Discrimination of drought tolerance in a worldwide collection of safflower (*Carthamus tinctorius* L.) genotypes based on selection indices

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Abstract: Improvement of elite safflower genotypes for drought-tolerance is hampered by a deficiency of effective selection criteria. The present study evaluated 100 genotypes of safflower in terms of their drought tolerance over a period of three years (2016–2018) under both non-stress and drought-stress conditions. The eight drought-tolerance indices of tolerance index (TOL), mean productivity (MP), geometric mean productivity (GMP), stress susceptibility index (SSI), stress tolerance index (STI), yield stability index (YSI), drought resistance index (DI), and harmonic mean (HARM) were calculated based on seed yield under drought (Y_s) and non-drought (Y_p) conditions. A high genetic variation was found in drought tolerance among the genotypes studied. The MP, GMP, and STI indices were able to discriminate between tolerant and drought-sensitive genotypes. Plots of the first and second principal components identified drought-tolerant genotypes averaged over the three study years. Cluster analysis divided the genotypes into three distinct groups using the drought tolerance indices. Ultimately, eight genotypes (namely, G_3, G_{11}, G_{13}, G_{24}, G_{33}, G_{47}, G_{58}, and G_{61}) from different origins were detected as more tolerant to drought stress suitable for use in safflower breeding programs in drought-affected areas. The most tolerant and susceptible genotypes could be exploited to produce mapping populations for drought tolerance breeding programs in safflower.

Key words: cluster analysis; drought stress; principal component analysis; selection index; yield; safflower

Abbreviations: TOL: Tolerance; MP: mean productivity; SSI: drought susceptibility index GMP: geometric mean productivity; YSI: yield stability index; DI: drought resistance index.

Odkrivanje tolerance na sušo v mednarodni zbirki genotipov žafranike (*Carthamus tinctorius* L.) na osnovi izbranih indeksov

Izvleček: Izboljšanje elitnih genotipov žafranike na prenašanje suše ovira pomanjkanje učinkovitih selekcijskih kriterijev. V raziskavi je bilo ovrednoteno 100 genotipov žafranike glede na njihovo prenašanje suše v obdobju treh let (2016–2018) v razmerah brez stresa in razmerah sušnega stresa. Izračunanih je bilo osem indeksov tolerance na sušni stres kot so tolernca na stres (TOL), poprečna produktivnost (MP), geometrijska poprečna produktivnost (GMP), indeks stresne občutljivosti (SSI), indeks stresne tolerance (STI), indeks stabilnosti pridelka (YSI), indeks odpornosti na sušo (DI), in harmonično poprečje na osnovi pridelka semena v sušnih (Y_s) in nesušnih (Y_p) razmerah. Med preučevanimi genotipi je bila ugotovljena velika genetska variabilnost v toleranci na sušo. Z indeksi MP, GMP, in STI je bilo mogoče razlikovati na sušo tolerantne in občutljive genotipe. Polja prve in druge glavne komponente so določila na sušo tolerantne genotipe v vseh treh letih raziskave. Klasterska analiza je z uporabo indeksov tolerance na sušo razdelila genotipe v tri jasno ločene skupine. Na koncu je bilo ugotovljenih osem genotipov (G_3, G_{11}, G_{13}, G_{24}, G_{33}, G_{47}, G_{58}, in G_{61}) različnega izvora, ki so bili bolj tolerantni na sušo in so primerni za uporabo v žlahtniteljskih programih žafranike na od suše ogroženih območjih. Na sušo najbolj prilagojene genotipe žafranike bi lahko uporabili za odkrivanje populacij, ki bi bile primerne pri žlatnjenju žafranike na sušo.

Ključne besede: klasterska analiza; sušni stres; analiza glavnih komponent; selekcijski indeks; pridelek; žafranika

Okrajšave: TOL: toleranca; MP: poprečna produktivnost; SSI: indeks občutljivosti na sušo; GMP: geometrijska poprečna produktivnost; YSI: indeks stabilnosti pridelka; DI: indeks odpornosti na sušo.

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1   INTRODUCTION

Droughts due to alterations in rainfall patterns and climate change form a most devastating factor in food production on a global scale (Blum, 2018; Anjum et al., 2017). This warrants a Blue Revolution in agriculture concentrated on increasing productivity per unit of water to produce more crops per drop of water. Recently, an important target in crop breeding programs is the development of drought-tolerant genotypes that possess a high capability for adaption to arid and semi- arid climates (Kirigwi et al., 2004; Basu et al., 2016).

The challenges in understanding the mechanisms involved in plant behavior under water scarcity include: 1) mutagenic control of drought tolerance, 2) genetic variability and differences among species in responding to changes in water availability, and 3) interactions with other factors such as drought stress duration and intensity (Varshney et al., 2018). Moreover, breeding programs are adversely affected by the high interaction of genotype × environment, low heritability of drought tolerance traits, lack of efficient selection particularly under field conditions, and the difficulties associated with simultaneous selection, sharp climate changes, and unpredictable rainfall in different regions (Ashraf, 2010; Rauf et al., 2016; Blum, 2018). Since the genotypes with a high yield under optimum conditions may not be drought tolerant (Blum, 2018), many studies preferred selection under both stress and non-stress conditions (Fernandez, 1992).

To have a high and durable yield in a drought-prone environment drought-tolerant genotypes are needed (Abdolshahi et al., 2012). The capacity of genotypes to perform reasonably well in drought-stressed environments is the paramount reason for their stable production (Raman et al., 2012). To decrease the impacts of abiotic stress without any substantial yield loss, researchers tend to develop drought-tolerant genotypes based on prior evaluation and identification of drought-tolerant germplasm. The high cost of drought soil amelioration has encouraged breeders to use selection indices as an economic and efficient method for resolving the problems associated with drought stress breeding (Vieira et al., 2016).

In this regard, a variety of selection indices to identify stress-tolerant cultivars have been proposed that some of the important and most applicable of them include: Tolerance (TOL) (Rosielle $ Hamblin, 1981) (Table 1) , mean productivity (MP) (Rosielle & Hamblin, 1981) (See Table 1), stress susceptibility index (SSI) (Fischer and Maurer, 1978) (See Table 1), geometric mean productivity (GMP) (Kristin et al., 1997) (See Table 1), stress tolerance index (STI) (Fernandez, 1992) (See Table 1), yield stability index (YSI) (Gavuzzi et al., 1997) (See Table 1), and drought resistance index (DI) (Lan, 1998) (See Table 1). Our literature review have reported on the efficiency of different selection indices for selecting drought-tolerant genotypes in different crops as like as rice (Raman et al., 2012); canola (Khalili et al., 2012);sunflower (Gholinezhad et al., 2014); maize (Hao et al., 2011) and bread wheat (Abdolshahi et al., 2012).

Safflower (*Carthamus tinctorius* L.) is an annual oil

Table 1: Different drought tolerance indices used for screening safflower genotypes

Index name	Equation⌐	Reference
Mean productivity	$MP = \dfrac{Y_s + Y_p}{2}$	Rosielle and Hamblin, 1981
Tolerance index (TOL)	$TOL = Yp - Ys$	Ficsher and Maurer, 1978
Geometric Mean Productivity (GMP)	$GMP = \sqrt{(Yp)(Ys)}$	Kristin et al., 1997
Stress Susceptibility Index (SSI)	$SSI = \dfrac{1 - \left(\frac{Ys}{Yp}\right)}{1 - \left(\frac{\overline{Ys}}{\overline{Yp}}\right)}$	Rosielle and Hamblin, 1981
Stress Tolerance Index (STI)	$STI = \dfrac{Ys \times Yp}{\overline{Yp}^{\,2}}$	Fernandez, 1992
Yield Stability Index (YSI)	$YSI = \dfrac{Ys}{\overline{Y}_P}$	Gavuzzi et al., 1997
Drought Resistance Index (DI)	$DI = \dfrac{Ys \times \left(\frac{Ys}{Yp}\right)}{\overline{Y}s}$	Lan, 1998
Harmonic Mean	$HARM = 2(Y_P \times Y_s)/(Y_p + Y_s)$	Kristin et al., 1997

seed crop with diverse industrial and pharmaceutical application that is grown commercially in Iran (Golkar & Karimi, 2019). The deep roots of safflower make it a drought-tolerant plant viable under the drought stress conditions in arid climates (Mirzahashemi et al., 2014; Hussain et al., 2016).

Drought stress is one of the most devastating abiotic stresses that poses a serious threat to worldwide safflower production (Hussain et al., 2016). Given the declining water resources in the arid and semi- arid regions of the world due to consecutive droughts, increased safflower cultivation can be an economic and valuable alternative to other drought-tolerant genotypes. In this regards, some studies is known about drought tolerance of local Iranian cultivars (Omidi et al., 2012; Bahrami et al., 2014; Mirzahashemi et al., 2014). Despite of current efforts intended for assessing tolerance criteria based on tolerance indices in safflower, little has been reported at maturity (Bahrami et al., 2014). Furthermore, this tolerance undoubtedly appears to be stage-dependent and must be evaluated at the yielding phase.

Variations in drought patterns such as differences in location, year, and drought intensity as well as genotypic differences call for safflower genotypes with different levels of drought tolerance to be cultivated in different areas. However, the differences in the genotypes recommended might have stemmed from the variability in the drought tolerance potential of safflower genotypes. Moreover, climate changes increase drought frequency in some regions but drought is a global issue. Given the broad distribution of safflower around the world, it is the objective of the present study to identify drought-tolerant genotypes from a new worldwide collection based on drought selection indices. The new identified genotypes could be used for cultivation in arid regions of the world.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

One hundred safflower genotypes originating from different geographical regions of the world were selected for screening drought tolerance (Table S1). The exotic genotypes were obtained from Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany. Iranian genotypes were taken from the genotype inventory at the Agricultural Research Center, Isfahan, Iran.

2.2 FIELD EXPERIMENT AND IRRIGATION RE-GIMES

An experiment was carried out in three consecutive years from early March 2016 to the end of 2018 at Lavark Research Farm, affiliated to Isfahan University of Technology, 40 Km southwest of Isfahan (32° 32′ N, 51° 23′ E, 1630 m above sea level), Iran. Mean annual precipitation and temperature at this site are 149 mm and 15.4°C, respectively. The soil was silty clay loam with a bulk density of 1.3 g cm^{-3} in the top 50 cm and a pH level of 7.4-7.9. The field experimental design was a square lattice design (10 by 10) with two replications for each different irrigation (drought stress and non-drought) regimes in each year. The seeds were planted in rows of 3 m long and spaced 25 cm from each other to yield a plant density of 40 plants m^{-2} in the plots. All the plants received the first irrigation before planting. After this period, irrigation was applied every week until the budding stage. From budding stage to full maturity stage, the non-stress treatment involved irrigation when 50 % of the total available water was depleted from the root zone, but in the drought stress conditions, irrigation was applied when 85 % of the total available water was depleted from the root zone.

The irrigation interval (number of days between two irrigations) during the growing season (budding to full maturity) was variable because of the variation in evapotranspiration (ET). Soil samples were taken from a depth of 0 to 60 cm of the soil from both drought and non- drought 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.

Then, irrigation depth was determined using the formulae: I = [(θ_{FC} - θ_i)/100] D × B); where, I represents irrigation depth (cm), θ_{FC} (–0.03 MPa) is soil gravimetric moisture percentage at field capacity (22 %), θi (–1.5 MPa) is soil gravimetric moisture percentage at irrigation time (10 %), D is root-zone depth (50 cm), and B is soil bulk density at the root zone (1.3 g cm^{-3}) (Clarke et al., 2008). The volume of irrigation water applied was monitored at each irrigation by calculating the depth of water over a Parshall flume which was calculated as: Id = I ×*p*, where *p* is the fraction of I that can be depleted from the root zone. Then I$_g$ = (I$_d$/ Ea) × 100, which E$_a$ is irrigation efficiency (%), assumed to be 65 % on the average. The differences in available water related to different mean for temperature in growing seasons across three years of study. No growth regulators or fungicides were applied. Surface application of 130 (kg ha^{-1}) N and 25 (kg ha^{-1}) P was carried out in both treatments with an additional 55 kg ha^{-1} of N during the rosette stage. Plants were harvested in the middle row at maturity and seed yield was recorded in each plot. Ten different selection indices were calculated using the equations reported in Table 1. In these equations, Y_s represents the

yield of genotypes under stress; Y_p, the yield of genotypes under normal conditions (kg ha^{-1}); and denote mean yields of all the genotypes under stress and non-stress conditions, respectively.

2.3 STATISTICAL ANALYSIS

A combined analysis of variance (ANOVA) was performed using SAS software (SAS. Ver. 9.3.1), for seed yield and selection indices using GLM procedure. Principal Component Analysis (PCA) and 3D biplot diagrams were exploited to identify tolerant and susceptible genotypes using R software (Ver. 3.6.1). Correlations between seed yield in the non-stress and drought-stress treatment as well as the relevant drought tolerance indices were determined using SAS PROC CORR and Heat Map Graph (R software ver 3.6.1). The safflower genotypes were classified using the seed yields obtained from each of the water treatments and drought tolerance indices data using the Ward algorithm based on the squared Euclidean distances in the R Software (Ver. 3.6.1).

3 RESULTS

Analysis of variance indicated the non-significant effect of year on all the studied traits (Table 2). A highly significant ($p < 0.01$) variation in seed yield was observed among the studied genotypes under both (stress and non-stress) conditions and for all the tolerance indices examined (Table 2). The genotype × year interaction effect was not significant for any of the indices, except for DI and HARM (Table 2). The significant genotype × environment interaction for both DI and HARM, indicating considerable variability among the genotypes across different years

and different irrigation treatments for these selection indices.

Table 3 reports the ten highest and the lowest seed yields, Y_p and Y_s, for the studied genotypes. Clearly, the highest Y_p values were obtained for G_{13} (5680 kg ha^{-1}) (from Iran) and G_{61} (5310 kg ha^{-1}) (from Morocco), but the least Y_p value was obtained for G_{79} (900 kg ha^{-1}). Under stress conditions, the highest seed yield (Y_s) was obtained in genotypes 47 (3038 kg ha^{-1}) and 24 (2670 kg ha^{-1}), but the lowest (590 kg ha^{-1}) was observed in G_{79} (Table 3). G_{13} recorded the highest values of TOL (4130), SSI (1.61), and HARM (1.14) indices, whereas G_{86} (from Tajikistan) recorded the least values for TOL (260), SSI (0.24), and HARM (0.13). The highest (0.87) and the lowest (0.27) values of YSI were obtained for G_{86} and G_{13}, respectively. Finally, the genotypes 47 and 25 had the highest (1.55) and lowest (0.20), respectively, mean values of the DI index (Table 3).

The correlation coefficients among Y_p, Y_s, and other drought tolerance selection indices were calculated to determine the most desirable drought tolerance criteria (Table 4). It was found that seed yield and YSI exhibited negative (- 0.5**) and positive (0.34**) correlations under the non-stress and stress conditions, respectively. Seed yield under the non-stress treatment showed positive and significant correlations with all the selection indices, except for YSI (−0.50**) and DI (Table 4). Seed yield under the stress treatment showed positive and significant correlations with MP, GMP, STI, and DI but negative and significant ones with SSI and HARM (Table 4).

Principal Component Analysis (PCA) as a representative for distinguish the relationships among the indices revealed that the first component (PC$_1$) explained 54 % of the total seed yield variation and exhibited positive correlations with Yp, MP, GMP, and STI (Figure 1). PC$_2$ explained 44 % of the total yield variation and had higher positive correlations with DI, YSI, and Ys but higher negative correlations with SSI and HARM (Figure 1).

Table 2: Combined analysis of variance for seed yield under non-stress (Y_p) and stress (Y_s) conditions and different susceptibility indices in safflower genotypes growing under drought stress and normal conditions evaluated in 2016 and 2018

S.O.V	DF	Y_p	Ys	SSI	YSI	TOL	MP	GMP	STI	DI	HARM
Year (Y)	2	3947374.2	77841	0.00019	0.05	133849.6	29145.2	303800.5	0.047	0.34	0.2
Block/ Year	3	282227.2	117545.25	0.36	0.06	696640.8	25726.0	14486.5	0.003	0.13	0.18
Genotype (G)	99	3305183.4**	844416.53**	0.40**	0.08**	2177268.3**	1530482.9**	1260048.7**	0.53**	0.34	0.23**
G × Y	198	89064.9	80535.49	0.071	0.01	143568.0	48908.2	55476.1	0.028	0.05**	0.54**
Residual	297	89064.9	154372.9	0.12	0.025	350774.0	122390.6	125950.5	0.06	0.09	0.07

* and **, significant at $p < 0.05$ and $p < 0.01$, respectively. Abbreviations: DF: degree of freedom; Y_p: seed yield under non-stress; Y_s: seed yield under stress; SSI: stress susceptibility index, YSI: yield stability index; TOL: stress tolerance; MP: mean productivity; GMP: geometric mean productivity; STI: stress tolerance index; DI: Drought Resistance Index; and HARM: Harmonic mean

Table 3: Ten highest and lowest values for seed yield under non-stress conditions (Y_p), Seed yield under stress conditions (Y_s), and different selection indices among the 100 different safflower genotypes investigated

Ten highest indices	YP[¥] (kg ha⁻¹)	YS (kg ha⁻¹)	SSI	YSI	TOL	MP	GMP	STI	DI	HARM
	5680(G13)	3080(G47)	1.613(G13)	0.8724(G86)	4130(G13)	3985(G47)	3820.2(G47)	2.2172(G47)	1.5516(G47)	1.1488(G13)
	5310(G61)	2670(G24)	1.58(G61)	0.8552(G50)	3770(G61)	3615(G13)	3233.2(G24)	1.6356(G11)	1.3097(G24)	1.1104(G61)
	4910(G58)	2390(G11)	1.5697(G25)	0.84(G99)	3140(G58)	3425(G61)	3159.8(G11)	1.5709(G24)	1.1934(G94)	1.1069(G76)
	4890(G47)	2385(G3)	1.5629(G76)	0.8148(G38)	2800(G76)	3380(G11)	3046.4(G3)	1.4443(G3)	1.1672(G97)	1.1029(G25)
	4520(G33)	2350(G97)	1.5285(G18)	0.8042(G81)	2760(G33)	3340(G58)	2957.2(G13)	1.33(G13)	1.15(G69)	1.0677(G18)
	4370(G11)	2167.5(G48)	1.5057(G59)	0.7948(G94)	2750(G18)	3305(G24)	2931(G58)	1.2916(G58)	1.1144(G48)	1.03(G59)
	3980(G27)	2160(G2)	1.4701(G27)	0.7841(G41)	2690(G27)	3142.5(G3)	2846.3(G61)	1.2356(G61)	1.1077(G99)	1.0095(G27)
	3980(G18)	2130(G63)	1.4483(G66)	0.7749(G12)	2450(G59)	3140(G33)	2824.3(G97)	1.215(G97)	1.0974(G63)	0.9724(G66)
	3940(G24)	2118.3(G94)	1.4284(G58)	0.7713(G69)	2440(G25)	2880(G97)	2817.8(G33)	1.2063(G33)	1.0941(G86)	0.97(G21)
	3900(G3)	2100(G69)	1.4258(G21)	0.7668(G37)	2300(G66)	2845(G2)	2759(G2)	1.1386(G2)	1.0793(G11)	0.9508(G58)

| Ten lowest indices	Y_p (kg ha⁻¹)	Y_s (kg ha⁻¹)	SSI	YSI	TOL	MP	GMP	STI	DI	HARM
1	2660(G8)	930(G78)	1.3358(G95)	0.7411(G10)	1915(G39)	2660(G57)	2568.3(G48)	1.0026(G48)	1.0463(G96)	0.8926(G95)
2	1510(G81)	920(G95)	0.5084(G69)	0.3546(G21)	410(G71)	1210(G67)	1167.2(G64)	0.209(G31)	0.3026(G79)	0.2604(G69)
3	1460(G64)	920(G87)	0.4939(G12)	0.3461(G66)	380(G99)	1200(G64)	1161.3(G31)	0.2038(G64)	0.3007(G95)	0.2553(G12)
4	1450(G67)	910(G37)	0.4824(G41)	0.3334(G27)	340(G38)	1175(G71)	1155.7(G71)	0.2032(G71)	0.3003(G66)	0.2536(G41)
5	1380(G89)	890(G21)	0.4545(G94)	0.3204(G59)	339(G5)	1160(G89)	1138.2(G89)	0.1942(G89)	0.2884(G18)	0.2367(G94)
6	1380(G71)	860(G31)	0.4392(G81)	0.3093(G18)	320(G81)	1150(G9)	1071.8(G9)	0.173(G9)	0.2834(G87)	0.2299(G81)
7	1350(G82)	801(G5)	0.4147(G38)	0.2939(G76)	310(G79)	1070(G82)	1046.6(G37)	0.1647(G37)	0.2647(G59)	0.208(G38)
8	1210(G37)	790(G82)	0.3429(G99)	0.2904(G25)	300(G50)	1060(G37)	1026(G82)	0.1582(G82)	0.2497(G21)	0.1803(G99)
9	1140(G5)	780(G9)	0.3284(G50)	0.2884(G61)	300(G37)	970.5(G5)	952.4(G5)	0.1363(G5)	0.2346(G76)	0.1587(G50)
10	900(G79)	590(G79)	0.2846(G86)	0.2719(G13)	260(G86)	745(G79)	718.9(G79)	0.0788(G79)	0.2024(G25)	0.1392(G86)

Table 4: Correlation coefficients between seed yield (kg ha⁻¹) of safflower genotypes under non-stress (Yp) and stress (Ys) conditions and each of the stress susceptibility indices averaged over three years

	Y_p	Y_s	SSI	YSI	TOL	MP	GMP	STI	HARM	DI
Y_p [¥]	1									
Y_s	0.59**	1								
SSI	0.50**	-0.34**	1							
YSI	-0.50**	0.34**	-0.99**	1						
TOL	0.86**	0.10	0.83**	-0.83**	1					
MP	0.95**	0.80**	0.24*	-0.24*	0.67**	1				
GMP	0.88**	0.89**	0.08	-0.08	0.52**	0.98**	1			
STI	0.86**	0.88**	0.06	-0.06	0.50**	0.96**	0.98**	1		
HARM	0.53**	-0.32**	0.99**	-0.99**	0.86**	0.27**	0.10	0.08	1	
DI	0.17	0.89**	-0.70**	0.70**	-0.31**	0.47**	0.61**	0.61**	-0.67**	1

* and ** Significant at $p < 0.05$ and $p < 0.01$; respectively; ns, not significant.
Abbreviations ¥: Y_p: Seed yield under non- stress condition; Y_s: Seed yield under stress condition; SSI: stress susceptibility index, YSI: yield stability index, TOL: stress tolerance, MP: mean productivity, GMP: geometric mean productivity, STI: stress tolerance index, HARM: harmonic mean, DI: drought resistance index.
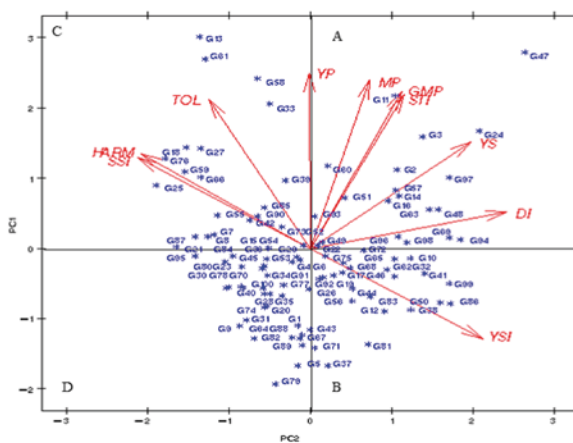
Figure 1: Biplot drawn based on the first and second components obtained from principal component analysis using seed yield of safflower genotypes under non stress (Y_p) and stress (Y_s) conditions. Abbreviations: stress susceptibility index (SSI), yield stability index (YSI), stress tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP), stress tolerance index (STI), drought resistance index (DI); harmonic mean (HARM), and conditions in 100 safflower genotypes.
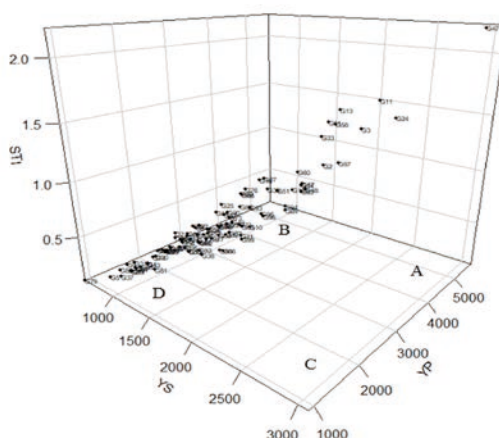

Figure 2: Three-dimensional diagram for identifying drought-tolerant genotypes based on seed yield under non-stress (Y_p) and stress (Y_s) conditions as well as the stress tolerance index (STI).

Because of the positive and significant correlation of STI with seed yield under both conditions, a three-dimensional graphs based on the STI index were drawn (Figure 2). These graphs split the genotypes into four groups, each of which represents one combination of the genotypes. The genotypes 47, 24, 97, 3, and 11 (Group A) are those with high yields under drought and non-stress environments. Those in Group B (e.g., G_{13}, G_{59}, and G_{61}) consisted of genotypes with high yields in a normal environment but low seed yields under drought conditions. No genotype was, however, detected as one with a high yield in a stressful environment (Group C). The genotypes with low yields under both environmental conditions were assigned to Group D (e.g., G_3, G_{11}, G_{79}, and G_5).

3.1 CLUSTER ANALYSIS

A dendrogram was drawn based on the cluster analysis using seed yield under drought and non-drought conditions along with the selection indices TOL, MP, GMP, STI, SSI, YSI, DI, and HARM (Figure 3). The cluster analysis performed classified the 100 genotypes of safflower investigated into three distinct groups consisting of 7, 44, and 49 genotypes. The genotypes in the smallest group (1) including G_{13}, G_{61}, G_{33}, G_{58}, G_{47}, G_{11}, G_{24}, and G_3 showed the highest seed yield under both non-stress and drought stress conditions (Figure 3). The genotypes clustered in Group 2 (i.e., G_{51}, G_{93}, G_{39}, G_{60}, G_2, G_{57}, G_{14}, G_{16}, G_{97}, G_{48}, G_{63}, G_{99}, G_{50}, G_{86}, G_{10}, G_{65}, and G_{81}) recorded medium levels of seed yield under drought stress. The
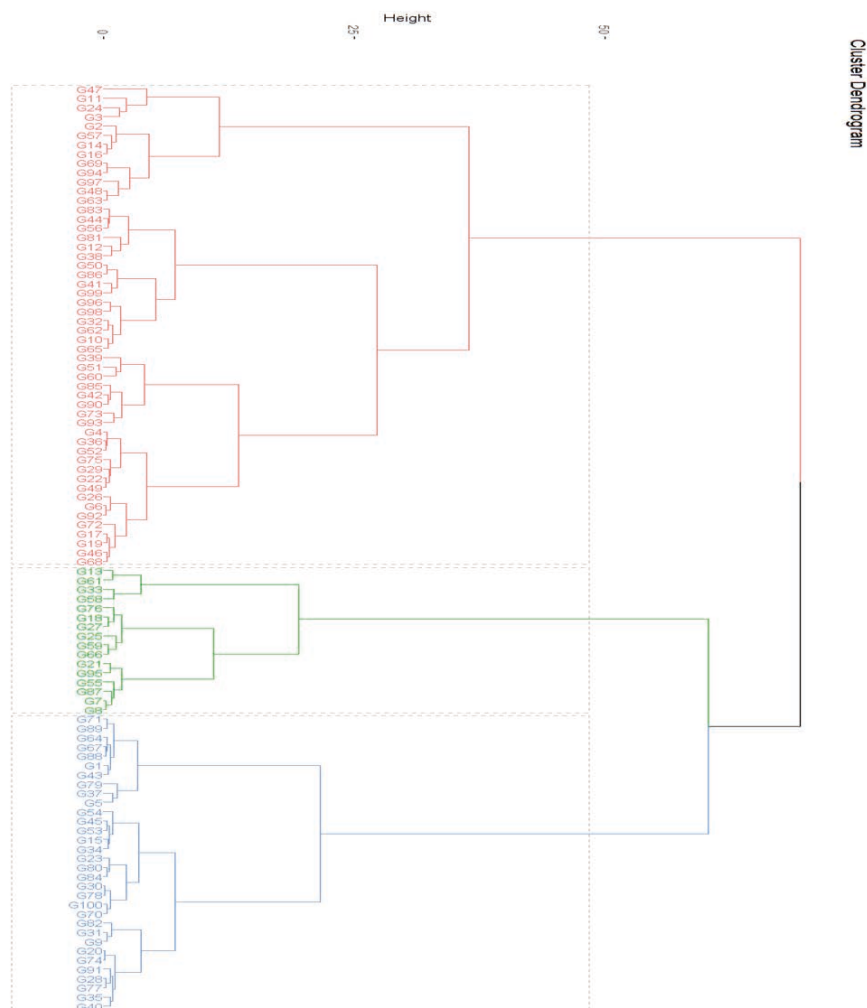
Figure 3: Discrimination of drought tolerance in a worldwide collection of safflower (*Carthamus tinctorius* L.) genotypes based on selection indices.

third group consisted of genotypes with a low productivity under either environmental conditions (i.e., G_{79}, G_{37}, G_{89}, G_{55}, G_{21}, and G_{25}).

4   DISCUSSION

This study evaluated drought tolerance in a world collection of safflower accessions under the effects of year and genotype. The analysis of variance showed a large genetic variation in drought tolerance among the accessions studied as an unpredictable factor affecting seed yield in the genotypes from different geographical regions. Year factor was not found to have any significant effect on seed yield or selection indices; hence, the indi-

ces selected for this germplasm can be effectively used if seed yield is adequately heritable. Considering the fact that traits involved in drought tolerance mechanisms are polygenic ones, the requirement to screen tolerant genotypes has encouraged plant breeders to seek a reliable index. In response to this need, the present study evaluated eight different selection indices (i.e., MP, GMP, TOL, SSI, STI, YSI, DI, and HARM) for use in the estimation of seed yield under drought stress. Based on the correlation analysis performed, the positive and significant correlation between TOL and Y_p (0.86**) implies that the genotypes superior in terms of seed yield (such as G_{79} and G_9) showed greater reductions in seed yield under drought conditions. Also, the non-significant correlation between TOL and Y_s (0.10) revealed the failure of the TOL index

to identify the most tolerant genotypes, confirming the results reported by Rizza et al. (2004). The greater TOL values indicated the higher sensitivity of the genotypes investigated to drought stress; thus, smaller values of this index is favored. The positive and significant correlation between Y_p and SSI (0.50^{**}) and that between Y_p and HARM (0.53^{**}) demonstrated that the genotypes with higher values for Y_p or the SSI index exhibited a higher sensitivity to drought stress (Table 2). On the other hand, the negative and significant correlation between SSI and Ys (-0.34^{**}) or that between HARM and Y_s (-0.32^{**}) implied that the superior genotypes under drought stress recorded lower values for SSI and HARM. Hence, the SSI and HARM indices are able to discriminate superior safflower genotypes (the ones with lower values of SSI or HARM indices) in drought prone areas. Studying spring wheat, Guttieri et al. (2001) maintained that SSI values >1 and <1 might indicate above-average and below-average susceptibility to drought stress, respectively. The most suitable index for selecting stress-tolerant genotypes is an index that establishes a positive and strong correlation with seed yield under both stress and non-stress conditions (Fernandez, 1992). To select drought-tolerant genotypes, based on the most desirable indices, use is made of the correlation coefficient of each index with Y_p and Y_s (Golabadi et al., 2006; Ebrahymian et al., 2012; Abdolshahi et al., 2012; Naghavi et al., 2013). Seed yield was found to have a highly significant positive correlation with GMP, MP, STI, and HARM indices under both the environmental (drought and non- drought conditions) conditions examined (Table 2). Based on the correlation analysis conducted in this study, GMP, STI, and MP were found to favor genotypes with a high-yield potential under stress conditions (Table 2), which agrees with the findings reported Sio-Se Mardeh et al. (2006), Hao et al. (2011), and Ebrahimiyan et al. (2012). Given the fact that G_{47} recorded the highest values for MP and STI, this genotype was identified as the most productive and stable safflower ones from among the ones investigated under both stress and non-stress conditions. The results of the present study indicating the capability of the selection indices GMP, MP, and STI to identify genotypes satisfactorily under both conditions are consistent with those reported for GMP and MP in mungbean (Fernandez, 1992); STI and GMP in rice (Raman et al., 2012); safflower (Bahrami et al., 2014) *Brassica napus* L. (Khalili et al., 2012) and durum wheat (Ilker et al., 2011); as well as GMP, STI, and MP in tall fescue (Ebrahymian et al., 2012) and maize (Hao et al., 2011). GMP is often used by plant breeders interested in calculating relative performance since drought stress might vary in severity both under field conditions and over different years (Fernandez, 1992). In the present study, GMP established

significant and positive correlations with TOL, Y_p, and Y_s (Table 3). DI, which is commonly accepted as an index to identify genotypes with high yields under both stress and non-stress conditions (Lan, 1998), showed only a highly significant and positive correlation with Y_s (0.89^{**}) (Table 4), demonstrating that selection of safflower genotypes with high DI values might be useful for severe drought-stricken regions but that the genotypes selected based on this index do not have very high yields or yields equivalent to those of genotypes currently cultivated under normal irrigation. Seed yield under non-drought conditions (Yp) was positively correlated with Y_s, confirming previous reports on safflower (Bahrami et al., 2014) other crop species such as bread wheat (El-Rawy and Hassan, 2014), corn (Naghavi et al., 2013) and bread wheat (Abdolshahi et al., 2012). It may also be noted that the satisfactory responses shown by some genotypes under stress conditions could be ascribed to the good adaptation mechanisms in these genotypes (Naghavi et al., 2013). The impacts of the different indices in each PC indicate that PC_1 and PC_2 could be identified as yield potential and stress susceptibility groups, respectively. The genotypes (such as G_{47}, G_{11}, G_3, and G_{24}) recording high values for both PC_1 and PC_2 may be considered as superior ones for seed yield under both experimental conditions; hence, they are designated as stable genotypes (Figure 1A). The genotypes recording low PC_1 but high PC_2 values included those also with high values of DI, YSI, and seed yield under drought stress, but low values of SSI and HARM values (Figure 1B). The genotypes (such as G_{13} and G_{61}) recording high PC_1 but low PC_2 values included genotypes with high values for GMP, STI, MP, and seed yields under non-stress conditions (Figure 1C). On the other hand, the majority of the genotypes with low PC_1 and PC_2 values were identified as susceptible genotypes; these included G_{79}, as the most tolerant one, and the genotypes G_9, G_{82}, and G_{89} (Figure 1D), that were recognized as unstable genotypes. The majority of the genotypes investigated (more than 60 %) were classified in Groups B and D (Figure 1). This biplot may also be used for identifying contrasting genotypes (genotypes in group A *versus* D) for planning fine mapping populations for safflower genome studies of drought tolerance. Based on our cluster analysis, the genotypes assigned to Group 3 were recognized as the most tolerant ones to be used as parents for improving drought tolerance in safflower breeding programs. Thus, the genotypes in Group 1 and Group 3 were identified as drought tolerant and drought susceptible, respectively. Cluster analysis has been widely used not only to discriminate high distance genotypes but also to determine genetic diversity based on similar traits under drought stress conditions (Golabadi et al., 2006; Mohammadi et al., 2011; Naghavi et al., 2013).The results of the

present cluster analysis of the genotypes investigated were consistent with the PCA results obtained. Thus, drought-tolerant genotypes recording high PC_1 and PC_2 values as well as those assigned to Groups of 1 and 3 in the cluster analysis can be used as extreme parental genotypes with the highest genetic distance to develop new hybrid varieties in safflower aimed at production of drought-tolerant cultivars. However, further evaluation of genotypes using drought tolerance indices across multiple locations is required to confirm their stability for developing improved safflower genotypes.

5 CONCLUSION

From the results obtained, it may be concluded that it is preferable to use simultaneously different drought tolerance indices for screening drought-tolerant safflower genotypes. The results of different multivariate analyses revealed that STI, MP, and GMP, in this descending order, were not only capable of efficient selection of high seed-yield genotypes under both the environmental conditions examined but also of discrete identification of drought-tolerant from drought-sensitive safflower genotypes. The G_{47} genotype (Spanish origin) was identified as the most drought-tolerant one with the highest seed yield under both drought and non-stress conditions. Based on the results obtained in this study, the elite genotypes (i.e., G_{24}, G_{13}, G_3, G_{11}, G_{33}, G_{58}, and G_{61}) may be recommended as promising cultivars for cultivation in drought-affected areas or as appropriate donor parents in safflower hybridization programs. These genotypes may also be exploited for improving seed yield and stability in safflower for cultivation in drought prone regions through appropriate selection methods.

6 ACKNOWLEDGMENT

7 REFERENCES

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Table supplementary 1: Characteristics of the 100 different genotypes of safflower used in this study

Genotype code	Genotype name	Geographical origin	Genotype code	Genotype name	Geographical origin	Genotype code	Genotype name	Geographical origin
G1	A2	Iran (Azerbayejan)	G34	Car159	Germany	G67	Car64	Slovakia
G2	Ac- Stirling	Canada	G35	Car160	Russia	G68	Car67	Germany
G3	AC-sunset	Canada	G36	Car161	Russia	G69	Car68	Germany
G4	Arak 2811	Iran (Markazi)	G37	Car169	Hungary	G70	Car70	Lybyan
G5	C111	Iran(Isfahan)	G38	Car175	India (Kusum)	G71	Car72	North Korea
G6	Car118	India	G39	Car181	India	G72	Car74	North Korea
G7	Car 116	India	G40	Car188	Poland	G73	Car75	North Korea
G8	Car 9	Slovaki	G41	Car19	Poland	G74	Car76	North Korea
G9	Car100	Italy	G42	Car190	Iran (Isfahan)	G75	Car77	North Korea
G10	Car106	Spain	G43	Car198	Azerbaijan	G76	Car78	Hungary
G11	Car114	India	G44	Car199	Korean republic	G77	Car79	Japan
G12	Car117	Sudan (tozi)	G45	Car200	unknown	G78	Car80	North Korea
G13	K21	Iran (Kordestan)	G46	Car201	Sudan	G79	Car83	Tajikistan
G14	Car124	Pakistan	G47	Car210	Spain	G80	Car86	Tunisia
G15	Car125	Russia	G48	Car211	Germany	G81	Car87	Romania
G16	Car126	Belgium	G49	Car214	Poland	G82	Car89	Tunisia
G17	Car127	Germany	G50	Car215	Germany	G83	Car94	Spain
G18	Car129	Germany	G51	Car216	Germany	G84	GE62918	Germany
G19	Car130	Morocco	G52	Car217	Germany	G85	Gila	USA
G20	Car131	Paraguay	G53	Car218	Germany	G86	Hartman	USA
G21	Car132	Germany	G54	Car219	Germany	G87	IL111	Iran (Aur-oumieh)
G22	Car135	Portugal	G55	Car221	Germany	G88	Isf-14	Iran (Isfahan)
G23	Car137	Pakistan	G56	Car224	Germany	G89	Isf28	Iran(Isfahan)
G24	Car138	Poland	G57	Car226	Germany	G90	K21	Iran (Kord-estan)
G25	Car146	Egypt	G58	Car227	Germany	G91	KMS 36	Iran (karaj)
G26	Car147	Pakistan	G59	Car228	Germany	G92	Mex.17-45	Mexico
G27	Car148	Pakistan	G60	Car230	Germany	G93	Mex.7-147	Mexico
G28	Car151	India	G61	Car24	Morocco	G94	Mex.7-38	Mexico
G29	Car152	Iraq	G62	Car37	Sudan	G95	Mex-13-216	Mexico
G30	Car155	Russia	G63	Car42	Sudan	G96	Mex2-138	Mexico
G31	Car156	Pakistan	G64	Car49	Spain	G97	Mex22-191	Mexico
G32	Car157	Morocco	G65	Car55	Poland	G98	Mex6-97	Mexico
G33	Car158	Paraguay	G66	Car56	Nebraska 8 (USA)	G99	PI 301055	Turkey
						G100	Saffire	Canada