and seed yield cultivars from other cultivars Safavi *et al*. (2013).

Table 4. Principal component analysis for drought tolerance indices in safflower cultivars

Indices	PC1	PC2
GMP	0.416	0.198
MP	0.424	0.121
SSI	0.346	-0.442
STI	0.416	0.191
TOL	0.385	-0.361
Yp	0.42	-0.112
Ys	0.167	0.76
Total Variation %	77.3	97.1
Variation %	77.3	19.81

For abbreviations, see Table1.

A biplot diagram from the first and second factor components is shown in Figure1. The biplot is divided into four classes named A, B, C and D based on the two first principle components. Lines which were located in zone A (2, 14, 15 and 11) demonstrated high yield under both moisture conditions. Hence, these cultivars can be used as tolerant varieties in breeding procedures for selection of drought tolerant and high-yielding cultivars under stress regime. Lines 13, 10 and 12 which were placed in region B, had suitable potential under both moisture conditions Safavi *et al.*, (2013) believed that some indices such as STI, GMP, HAR and MP were more able to screen drought tolerant varieties and based on correlations between mentioned indices and Yp and Ys vectors (the angle between the vectors) in the biplot graph, STI was the favorable index for identifying drought tolerant cultivars in safflower.

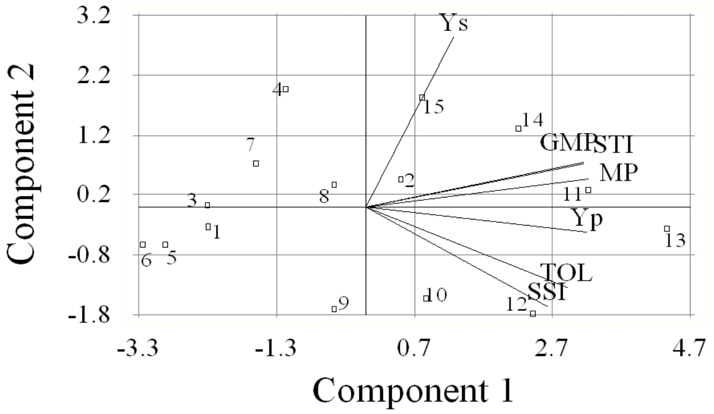

Figure 1. Biplot graphical display of 15 safflower varieties and 7 drought indices (See Tables 1 and 2 for abbreviations and genotype codes).

On the other hand, genotypes that were located in zone D (genotypes 1, 5, 6 and 9) had the lowest yields under stress and normal conditions. Genotype, number 3, 4, 7 and 8 were located in C area and had low and high-yield under normal and stress regimes, respectively. Accordingly, genotypes of the area A were classified as high-yielding and drought resistance groups. Majidi *et al.* (2011) indicated that wild genotypes had a low yield but their seed yield was stable when the environment changed. Therefore these landraces make a favorable genetic source for transferring drought tolerant genes to other genotypes. Cluster analysis of drought tolerance indices classified the mentioned 7 indices into three groups with 4, 2 and 1 indices, respectively (Figure 2). Group 1 consisted of indices with high positive values for first principle components (GMP, STI, MP and Yp indices). These results were verified by the biplot graph analysis which could locate genotypes 15, 14 and 11 with high GMP, STI and MP values into group A. Group 2 included indices with negative SSI and TOL values in second principle components. Ys index (yield under stress conditions) with lowest and positive high values for first and second principle components was located into group 3 (Figure 2).

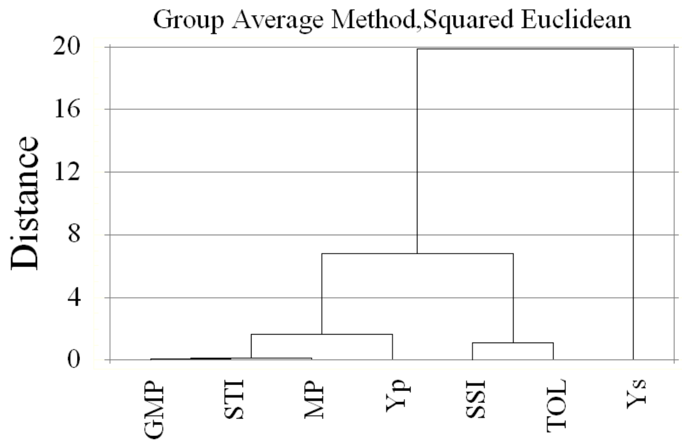

Figure 2. Dendrogram from cluster analysis of drought tolerance indices based on WARD's method

Cluster analysis based on yield under both moisture regimes and drought tolerance indices classified the cultivars into six groups with 5, 3, 2, 2, 2 and 1 genotype, respectively (Figure 3). Group 3 included genotypes with high Ys, Yp, MP and GMP values, and is considered as a drought tolerant group with high-yielding under normal and stress conditions. Genotypes 14 and 15 (Gila) with high drought resistance and high GMP values were located in the same group. Roudbari *et al.* (2012) concluded that Gila genotype is more suitable genotype for drought stressed conditions. Grain yield, as a gross selection criterion for drought tolerance, is a complex characteristic that is defined by several metabolic, biochemical and physiological plant operations.

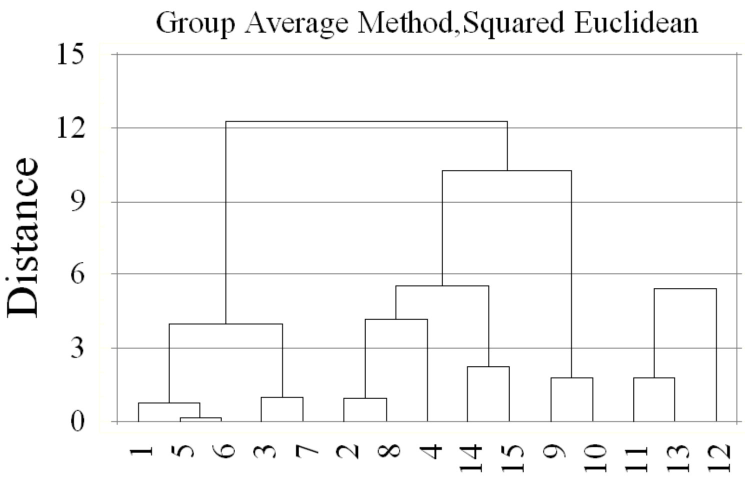

Figure 3. Dendrogram from WARD cluster analysis of safflower cultivars based on drought tolerance indices (See Tables 2 for abbreviations and genotype codes).

Group 4 included genotypes 9 and 10 with low seed yield under drought regime. Genotypes 1, 3, 5, 6 and 7 were classified into group 5. These lines showed lower drought tolerance than the genotypes of group 4. The last group consisted of line 12 that had the lowest and high yield under drought and normal conditions respectively, and classified into susceptible group.

noisulcnoc nI, the results of yduts tneserp, showed that moisture regimes had a clear impact on yield of safflower genotypes, so that drought conditions could decline yield up to

1657 kg ha^{-1}. This reduction is 27% compared to the normal treatment. Gila and S149 had higher yields in during stress and normal conditions, respectively. Genotypes Semnan and Lordegan landrace had the lowest seed yields under both moisture conditions. According to the results, GMP and STI were correlated with Yp and Ys, so they were determined as the best drought tolerance indices to select drought tolerant safflower cultivars. Selection based on these indices may be useful for determining a genotype with good seed yield under both stress and normal regimes. We can suggest that the genotypes Gila and U.S.10 can be recommended as candidate cultivars for drought tolerance in arid area.

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