

Evaluation of Yield-Based Drought Tolerance Indices for Screening Safflower Genotypes

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ABSTRACT

Drought tolerance is not often considered an independent trait by plant breeders because the collective result of many traits of a plant with positive or negative interactions may be fairly general and polygenic in nature. The objective of this study was to evaluate six drought tolerance indices, namely the stress susceptibility index (SSI), yield stability index (YSI), tolerance index (TOL), mean productivity (MP), geometric mean productivity (GMP), and stress tolerance index (STI), to be used in screening safflower (*Carthamus tinctorius* L.) genotypes. For this purpose, 64 genotypes were grown during two growing seasons (2011–2012) under both normal and drought-stress field conditions at the Research Farm of Isfahan University of Technology located at Lavark, Najaf-Abad, Iran. The drought tolerance indices were calculated based on seed yield under drought stress and nonstress conditions. Results of combined analysis of variance showed the significant influences of drought stress on seed yield as well as significant differences among genotypes for seed yield and the indices. Results of calculated correlation coefficients and multivariate analyses showed that GMP and STI indices were able to discriminate between drought-sensitive and -tolerant safflower genotypes. Cluster analysis using the drought-tolerance indices divided the 64 genotypes into tolerant and susceptible groups. Based on multivariate analyses using the indices singly or in combinations, it was possible to identify the most yield-stable genotypes across the environments. Overall, we concluded that GMP and STI indices can be efficiently exploited not only to screen drought tolerance but also to identify superior genotypes for both stress and nonstress field conditions in safflower.

Drought is a major abiotic stress limiting the growth and production of crop plants and has recently attracted increasing attention by researchers due to it being exacerbated by climate change. Understanding drought effects on plants will help improve crop management practices, guide breeding efforts in agriculture, and predict the fate of natural vegetation under periods of climate change (Chaves et al., 2003). Drought tolerance is controlled by multiple genes having additive effects. Moreover, the genes responsible for drought tolerance interact with those affecting yield potential to limit improvements in crop drought tolerance. Thus, research is needed to identify crop genotypes that are better adapted to drought tolerance.

Safflower is an important annual oilseed crop grown in arid and semiarid regions (Weiss, 2000, p. 93–129). Its deep roots can absorb moisture stored in the subsoil (Singh et al., 1995; Smith, 1996). Because seed yield can be positively influenced by the availability of deep soil moisture, safflower's ability to set deep roots makes it an efficient drought-tolerant plant capable of enduring periods of drought in arid regions.

A reduction of seed yield under stress conditions compared with yield under nonstress conditions is usually a criterion for evaluating the drought susceptibility of genotypes (Fernandez, 1992; Blum, 2011). Several indices have been utilized to evaluate the drought tolerance of genotypes based on seed yield in different environments. Rosielle and Hamblin (1981) introduced TOL as the differences in yield between stress (Y_s) and nonstress (Y_p) environments and MP as the average yield of Y_s and Y_p . The SSI of the cultivar was proposed by Fischer and Maurer (1978). Genotypes with lower SSI and TOL values are considered to be less drought sensitive. Selection based on these criteria leads to the selection of genotypes with low and high yield potentials under nonstress and stress conditions, respectively. Guttieri et al. (2001) used the SSI in spring wheat (*Triticum aestivum* L.) and concluded that SSI values >1 and <1 might indicate above-average and below-average susceptibility to drought stress, respectively. The YSI is another index proposed by Bousslama and Schapaugh (1984) for evaluating stress tolerance in soybean [*Glycine max* (L.) Merr.].

Fernandez (1992) defined the STI and GMP to distinguish genotypes with higher levels of yield potential and stress tolerance under both conditions. In studying the evolution of wild safflower genotypes under water-deficit stress and nonstress conditions, Majidi et al. (2011) found that STI, GMP, and HM (the harmonic mean) were superior criteria

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Abbreviations: GMP, geometric mean productivity; MP, mean productivity; PCA, principal component analysis; SSI, stress susceptibility index; STI, stress tolerance index; TOL, stress tolerance; Y_p , seed yield under non-stress condition; Y_s , seed yield under drought stress condition; YSI, yield stability index.

for selecting high-yield genotypes. Mohammadi et al. (2011) evaluated bread wheat genotypes under dryland and supplemental irrigation conditions for 2 yr and reported that a biplot display and cluster analysis could be used to identify the superior genotypes.

Various approaches have been suggested to improve the efficiency of plant breeding for increased plant productivity under dry environments. Breeding for drought-tolerant crops largely depends on the availability of the genetic resources for tolerance, reliable screening techniques, identification of genetic components of tolerance, successful genetic manipulation of the desired genetic backgrounds, and ultimate development of drought-tolerant cultivars with acceptable agronomic and quality-related traits. Nevertheless, the screening methods have a great bearing on how the germplasm can be incorporated into breeding programs for drought tolerance. Hence, the objective of the present study was to identify drought-tolerant genotypes based on tolerance indices that can be used in large-scale screening of safflower genotypes.

MATERIALS AND METHODS

Sixty-four safflower genotypes consisting of 46 native and 18 exotic genotypes were evaluated during two growing seasons (2011–2012). The field experimental design was a square lattice design (8 by 8) with two replications under two irrigation (nonstress and stress) regimes conducted at the research farm of Isfahan University of Technology located at Lavark, Iran (40 km south west of Isfahan, 32°32' N, 51°23' E, 1630 m asl). The soils at the experimental field are Typic Haplargids of the arid tropics with a silty clay loam texture. Mean annual precipitation and temperature at this site are 149 mm and 15.4°C, respectively. Plant materials were planted in plots comprised of three 4-m-long rows spaced 30 cm apart in early March 2011 and 2012. The plant density was adjusted to 40 plants m⁻² in the plots. Cumulative precipitation values at the experimental site were 20 and 50 mm from sowing to harvest in 2011 and 2012, respectively.

Plants were irrigated uniformly under both conditions until the branching growth stage, after which soil-moisture regimes were established based on 50% soil moisture depletion of soil available water (SAW) from the root zone for the nonstress fields and 80% for the drought-stress fields. Irrigation intervals were variable because of the evapotranspiration variation throughout the growing season. Soil samples were taken from a soil depth of 0 to 60 cm from both the nonstress and stress plots to determine the soil water content and calculate the irrigation water content on the basis of a 60-cm rooting depth. Soil samples were taken before each irrigation when evaporation from a Class A pan indicated 70 and 140 mm of evaporation under normal and drought-stress conditions, respectively.

The SAW was computed as the difference between the volumetric water content at field capacity (θ_{FC}) and at the permanent wilting point (θ_{PWP}). In this study, volumetric soil water content at FC (–0.03 MPa) and PWP (–1.5 MPa) were 25 and 15%, respectively.

The amount of irrigation water applied was monitored at each irrigation by measuring the depth of water over a Parshall flume and calculated as

$$I_d = SAW \times p \quad [1]$$

$$SAW = (\theta_{FC} - \theta_{PWP}) \times D \times 100 \quad [2]$$

$$I_g = \frac{I_d}{Ea} \times 100 \quad [3]$$

where I_d is irrigation depth (cm), p is the fraction of SAW that can be depleted from the root zone, D is the soil layer depth (60 cm), I_g is the gross irrigation depth (cm), and Ea is irrigation efficiency (%), assumed to be 65% on the average.

The total amount of irrigation water applied was 6915 and 4149 m³ ha⁻¹ in 2011 as well as 6545 and 3688 m³ ha⁻¹ in 2012 under nonstress and stress conditions, respectively.

Weeds were controlled by trifluralin (2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl)benzenamine) herbicide at a rate of 2 L ha⁻¹ before sowing and by hand weeding throughout the growing season.

Seed yield was determined by harvesting the entire middle row. Seed common moisture content was about 15% at harvest.

Six selection indices including SSI (Rosielle and Hamblin, 1981), YSI (Bouslama and Schapaugh, 1984), TOL and MP (Fischer and Maurer, 1978), and GMP and STI (Fernandez, 1992) were calculated based on seed yield under nonstress and drought-stress conditions:

Stress susceptibility index

$$SSI = \frac{1 - Y_s/Y_p}{SI}$$

$$SI = 1 - \frac{Y_{ms}}{Y_{mp}}$$

Yield stability index

$$YSI = \frac{Y_s}{Y_p}$$

Tolerance index

$$TOL = Y_p - Y_s$$

Mean productivity

$$MP = \frac{Y_p + Y_s}{2}$$

Geometric mean productivity

$$GMP = (Y_p \times Y_s)^{0.5}$$

Stress tolerance index

$$STI = \frac{Y_p \times Y_s}{(Y_{mp})^2}$$

Table 1. Combined analysis of variance for seed yield under nonstress (Y_p) and stress (Y_s) conditions, stress susceptibility index (SSI), yield stability index (YSI), stress tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP), and stress tolerance index (STI) in safflower genotypes grown under drought stress and normal conditions in two growing seasons in 2011 and 2012.

Source of variation	df	Mean square							
		Y_p	Y_s	SSI	YSI	TOL	MP	GMP	STI
		— kg ha ⁻¹ —							
Year (Y)	1	0.001 ns	0.002 ns	0.003 ns	0.0003 ns	1.56 ns	0.001 ns	0.002 ns	0.0001 ns
Block/Y	2	0.08**	0.013 ns	0.27 ns	0.03 ns	653,080*	0.05**	0.04**	0.41**
Genotype (G)	63	0.026**	0.052**	1.35**	0.12**	620,828**	0.027**	0.03**	0.39**
G × Y	63	0.003 ns	0.008 ns	0.03 ns	0.002 ns	0.77 ns	0.004*	0.005*	0.04 ns
Residual	126	0.004	0.009	0.3	0.02	146,301	0.003	0.003	0.04

* Significant at $P < 0.05$; ns, not significant.

** Significant at $P < 0.01$.

Table 2. Correlation coefficients between seed yield of safflower genotypes under nonstress (Y_p , kg ha⁻¹) and stress (Y_s , kg ha⁻¹) conditions, stress susceptibility index (SSI), yield stability index (YSI), stress tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP), and stress tolerance index (STI).

Parameter	Y_p	Y_s	SSI	YSI	TOL	MP	GMP	STI
Y_p	1							
Y_s	0.50**	1						
SSI	0.25*	-0.71**	1					
YSI	-0.25*	0.71**	-0.99**	1				
TOL	0.56**	-0.43**	0.92**	-0.91**	1			
MP	0.75**	0.68**	-0.25*	0.24*	0.1 ns	1		
GMP	0.82**	0.89**	-0.33**	0.33**	0.01 ns	0.79**	1	
STI	0.84**	0.88**	-0.30**	0.29**	0.03 ns	0.62**	0.96**	1

* Significant at $P < 0.05$; ns, not significant.

** Significant at $P < 0.01$.

where Y_s and Y_p are the yields of each genotype under stress and nonstress conditions, respectively, and Y_{ms} and Y_{mp} are the yield means for all genotypes under drought-stress and nonstress conditions, respectively.

Statistical Analysis

A combined analysis of variance (ANOVA) was performed for seed yield and the indices using the GLM procedure of SAS. The genotypic means of seed yield and the indices were compared using Fisher's LSD test at 5%. The ANOVA indicated only slight gains in efficiency using a lattice over a randomized complete block design for the majority of the traits studied.

In this study, multivariate analysis was also used for distinguishing drought tolerance genotypes. Principle component analysis (PCA) and the biplot diagrams were exploited to identify tolerant and susceptible genotypes using Stat Graphics (Stat Graphics, 1996). For identifying the drought-tolerant and high-yielding genotypes in both environments, three-dimensional graphs based on the best drought tolerance index and genotype yield under stress and nonstress conditions was drawn using Sigma Plot software. Cluster analysis was performed on the drought indices by Ward's method using squared Euclidean distance as a measure of similarity. The P value obtained from Hotelling's T -square (T^2) test was used to estimate the optimal number of clusters. Mean comparisons of groups obtained from the cluster analysis were conducted using Fisher's LSD test at 5%.

RESULTS AND DISCUSSION

Results of the combined ANOVA indicated a significant influence of drought stress on safflower seed yield (data not

shown). A highly significant ($P < 0.01$) variation was observed in seed yield and tolerance indices among the genotypes (Table 1). There were no significant differences between the two study years in terms of seed yield and tolerance indices (Table 1); hence, data averaged across the two seasons were used for means comparisons (data not shown).

Among the 64 cultivated genotypes used in this study, C411 and Kordestan3 had the lowest values of SSI and TOL as well as the highest values of YSI and were suggested as the most drought-tolerant genotypes under drought-stress conditions (data not shown). This indicated that SSI, TOL, and YSI indices were able to identify genotypes with higher yields under drought stress rather than under nonstress conditions. The highest values of STI, GMP, and MP were recorded for Kermanshah47, IL, Khorasan62, and Hamedan38 genotypes. Hence, they were introduced as the most stable and productive genotypes among the cultivated genotypes under both environmental conditions.

Tolerance is not often considered an independent trait by plant breeders because tolerance mechanisms can be fairly general and polygenic in nature. Therefore, the requirement to screen for tolerance has encouraged plant breeders to look for a reliable index. Seed yield was found to have a highly significant positive correlation with GMP and STI under both environmental conditions. They therefore seem to yield similar results and can thus be used as suitable criteria for identifying drought tolerance in genotypes (Table 2). The capability of the GMP and STI indices to identify genotypes satisfactorily under both conditions observed in this study is consistent with the results reported by Fernandez (1992) with mungbean [*Vigna radiata* (L.) R. Wilczek var. *radiata*], Nouri et al. (2011) with durum wheat [*Triticum turgidum* L. ssp. *durum*

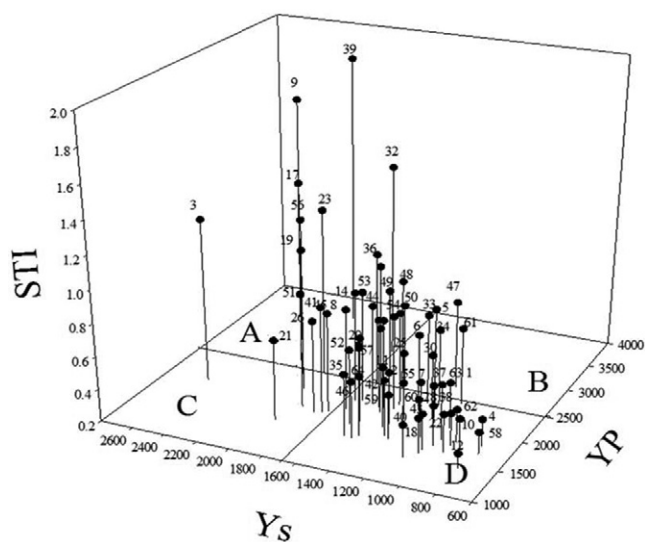


Fig. 1. Three-dimensional diagram for identifying drought-tolerant genotypes based on seed yield under nonstress (Yp) and stress (Ys) conditions as well as the stress tolerance index (STI): genotypes with high yields under both environments (Group A), high yield in a normal environment (Group B), high yield in a stressful environment (Group C), and low yield under both environmental conditions (Group D). Numbers assigned to the genotypes are: (1) C111; (2) C116; (3) C411; (4) C444; (5) C4110; (6) S6-58/41-168; (7) S6-697-307; (8) S6-697-324; (9) IL; (10) N/27; (11) 73-14-34; (12) PI-405985; (13) LRV-51-51; (14) LRV-55-295; (15) Hamedan17; (16) Hamedan21; (17) Hamedan38; (18) Hamedan40; (19) Kordestan1; (20) Kordestan2; (21) Kordestan3; (22) Kordestan4; (23) Kordestan5; (24) Kordestan6; (25) Kordestan7; (26) Kordestan8; (27) Kordestan9; (28) Darab1; (29) Darab2; (30) Darab4; (31) Darab9; (32) Khorasan62; (33) Khorasan330; (34) Khorasan376; (35) Khorasan508; (36) Kermanshah; (37) Kermanshah44; (38) Kermanshah46; (39) Kermanshah47; (40) Kermanshah60; (41) Esfahan4; (42) Esfahan Kuse; (43) Marand; (44) Zarghan; (45) Sina; (46) Arak; (47) Dincer; (48) Yinice; (49) CI055; (50) PI-198844; (51) PI-253384; (52) PI-250190; (53) PI-250537; (54) PI-506426; (55) Cyprus Bregon; (56) Syrian; (57) PI-258417; (58) Hartman; (59) Gila; (60) CW-4440; (61) S-541; (62) PI-537636-S; (63) PI-537636; and (64) Kino-76.

(Desf.) Husn.), and Majidi et al. (2011) with wild safflower. Fernandez (1992) reported that selection based on STI, GMP, and MP would identify genotypes with higher levels of yield potential and stress tolerance, whereas SSI and TOL indices had a significant negative correlation with seed yield under stress conditions but a positive correlation with seed yield under nonstress conditions (Table 2). Therefore, selection based on SSI and TOL decreases seed yield under normal conditions but increases it under drought-stress conditions. The restricted ability of the SSI and TOL indices in identifying tolerant genotypes has also been reported with wheat (Mohammadi et al., 2011) and potato (*Solanum tuberosum* L.; Cabello et al., 2013). In the present study, seed yield and the YSI index exhibited negative and positive correlations, respectively, under nonstress and stress conditions (Table 2).

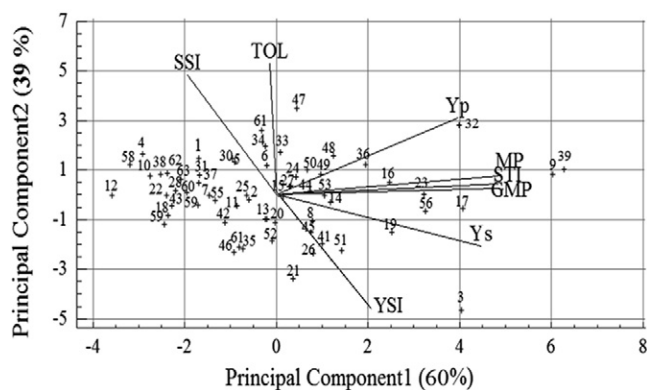


Fig. 2. Biplot drawn based on the first and second components obtained from principal component analysis using the stress susceptibility index (SSI), yield stability index (YSI), stress tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP), stress tolerance index (STI), and seed yield of safflower genotypes under nonstress (Yp) and stress (Ys) conditions in 64 safflower genotypes. The numbers assigned to the genotypes are: (1) C111; (2) C116; (3) C411; (4) C444; (5) C4110; (6) S6-58/41-168; (7) S6-697-307; (8) S6-697-324; (9) IL; (10) N/27; (11) 73-14-34; (12) PI-405985; (13) LRV-51-51; (14) LRV-55-295; (15) Hamedan17; (16) Hamedan21; (17) Hamedan38; (18) Hamedan40; (19) Kordestan1; (20) Kordestan2; (21) Kordestan3; (22) Kordestan4; (23) Kordestan5; (24) Kordestan6; (25) Kordestan7; (26) Kordestan8; (27) Kordestan9; (28) Darab1; (29) Darab2; (30) Darab4; (31) Darab9; (32) Khorasan62; (33) Khorasan330; (34) Khorasan376; (35) Khorasan508; (36) Kermanshah; (37) Kermanshah44; (38) Kermanshah46; (39) Kermanshah47; (40) Kermanshah60; (41) Esfahan4; (42) Esfahan Kuse; (43) Marand; (44) Zarghan; (45) Sina; (46) Arak; (47) Dincer; (48) Yinice; (49) CI055; (50) PI-198844; (51) PI-253384; (52) PI-250190; (53) PI-250537; (54) PI-506426; (55) Cyprus Bregon; (56) Syrian; (57) PI-258417; (58) Hartman; (59) Gila; (60) CW-4440; (61) S-541; (62) PI-537636-S; (63) PI-537636; and (64) Kino-76.

Considering the high positive correlation between the STI index and seed yield under drought-stress and nonstress conditions, three-dimensional graphs based on this index were drawn to categorize the 64 safflower genotypes according to their yield performance (Fig. 1). These graphs divide the genotypes into four groups each of which represents one combination of the genotypes with high yields under both environments (Group A), high yield in a normal environment (Group B), high yield in a stressful environment (Group C), and low yield under both environmental conditions (Group D). The following genotypes are included in Group A: Kermanshah47, IL, Hamedan38, Khorasan62, Syrian, and Kordestan5, all of which had superior performance under both stress and nonstress conditions. Based on this plot, it may then be possible to identify and select genotypes with the most stable yields across all the environments. Likewise, Fernandez (1992) used the same approach to classify the genotypes into four groups based on their performance under stress and nonstress conditions.

A PCA was conducted using the tolerance indices, and the genotypes were then subjected to biplot analysis to obtain the

Table 3. Results of principal component analysis for seed yield of safflower genotypes under nonstress (Yp) and stress (Ys) conditions, stress susceptibility index (SSI), yield stability index (YSI), stress tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP), and stress tolerance index (STI).

	Contribution to variation	Cumulative percentage	Yp	Ys	SSI	YSI	TOL	MP	GMP	STI
	%	%	kg ha ⁻¹							
PC1	60	60	0.29	0.44	-0.26	0.26	-0.12	0.42	0.44	0.43
PC2	39.4	99.4	0.42	-0.1	0.46	-0.46	0.54	0.19	0.15	0.17

Table 4. Mean comparison of three groups obtained from cluster analysis for seed yield of safflower genotypes under non-stress (Yp) and stress (Ys) conditions, mean productivity (MP), geometric mean productivity (GMP), and stress tolerance index (STI), 1000 seed weight (SW), number of seed per capitula (NSC), and number of capitula per plant (NCP) based on cluster analysis.

Trait	Group means		
	Group 1	Group 2	Group 3
Yp, kg ha ⁻¹	1740.1 c†	2151.9 b	2626.5 a
Ys, kg ha ⁻¹	1168.4 c	1520.3 b	2014.2 a
MP	1454.3 c	1836.1 b	2320.4 a
GMP	1414.1 c	1787.1 b	2277.5 a
STI	0.5 c	0.8 b	1.31 a
SW, g	29.1 c	32.7 b	39.1 a
NSC	29.3 b	29.5 b	32.1 a
NCP	14.1 c	17.5 b	20.6 a

† Means in each column followed by the same letter are not statistically different at $P < 0.01$.

relationships among the indices (Table 3; Fig. 2). Results of the PCA revealed that the first component (PC1) explained 60% of the total yield variation and exhibited a positive correlation with Yp, Ys, YSI, MP, GMP, and STI. The PC2 explained 39.4% of the total yield variation and had a higher positive correlation with TOL and SSI. Therefore, PC1 and PC2 were named *yield potential* and *stress susceptibility*, respectively. Based on this criterion, stable genotypes possessed greater PC1 but lower PC2 values and vice versa (Kaya et al., 2002). The results of a biplot drawn based on the PC1 and PC2 data for the 64 cultivated genotypes showed the five genotypes Kermanshah47, IL, Hamedan38, Syrian, and Kordestan5 closely located to the best drought tolerance indices with high PC1 but low PC2 values. On the other hand, the majority of genotypes with low PC1 and high PC2 values were identified as susceptible genotypes. These included S6-697-307, Kermanshah44, PI-537636, Hartman, C444, Kordestan4, Kermanshah46, and PI-405985 (Fig. 2).

The results of the three-dimensional graphs and biplot showed that C411 and Kordestan3 had the best performance in stressful environments (Group C) and a lower sensitivity among the genotypes (Fig. 1 and 2). Thus, these genotypes seem to be sensitive to the irrigation water content in nonstress plots and to have the ability to adapt under drought-stress conditions. Deeper roots due to a higher root elongation rate is one of the drought avoidance mechanisms and a key plant mechanism for adaptation to drought because the root system allows the maintenance of a more favorable plant water status. One of the benefits is that deeper plant roots may capture more water and nutrients that might otherwise escape toward the groundwater. Subbarao et al. (1995) found that selection for a deep and extensive root system increased the productivity of food legumes under water deficiency conditions by optimizing the capacity to acquire water. We also hypothesize that lower seed production in these two genotypes under irrigated conditions may be a result of the excessive vegetative growth, caused by the favorable water and nutrient supplies, which competes with reproductive growth and thus can also lead to a lower harvest index. It may, therefore, be presumed that a higher rate of vegetative growth caused by an irrigation treatment is not always associated with higher safflower yields

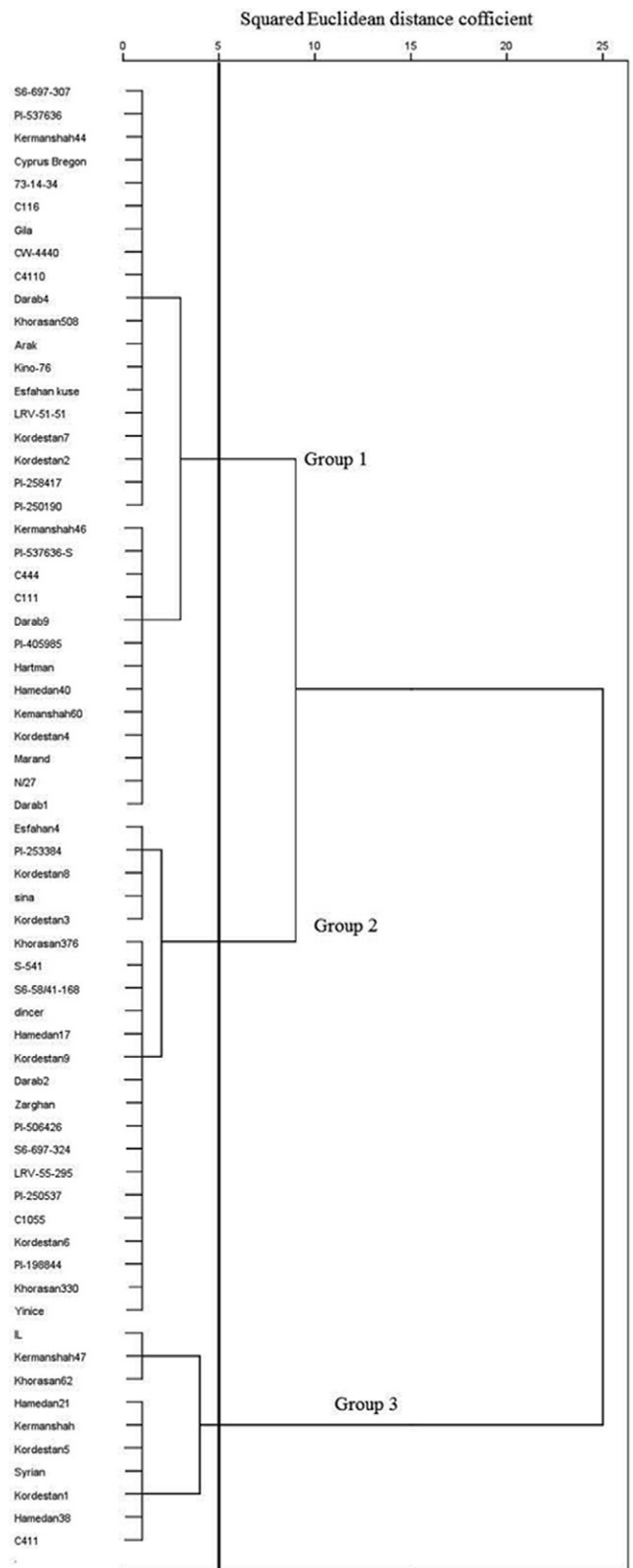


Fig. 3. Dendrogram generated for safflower genotypes based on seed yield under nonstress (Yp) and stress (Ys) conditions, the geometric mean productivity (GMP) index, and the stress tolerance index (STI) using Ward's method.

due to the investment of photosynthate into structural rather than seed storage compounds.

Cluster analysis was performed on the basis of Yp, Ys, GMP, and STI indices to classify the genotypes into three groups (Fig. 3). The means of the indices and yield components of the genotype groups obtained from cluster analysis are presented in Table 4. The clustering results of the genotypes were consistent with the PCA results. Out of the 64 genotypes, 32 with low productivity under both environmental conditions were located in the first group, whereas the third group included genotypes possessing the highest yield potential and yield components (Table 4). Thus, the genotypes in Groups 1 and 3 were identified as drought susceptible and tolerant genotypes, respectively. The ability of drought tolerance indices to identify genotypes with good performance under both stress and nonstress conditions has also been evaluated using both multivariate analysis and the correlations of the indices with yield in other plant species such as wheat (Mohammadi et al., 2011), durum wheat (Golabadi et al., 2006), maize (*Zea mays*L.; Khodarahmpour et al., 2011), wild safflower (Majidi et al., 2011) and potato (Cabello et al., 2013).

CONCLUSIONS

The results of multivariate analysis revealed that the GMP and STI indices were capable of not only more efficiently selecting the high-yield genotypes under both environmental conditions but also discretely identifying drought-sensitive and -tolerant safflower genotypes. Although either yield potential or yield loss is generally used to screen plant genotypes for drought stress under field conditions, neither can fully capture the drought-tolerance trait of crop plants. According to the results of the present study, the GMP and STI indices could be efficiently exploited not only to screen drought-tolerant genotypes but also to identify superior genotypes for both stress and nonstress field conditions in safflower.

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