

## Wild safflower species (*Carthamus oxyacanthus* Bieb.): A possible source of drought tolerance for arid environments

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### Abstract

The detection and incorporation of genes from wild plant species provides a means for sustaining genetic improvement in plants cultivated in arid environments. This research measured relative levels of drought tolerance in several genotypes of cultivated (*Carthamus tinctorius*) and wild (*Carthamus oxyacanthus*) species. These plants were collected from different regions of Iran and evaluated. Their drought tolerance indices were assessed at three moisture levels. Five drought tolerance indices were calculated based on relative grain yield under drought and normal conditions. There were significant differences between the two species (cultivated and wild). The wild plants sustained more moisture stress tolerance than cultivated species. Increasing water stress levels caused significantly more reductions in the seed yield of cultivated genotypes as compared with wild genotypes. Calculated correlation coefficients revealed that geometric mean productivity (GMP), stress tolerance index (STI) and Harmonic mean (HM) indices are superior criteria for selecting high-yield genotypes under stress and non-stress conditions. Biplot analysis, according to principle component analysis (PCA), indicated that wild genotypes had low yields, but their production was stable when the environment changed. High drought tolerance makes wild *Carthamus oxyacanthus* safflower a suitable source for transferring drought tolerant genes to cultivated species.

**Key words:** moisture stress, multivariate analysis, stability, wild species.

**Abbreviations:** GMP=geometric mean productivity, STI =stress tolerance index, HM=Harmonic mean, PCA=principle component analysis.

### Introduction

Safflower (*Carthamus tinctorius* L.), due to cold, drought and salinity tolerance (Weiss, 2000), is an important oilseed crop in arid and semi-arid regions of the world. The crop has traditionally been grown for its flower used in coloring and flavoring foods, for making dye, and in medicinal applications. In recent years, this crop has been grown as a source for vegetable oil used for human consumption and industrial purposes (Li and Mundel, 1996). Among the wild relatives of safflower, only two species (*Carthamus oxyacanthus* Bieb. and *Carthamus palaestinus* Eig.) are easily crossable with cultivated species (Ashri and Knowles, 1960). These two species are suitable for safflower improvement. The wild species, (*C. oxyacanthus*), is widely spread through Turkey, Western Iraq, Iran (Dittrich et al., 1979), Northwest India, Kazakhstan, Turkmenistan, and Uzbekistan (Knowles and Ashri, 1995). This species has considerable genetic diversity in Iran (Sabzalian et al., 2009) and an oil quality comparable with cultivated safflower (Mundel and Bergman, 2009, Sabzalian et al., 2008). A

strong relationship has been found between the brown-black seed coat color of *C. oxyacanthus* and resistance to safflower fly indicating a possibility of using this trait in safflower breeding programs (Sabzalian et al., 2010). Drought, among the different environmental stresses, has a highly negative impact on crop production. Plants possess a variety of morphological and physiological mechanisms which allow them to adapt to water stress (Karkanis et al., 2011). The development of cultivars with improved productivity under water stress is important because of severe limitations imposed by drought in specific regions (McWilliam, 1989). Low heritability for drought tolerance and few effective selection approaches has limited the development of resistant crop cultivars (Kirigwi et al., 2004). Breeding for drought resistance is problematic due to a lack of fast, reproducible screening techniques and an inability to routinely create (defined and repeatable) water stress conditions for efficient evaluation of large populations (Ramirez and Kelly, 1998). Fernandez (1992) classified plants (according to their

performance in water-stress and stress-free environments) into four groups: genotypes with good performance in both environments (Group A), only in non-stress environments (Group B), in stressful environments (Group C); and genotypes with weak performance in both environments (Group D). The question is, should breeding for stress-prone environments rely on selection under both potential and stress conditions or in either environment alone?

Several selection indices, which provide a measure of drought tolerance based on loss of yield under drought-conditions in comparison with normal conditions, have been suggested for screening drought tolerant genotypes (Clarke et al., 1992; Mitra, 2001). Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the differences in yield between stress (Ys) and non-stress (Yp) environments and mean productivity (MP) as the average yield of Ys and Yp. Fischer and Maurer (1978) proposed a stress susceptibility index (SSI) of the cultivar. Fernandez (1992) defined a new advanced index (STI= stress tolerance index) which can be used to identify genotypes that produce high yield under both stress and non-stress conditions. The geometric mean productivity (GMP) is often used by breeders interested in relative performance since field drought stress severity can increase over a number of years (Ramirez and Kelly, 1998; Azizi Chakherchaman et al., 2009). Among stress tolerance indicators, larger values of TOL and SSI represent relatively more sensitivity to stress. Smaller values of TOL and SSI are favored. Selection based on these criteria favors genotypes with low yield potential under non-stress conditions and high yield under stress conditions. Selection based on STI and GMP will result in genotypes with higher stress tolerance and yield potential (Fernandez, 1992).

Ramirez and Kelly (1998) reported that selection based on a combination of both SSI and GM indices may provide a more desirable criterion for improving drought resistance in common beans. Guttieri et al. (2001), using SSI criterion in spring wheat, suggested that more than 1 unit of SSI value may indicate above-average susceptibility for drought stress and less than 1 unit has below-average susceptibility. Golabadi et al. (2006) found that STI, MP, and GMP are superior indices for selecting high yield durum wheat genotypes both under moisture stress and non-stress field environments. Pourdad (2008) reported that STI was the best index to identify superior cultivated safflower genotypes in conditions both with and without drought stress. Wild safflower *C. oxyacanthus* has large genetic diversity, with distribution in some regions of Iran which has very low precipitation (less than 100 mm). The oil quality of *C. oxyacanthus* is comparable with cultivated species. This wild species may be a useful source of genes which can improve cultivated safflower. The identification of selection indices which help delineate high- yielding genotypes in water stress situations greatly facilitates the improvement of safflower cultivars. The suitability of specific indicators depends on the timing and severity of stress in drought-prone environments. In this study we evaluated seven accessions of wild safflower (*C. oxyacanthus*) and 13 accession of cultivated safflower (*C. tinctorius*) under three moisture stress conditions. The objectives of this study were (1) to estimate the level of drought tolerance of wild safflower of *C. oxyacanthus* as compared with cultivated species, (2) to assess the efficiency of different selection indices in mild and intense water stress

field conditions, and (3) to identify drought tolerant genotypes of cultivated safflower and the most relatively wild species of *C. oxyacanthus*.

## Result and discussion

### Analysis of variance

A combined analysis of variance over environments (different moisture conditions) indicated variability among the genotypes, significant influence of moisture conditions, and differential responses of genotypes over environments for all criteria (data not shown). A separate analysis of mean comparison, correlation, clustering, and biplot were performed for each moisture stress. The results of analysis of variance indicated highly significant differences among genotypes for all indices, demonstrating high diversity among studied germplasm. But there were no significant differences among wild genotypes for TOL index (Table 2).

### Moisture treatment means

The mean grain yield of both species under mild stress condition was 189 g/m<sup>2</sup>. This indicated a reduction of 43% compared with that of non-stress (control) conditions. Under an intense stress environment, the reduction of grain yield was about 56% as compared with the control treatment. Mean comparison for interaction of species and moisture treatments (Fig.1) showed that increasing water stress level caused significant reduction in the seed yield of cultivated genotypes, while wild genotype seed yields were not significantly affected. Fig.1 shows that when each level of drought increased; there was almost a two-fold decrease in the grain yield of cultivated genotypes while the wild genotypes were not significantly affected. At the intense water stress level, the wild genotypes performed much as they did in mild stress conditions. This indicates general yield stability in wild safflowers in response to drought stress. This constancy makes *C. oxyacanthus* a suitable source for transferring drought tolerance genes to cultivated safflower. Alleles for better performance to drought under field conditions have been found in wild barley (*Hordeum spontaneum*), the closest relative of cultivated barley (*Hordeum vulgare*) (Baum et al., 2003; Talame et al., 2004) and exotic germplasm has been considered a potential source for improving drought adaptive mechanisms in wheat (Reynolds et al., 2007).

### Mean comparison of genotypes

Cultivated genotypes, C4110 (YP =619.5 g/m<sup>2</sup>) and wild genotypes Azari (YP=344.1 g/m<sup>2</sup>) had the highest yields in optimal conditions (Table 3). With mild and intense moisture conditions, cultivated genotypes of Kashan had the highest yield. Among wild accessions, Azari and Arakva showed better yield performance in mild and intense stress (Table 3 and 4). Fig.2 shows a pattern of reaction for all genotypes under normal and moisture stress conditions. Since there is no clear pattern of drought stress response among genotypes of both species for yield performance, selection based on drought indices may be more effective for drought tolerance.

**Table 1.** Information of cultivated and wild safflower evaluated in two drought environment conditions

Num	Genotype	Species	Origin
1	Acst (cultivated)	<i>C. tinctorius</i>	Canada
2	C111(cultivated)	<i>C. tinctorius</i>	Line selected from Koseh landrace, Iran
3	C4110 (cultivated)	<i>C. tinctorius</i>	Line selected from Koseh landrace, Iran
4	M113(cultivated)	<i>C. tinctorius</i>	Line selected from Markazi landrace, Iran
5	M115 (cultivated)	<i>C. tinctorius</i>	Line selected from Markazi landrace, Iran
6	S149 (cultivated)	<i>C. tinctorius</i>	Line selected from Isfahan landrace, Iran
7	S144 (cultivated)	<i>C. tinctorius</i>	Line selected from Isfahan landrace, Iran
8	Arak2811 (cultivated)	<i>C. tinctorius</i>	Line selected from Arak landrace, Iran
9	Kashan (cultivated)	<i>C. tinctorius</i>	Kashan, Iran
10	Kordes (cultivated)	<i>C. tinctorius</i>	Kordestan, Iran
11	Koseh (cultivated)	<i>C. tinctorius</i>	Isfahan, Iran
12	Saffire (cultivated)	<i>C. tinctorius</i>	Canada
13	Shiraz (wild)	<i>C. oxyacanthus</i>	Shiraz, Iran
14	Aligod (wild)	<i>C. oxyacanthus</i>	Aligodarz, Iran
15	Arakva (wild)	<i>C. oxyacanthus</i>	Arak, Iran
16	Azari (wild)	<i>C. oxyacanthus</i>	Azarbaijan, Iran
17	Hamedan (wild)	<i>C. oxyacanthus</i>	Hanmadan, Iran
18	Kermansh (wild)	<i>C. oxyacanthus</i>	Kermanshh, Iran
19	Lavark (wild)	<i>C. oxyacanthus</i>	Lavark, Isfahan, Iran
20	Shirazva (wild)	<i>C. oxyacanthus</i>	Shiraz, Iran

**Table 2.** An analysis of variation for grain yield and drought tolerance indices in safflower genotypes evaluated under two moisture stress environments

Stress	Variation sources	DF	Mean Square							
			YP (g/m <sup>2</sup> )	YS(g/m <sup>2</sup> )	TOL	MP	GMP	SSI	STI	HM
Mild	Replication	2	6666.4 <sup>ns</sup>	760.9 <sup>ns</sup>	194.9 <sup>ns</sup>	2085.3 <sup>ns</sup>	1757.7 <sup>ns</sup>	.05 <sup>ns</sup>	.05 <sup>ns</sup>	1724.4 <sup>ns</sup>
	Genotype	19	84798 <sup>**</sup>	17335.4 <sup>**</sup>	22518.8 <sup>**</sup>	38683.5 <sup>**</sup>	31928.5 <sup>**</sup>	0.32 <sup>**</sup>	0.57 <sup>**</sup>	27072.0 <sup>**</sup>
	Cultivated	12	50570 <sup>**</sup>	163724 <sup>**</sup>	15970.6 <sup>**</sup>	20252 <sup>**</sup>	16691 <sup>**</sup>	0.19 <sup>**</sup>	0.42 <sup>**</sup>	14713.3 <sup>**</sup>
	Wild	6	39017 <sup>**</sup>	34167 <sup>**</sup>	8851 <sup>ns</sup>	107421 <sup>**</sup>	93234 <sup>**</sup>	0.35 <sup>**</sup>	0.82 <sup>**</sup>	13537.9 <sup>**</sup>
	Cultivated vs Wild	1	553066 <sup>**</sup>	92191 <sup>**</sup>	119104 <sup>**</sup>	274215 <sup>**</sup>	221040 <sup>**</sup>	1.38 <sup>**</sup>	4.1 <sup>**</sup>	179338 <sup>**</sup>
	Error	38	4134.9	2374.8	4758.7	1397.2	1371.5	.07	.05	1630.4
	CV (%)		19.3	25.7	38.0	14.33	15.1	26.1	33.1	17.5
Intense	Replication	2	6666.4 <sup>ns</sup>	208.1 <sup>ns</sup>	3148.5 <sup>ns</sup>	2264.4 <sup>ns</sup>	1975.6 <sup>ns</sup>	.03 <sup>ns</sup>	.04 <sup>ns</sup>	13099.9 <sup>ns</sup>
	Genotype	19	84798 <sup>**</sup>	10245.5 <sup>**</sup>	43444.3 <sup>**</sup>	24985.3 <sup>**</sup>	16568.4 <sup>**</sup>	0.22 <sup>**</sup>	0.24 <sup>**</sup>	1649.1 <sup>**</sup>
	Cultivated	12	50570 <sup>**</sup>	11374.3 <sup>**</sup>	42194.6 <sup>**</sup>	15474.4 <sup>**</sup>	11439.2 <sup>**</sup>	0.14 <sup>**</sup>	2094.1 <sup>**</sup>	11196.7 <sup>**</sup>
	Wild	6	39017 <sup>**</sup>	9505.5 <sup>**</sup>	13235.8 <sup>ns</sup>	13562.8 <sup>**</sup>	10823.3 <sup>**</sup>	0.09 <sup>**</sup>	888.6 <sup>**</sup>	8973.3 <sup>**</sup>
	Cultivated vs Wild	1	553066 <sup>**</sup>	577.3 <sup>ns</sup>	235157.9 <sup>**</sup>	129478 <sup>**</sup>	62908.4 <sup>**</sup>	1.47 <sup>**</sup>	9760.1 <sup>**</sup>	29089.7 <sup>**</sup>
	Error	38	4134.9	1903.6	4274.1	1510.7	1365.1	.03	.03	1429.5
	CV (%)		19.3	30.1	25.6	16.3	17.8	16.1	32.6	20.8

ns, \* and \*\* not significant and significant at the 5% and 1% levels of probability, respectively.

YP= grain yield under normal condition, YS= grain yield under drought condition, TOL= stress tolerance, MP= mean productivity, GMP= geometric mean productivity, SSI= stress susceptibility index, STI= stress tolerance index, HM= Harmonic mean

Larger values of water stress tolerance indices TOL and SSI represent relatively more sensitivity to stress. The lowest values of TOL were recorded in wild genotypes of Kermanshah (TOL= -31.64) and Arakva (TOL= -119.2) for mild and intense water stress levels. This shows that a low irrigation condition had negative impacts on yield for some wild genotypes (Fig. 2, Table 3 and 4) and TOL was able to highlight genotypes with high yield under stress condition. But for both species, most genotypes with a low value of TOL had low yield in normal moisture conditions. TOL was less helpful in selecting high yield, drought tolerant genotypes. Based on GMP and STI values, the line M113 and cultivar Kashan could be considered relatively drought tolerant among cultivated genotypes in both stress conditions (Table 3 and 4).

### Correlation of indices

The correlation coefficient between YP, YS and other quantitative indices of drought tolerance were calculated based on both species (Table 5) to determine the most desirable drought tolerance criteria. There were positive and significant correlations between YP and YS in mild stress condition, while no correlation was found under intense stress condition indicating that selection based on the drought tolerance indices would be more effective. The results under both stress environments indicated positive and significant correlations between YP with all selection indices. Correlations between YS with GMP, STI, and HM indicated that selection based on these indices may increase yield in stress and non stress conditions. The observed correlation

**Table 3.** Average yields of safflower genotypes under optimal (YP) and mild stress (YS) conditions, and calculated different drought tolerance indices

Species	Genotypes	YP(g/m <sup>2</sup> )	YS (g/m <sup>2</sup> )	TOL	MP	GMP	SSI	STI	HM
<i>C. tinctorius</i>	Acst	218.4	106.4	112.0	162.4	151.559	1.2	0.2	141.7
	C111	347.5	174.1	173.3	260.8	232.1	0.9	0.5	208.2
	C4110	619.5	204.5	414.9	412.0	354.3	1.5	1.2	305.4
	M113	533.6	287.5	246.13	410.5	390.4	1.01	1.4	371.5
	M115	466.8	269.6	197.2	368.2	353.7	1.0	1.2	340.0
	S149	535.1	217.9	317.2	376.5	337.3	1.4	1.1	303.8
	S144	424.5	194.4	230.1	309.5	285.5	1.2	0.7	263.7
	Arak2811	386.9	228.9	158.1	307.9	297.1	0.9	0.8	286.7
	Kashan	370	360	10	365	364.9	0.06	1.2	365
	Kordes	269.9	237.6	32.27	253.7	250.0	0.2	0.6	246.5
	Koseh	561.6	221.9	339.73	391.7	352.5	1.4	1.1	317.4
	Saffire	250.7	127.5	123.2	189.1	177.9	1.2	0.3	167.6
	Shiraz	479.5	276.5	202.93	378.0	359.6	0.9	1.2	342.8
<i>C. oxycantha</i>	Aligod	279.3	133.3	145.98	206.3	190.8	1.2	0.3	177.1
	Arakva	98.86	88.3	10.55	93.59	92.9	0.2	0.1	62.2
	Azari	344.1	196.0	148.12	270.1	259.3	1.0	0.6	249.0
	Hamedan	92.4	95.2	-2.80	93.8	93.8	-0.1	0.1	93.8
	Kermansh	94.5	126.1	-31.64	110.3	108.1	-0.7	0.1	105.9
	Lavark	266.9	164.3	102.56	215.62	207.5	0.8	0.4	199.9
	Shirazva	69.7	75.0	-5.28	72.37	72.0	-0.2	0.1	71.7
LSD (0.05%)	107.0	81.1	114.7	62.2	61.5	0.4	0.4	67.3	

YP= grain yield under normal condition, YS= grain yield under drought condition, TOL= stress tolerance, MP= mean productivity, GMP= geometric mean productivity, SSI= stress susceptibility index, STI= stress tolerance index, HM= Harmonic mean

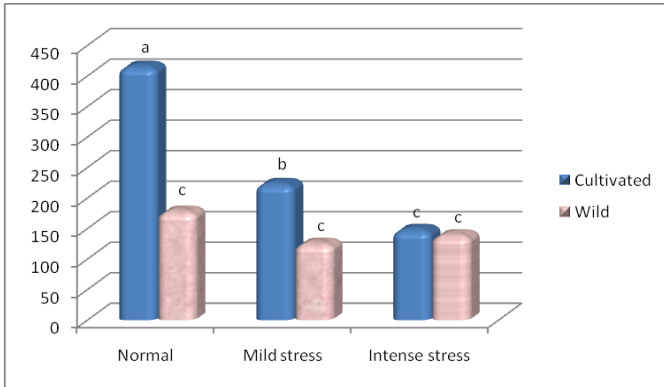
**Table 4.** Average yields of safflower genotypes under optimal (YP) and intense stress (YS) conditions, and calculated different drought tolerance indices

Species	Genotypes	YP(g/m <sup>2</sup> )	YS(g/m <sup>2</sup> )	TOL	MP	GMP	SSI	STI	HM
<i>C. tinctorius</i>	Acst	218.4	77.1	141.3	147.7	127.7	1.17	0.2	111.1
	C111	347.5	196.2	151.2	271.9	252.9	0.6	0.6	236.0
	C4110	619.5	136.7	482.8	378.1	289.8	1.4	0.8	222.9
	M113	533.6	184.5	349.1	359.1	313.3	1.2	0.9	273.5
	M115	466.8	185.9	280.9	326.3	294.5	1.1	0.8	265.8
	S149	535.1	124.4	410.7	329.7	257.4	1.4	0.6	201.3
	S144	424.5	97.1	327.5	260.8	201.7	1.4	0.4	156.7
	Arak2811	386.9	174.1	212.8	280.5	254.3	0.9	0.6	232.4
	Kashan	370	295.2	74.8	332.6	382.4	0.35	0.99	328.5
	Kordes	269.9	110.7	159.2	190.3	171.1	1.0	0.3	154.4
	Koseh	561.6	159.2	402.4	360.4	296.6	1.3	0.8	245.4
	Saffire	250.67	125.6	125.1	188.1	177.3	0.9	0.3	167.1
	Shiraz	479.5	60.1	419.3	269.8	169.5	1.6	0.3	106.7
<i>C. oxycantha</i>	Aligod	279.3	107.0	172.3	193.1	172.4	1.1	0.3	154.0
	Arakva	98.9	218.0	-119.2	158.5	145.3	-2.1	0.2	133.6
	Azari	344.1	123.4	220.7	233.7	205.2	1.1	0.4	180.4
	Hamedan	92.4	110.4	-18.0	101.4	100.4	-0.4	0.1	99.5
	Kermansh	94.5	123.0	-28.5	108.7	105.4	-0.6	0.1	102.1
	Lavark	266.9	217.7	49.16	242.3	236.0	0.2	0.5	230.0
	Shirazva	69.7	74.1	-4.3	71.9	70.9	-0.3	0.0	69.9
LSD (0.05)	107.0	72.6	108.8	64.6	61.5	0.2	0.2	63.0	

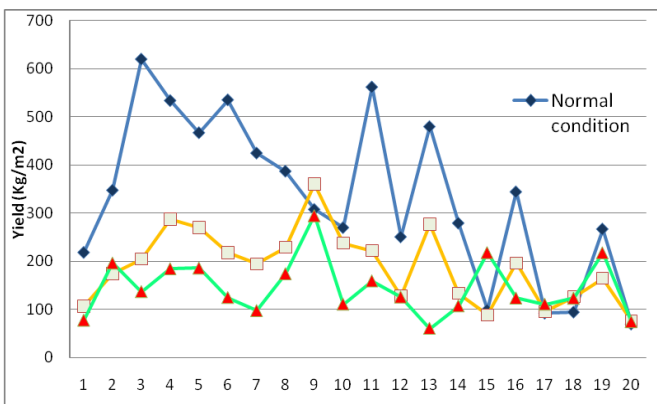
YP= grain yield under normal condition, YS= grain yield under drought condition, TOL= stress tolerance, MP= mean productivity, GMP= geometric mean productivity, SSI= stress susceptibility index, STI= stress tolerance index, HM= Harmonic mean

between YP and STI and also YS and STI are in agreement with those reported in durum wheat by Golabadi et al (2006) and in *mungbean* by Fernandez (1992). Ramirez and Kelly (1998) observed positive and significant correlation of some yield components with geometric mean yield (GMP) in common bean. In the current study, the correlation coefficient for stress tolerance (TOL) and grain yield (YS) was  $r=-0.25$  and  $r=-0.11$  in two moisture environments. Selection based on TOL should decrease yield in the moisture stress

environment, and increase grain yield under non-moisture stress (YP), as indicated by  $r=0.94$  and  $r=0.90$  between TOL and YS in the two water stress conditions. SSI does not differentiate between potentially drought-tolerant genotypes and those that possess low overall yield potential. Limitations of using SSI and TOL indices have already been described in wheat (Golabadi et al, 2006; Clarke et al., 1992) and in common bean (Ramirez and Kelly, 1998).



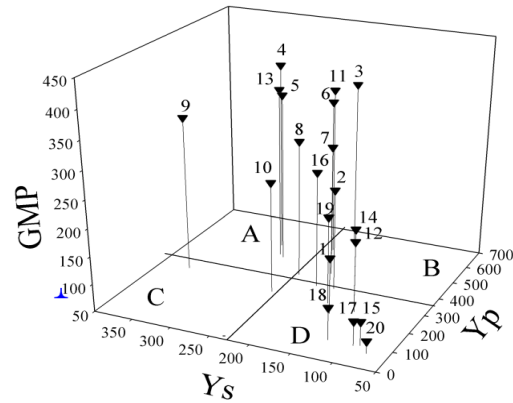
**Fig 1.** Mean comparison of yield under different moisture stress conditions for cultivated and wild safflower genotypes



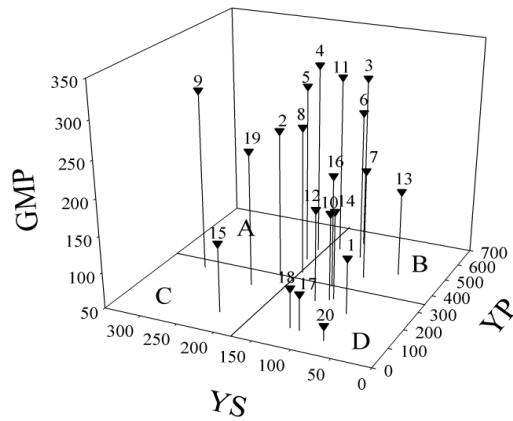
**Fig 2.** Mean comparison for grain yield ( $\text{gr/m}^2$ ) of 20 genotypes (1-13, cultivated and 14-20, wild safflower) under three moisture stress conditions ( $\text{LSD}_{5\%}=89.45 \text{ gr/m}^2$ ).

### Classifying genotypes based on GMP index

3-dimensional graphs based on GMP index and grain yield under stress and non stress condition were prepared (Fig. 3 and 4) for classifying all genotypes of both species according to their performance in stressful and stress free environments. Based on these graphs, cultivated genotypes number 4 and 5 (M113 and M115) were located in group A: genotypes with highest yield in both mild and intense drought stress (Fig. 3 and 4). The yield reduction for these two genotypes under water stress situation was significant but they still had the highest yield production as compared with other genotypes. No wild genotypes, due to low yield production under both stress and non-stress condition, were located in group A. Most were placed in group D. In contrast to cultivated genotypes, the wild genotypes of group D had minimal yield reduction under stress conditions and were considered stable genotypes. Although the wild species have low rates of growth and yield potential, they have developed mechanisms to allow them to tolerate extreme growing conditions in natural environments. Plant breeders can use these potentials to introduce desired traits into the gene pool of cultivated species after a period of pre-breeding activity (Araus et al., 2008). Rizza et al. (2004) used linear regression between relative yield under irrigation and relative yield under rain-fed conditions and observed four main types of genotypic



**Fig 3.** 3-D diagram for specifying the drought tolerance genotypes based on YP, YS and GMP index for mild moisture stress condition



**Fig 4.** 3-D diagram for specifying the drought tolerance genotypes based on YP, YS and GMP index for intense moisture stress condition

response in barley. Fernandez (1992) suggested that genotypes can be classified into four groups base on their performance in water stress and stress free environments.

### Principle component analysis

GMP and STI, separately, are effective indices for selecting drought tolerant genotypes but selection based on a combination of indices may provide a more useful criterion for improving safflower drought resistance. Principle component analysis (PCA) was performed on the basis of all attributes (Table 6) and genotypes were subjected to biplot analysis for assessing the relationships between all of attributes at once and their comparisons in each stress intensity (Fig. 5 and Fig. 6).

A similar pattern in both drought environments was observed. Principal component analysis (PCA) revealed that the first component explained 82% and 70% of the variation in mild and intense stress, respectively, and had higher correlation with YP, YS, MP, GMP, STI and HM. Thus, the first dimension (PC1) can be named as the yield potential and drought tolerance. Considering the high and positive value of this PC on biplot, selected genotypes will be high yielding under stress and non-stress environments.

**Table 5.** Correlation coefficients between YP, YS and drought tolerance indices for mild moisture stress (above diameter) and intense moisture stress (below diameter)

	YP	YS	TOL	MP	GMP	SSI	STI	HM
YP	1	0.69 <sup>***</sup>	0.90 <sup>***</sup>	0.87 <sup>***</sup>	0.94 <sup>***</sup>	0.72 <sup>***</sup>	0.93 <sup>***</sup>	0.91 <sup>***</sup>
YS	0.08 <sup>ns</sup>	1	-0.11 <sup>ns</sup>	0.54 <sup>*</sup>	0.89 <sup>***</sup>	0.11 <sup>ns</sup>	0.87 <sup>***</sup>	0.82 <sup>***</sup>
TOL	0.94 <sup>***</sup>	-0.25 <sup>ns</sup>	1	0.77 <sup>***</sup>	0.71 <sup>***</sup>	0.88 <sup>***</sup>	0.70 <sup>***</sup>	0.64 <sup>**</sup>
MP	0.95 <sup>***</sup>	0.40 <sup>ns</sup>	0.79 <sup>***</sup>	1	0.99 <sup>***</sup>	0.57 <sup>**</sup>	0.98 <sup>***</sup>	0.98 <sup>***</sup>
GMP	0.83 <sup>***</sup>	0.60 <sup>**</sup>	0.60 <sup>**</sup>	0.96 <sup>***</sup>	1	0.52 <sup>*</sup>	0.98 <sup>***</sup>	0.99 <sup>***</sup>
SSI	0.76 <sup>***</sup>	-0.36 <sup>ns</sup>	0.86 <sup>***</sup>	0.59 <sup>**</sup>	0.47 <sup>*</sup>	1	0.37 <sup>ns</sup>	0.37 <sup>ns</sup>
STI	0.81 <sup>***</sup>	0.61 <sup>**</sup>	0.58 <sup>**</sup>	0.94 <sup>***</sup>	0.98 <sup>***</sup>	0.42 <sup>ns</sup>	1	0.98 <sup>***</sup>
HM	0.65 <sup>**</sup>	0.56 <sup>**</sup>	0.37 <sup>ns</sup>	0.84 <sup>***</sup>	0.96 <sup>***</sup>	0.31 <sup>ns</sup>	0.95 <sup>***</sup>	1

YP= grain yield under normal condition, YS= grain yield under drought condition, TOL= stress tolerance, MP= mean productivity, GMP= geometric mean productivity, SSI= stress susceptibility index, STI= stress tolerance index, HM= Harmonic mean

**Table 6.** Principal component loadings for the traits measured on safflower genotypes for mild and intense moisture stress

Traits	Mild moisture stress		Intense moisture stress	
	PC1	PC2	PC1	PC2
YP	0.48	0.14	0.39	-0.22
YS	0.42	-0.11	0.47	0.12
TOL	0.12	0.59	0.13	0.53
MP	0.39	-0.06	0.42	-0.01
GMP	0.39	-0.13	0.41	0.16
SSI	0.15	0.34	0.17	0.57
STI	0.48	-0.15	0.49	0.18
HM	0.38	-0.20	0.35	-0.11
Eigen value	6.52	1.32	5.63	2.11
Cumulative percentage	0.82	0.98	0.70	0.97

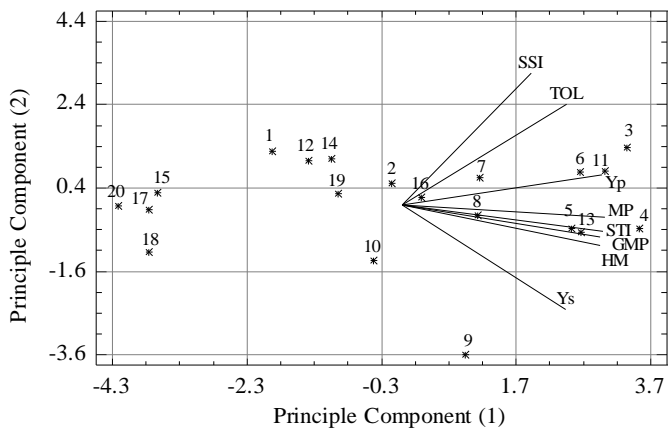
YP= grain yield under normal condition, YS= grain yield under drought condition, TOL= stress tolerance, MP= mean productivity, GMP= geometric mean productivity, SSI= stress susceptibility index, STI= stress tolerance index, HM= Harmonic mean

The second component (PC2) explained 16% and 27% of the total variability in mild and intense stress, respectively and had positive correlation with TOL and SSI. The second component was named as a stress-tolerant dimension which separates stress-tolerant genotypes from non-stress tolerant types. Selection of genotypes that have high PC1 and low PC2 are suitable for both stress and non stress environments. In the present study among cultivated genotypes, number 4, 5, 9, and 13 in mild stress condition and 4, 5, 9 and 11 in intense stress condition had high PC1 and low PC2 and identified as the superior genotypes for stress and non stress conditions. According to biplot analysis (Fig. 5 and 6) wild genotypes number 14, 16 and 19 were located in the center of biplot (moderate value of PC1 and PC2) and were more similar to cultivated genotypes. Other wild genotypes (numbers 15, 17, 18 and 20) had low loads of PC1 and moderate loads of PC2.

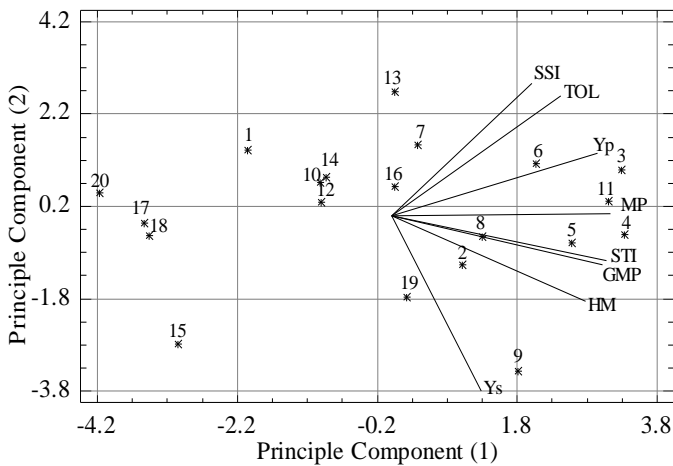
#### Cluster analysis

Cluster analysis provided a better illustration of genetic similarities between accessions based on YP, YS and calculated indices and confirmed the result of PCA (Fig. 7 and 8). Cluster analysis categorized genotypes into three groups for mild stress environment (Fig.7). Group one included 4 wild accessions, all with low yield under non stress condition but high drought tolerance having small TOL and SSI (Table 3). The 3 remained wild accessions with

higher yield were located in group two together with 6 cultivated genotypes. Group three consisted of 7 cultivated accessions with high yield potential (Table 3), high PC1 and low PC2 (Fig.5). For intense water stress condition, cluster analysis also categorized genotypes into three groups (Fig. 7). This was generally consistent with results from the PCA in grouping the accessions. Thomas et al. (1996) observed that accessions of meadow fescue from seven countries that investigated in four experiments could be distinguished based on biplot display. Kaya et al. (2002) reported that genotypes with larger PC1 and lower PC2 scores gave high yields (stable genotypes), and genotypes with lower PC1 and larger PC2 scores had low yields (unstable genotypes). Multivariate analysis was used for distinguishing drought tolerance genotypes in soybean (Yan and Rajcan, 2002) and wheat (Golabadi et al, 2006). Drought stress significantly reduced the yield of cultivated safflower genotypes where drought stress intensity was 0.43 and 0.56 in mild and intense stress conditions, respectively. In these conditions, wild genotypes of *C. oxyacanthus* showed high degrees of drought tolerance indicating they have general stability in water stress environments and may be considered a useful source for drought stress tolerance. GMP, STI and HM indices were similarly able to separate drought sensitive and tolerant genotypes of safflower in both mild and intense water stress environments.



**Fig 5.** The genotype by trait biplots for mild moisture stress. The traits are spelled out in capital letters and genotype are represented by numbers. YP= grain yield under normal condition, YS= grain yield under drought condition, TOL= stress tolerance, MP= mean productivity, GMP= geometric mean productivity, SSi= stress susceptibility index, STI= stress tolerance index, HM= Harmonic mean

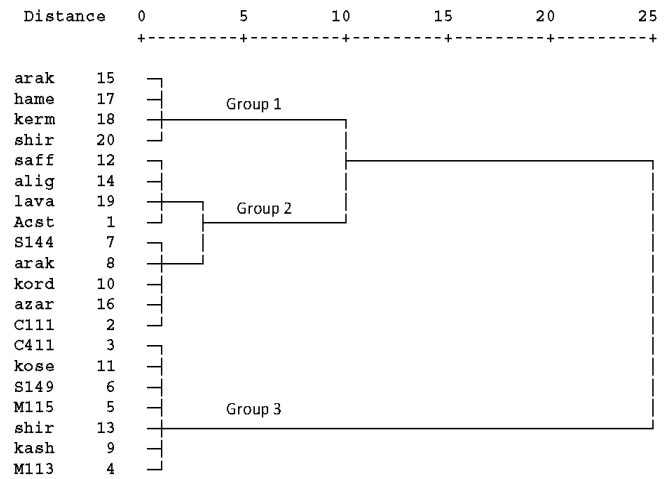


**Fig 6.** The genotype by trait biplots for intense moisture stress. The traits are spelled out in capital letters and genotypes are represented by numbers. YP= grain yield under normal condition, YS= grain yield under drought condition, TOL= stress tolerance, MP= mean productivity, GMP= geometric mean productivity, SSi= stress susceptibility index, STI= stress tolerance index, HM= Harmonic mean

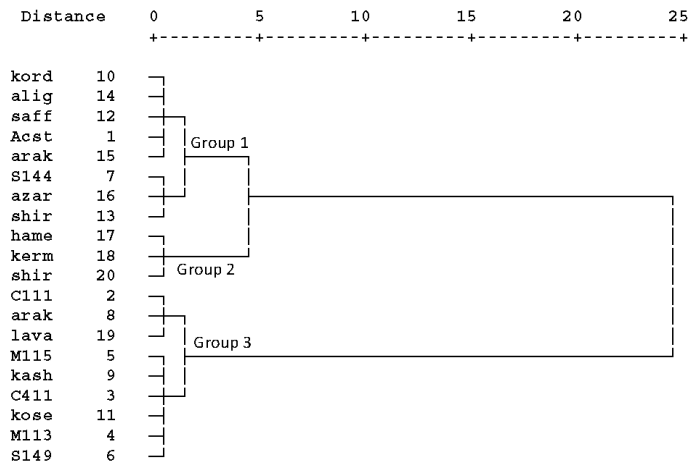
## Materials and methods

### Plant materials

Plant materials consisted of 20 accessions. These included seven populations of *C. oxyacanthus* collected from Western, Central, and Southern regions of Iran and 13 cultivated safflower genotypes: 4 Iranian landraces, 7 Iranian breeding lines, and 2 Canadian cultivars (Saffire and AC-Stirling (Table 1).



**Fig 7.** Cluster analysis of safflower genotypes on the basis of GMP, MP, HM and STI indices for mild stress



**Fig 8.** Cluster analysis of safflower genotypes on the basis of GMP, MP, HM and STI indices for intense stress

## Experimental site

The experiment was conducted, during 2008, at the Isfahan University of Technology Research Farm, located at Lavark, Iran (40 km southwest of Isfahan, 32° 32' N and 51° 23' E, 1630m asl). Mean annual precipitation was 140 mm and mean annual temperature was 15° C. Each plot consisted of five rows: 40 cm apart and 3 m in length. The experiment was conducted on a Typic Haplargid soil with clay loam texture, pH 7.5, and 1% organic matter content. Fertilizers were applied at 100 kg N/ha and 100 kg P/ha prior to sowing and at 75 kg N/ha, top dressed at shooting stage.

## Treatments

Accessions were evaluated using randomized complete block design with three replications under normal, mild and intense drought stress field conditions. The time of each irrigation was based on the evaporation from class A pan: 80, 120 and 180 mm of evaporation were considered as normal (no stress), mild stress and intense stress. Irrigation depth was calculated based on the average of soil moisture gravimetric percent in the rooting zone (maximum to 50 cm) using Eq. 1 (Walker and Skogerboe, 1987):

$$I = [(FC - \theta) / 100] DB_d \quad [\text{Eq. 1}]$$

Where I is irrigation depth in cm, FC is soil gravimetric moisture percent at field capacity,  $\theta$  is soil gravimetric moisture percent at irrigating time, and  $B_d$  is soil bulk density at root zone in  $\text{gr cm}^{-3}$ .

## Selection indices

Five selection indices: stress susceptibility index (SSI, Fischer and Maurer, 1978), stress tolerance index (STI, Fernandez, 1992), tolerance (TOL, Rosielle and Hamblin, 1981), mean productivity (MP, Rosielle and Hamblin, 1981), geometric mean productivity (GMP, Fernandez, 1992) and harmonic mean (HM) were calculated based on grain yield under mild and intense drought-stressed conditions according to the following formulas:

### 1. Stress susceptibility index (SSI)

$$SSI = (1 - (Y_{si} / Y_{pi})) / SI \quad \text{where } SI = 1 - (Y_{ms} / Y_{mp})$$

### 2. Stress tolerance index (STI)

$$STI = [(Y_p) \times (Y_s) / (Y_{mp})^2]$$

### 3. Tolerance index (TOL)

$$TOL = Y_{pi} - Y_{si}$$

### 4. Geometric mean productivity (GMP)

$$GMP = (Y_{pi} \times Y_{si})^{0.5}$$

### 5. Mean productivity (MP)

$$MP = (Y_{pi} + Y_{si}) / 2$$

### 6. Harmonic mean productivity (HM)

$$HM = 2(Y_{pi} \times Y_{si}) / (Y_{pi} + Y_{si})$$

Where  $Y_{si}$  is the yield of each genotype in the stress condition,  $Y_{pi}$  is the yield of each genotype in normal condition,  $Y_{ms}$  is the yield mean over all genotypes in stress condition, and  $Y_{mp}$  is the yield mean over all genotypes in normal condition.

## Statistical analysis

An analysis of variances for calculated indices was performed for each of two levels of drought stress using an SAS statistical program. The CORR SAS procedure was used to estimate correlations among traits. For specifying the drought tolerant genotypes with high yielding potential in both normal and stress environments, a 3-D diagram based on  $Y_P$ ,  $Y_S$  and the best drought tolerance indices were drawn (using Sigma plot ver11). Principal component analysis was performed using SAS, and the biplot was drawn using Stat

Graphics software. Cluster analysis of the genotypes was conducted for each level of drought conditions using Ward method (hierarchical cluster analysis, SPSS 10 for windows).

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## References

- Araus JL, Slafer GA, Conxita R, Serret MD (2008) Breeding for yield potential and stress adaptation in cereals. *Crit Rev Plant Sci* 27: 377–412
- Ashri A, Knowles PF (1960) Cytogenetics of safflower (*Carthamus L.*) species and their hybrids. *Agron J* 52: 11–17
- Azizi Chakherchaman SH, Mostafaei H, Imanparast L, Eivazian MR (2009) Evaluation of drought tolerance in lentil advanced genotypes in Ardabil region, Iran. *J Food Agric Environ* 7: 283–288
- Baum M, Grando S, Backes G, Jahoor A, Sabbagh A, Ceccarelli S (2003) QTLs for agronomic traits in the Mediterranean environment identified in recombinant inbred lines of the cross 'Arta' x H-spontaneum 41-1. *Theor Appl Genet* 107: 1215–1225
- Clarke, JM, De-Pauw RM, Townley-Smith TM (1992) Evaluation of methods for quantification of drought tolerance in wheat. *Crop Sci* 32: 728–732
- Dittrich M, Petrak F, Rechinger KH, Wagenitz G (1979) *Compositae III –Cynareae*. In: Rechinger, KH (ed), *Flora Iranica* No. 139, p 468
- Fernandez GCJ, (1992) Effective selection criteria for assessing stress tolerance. In: Kuo CG (ed), *Proceedings of the international symposium on adaptation of vegetables and other food Crops in temperature and water stress publication*, Tainan, Taiwan, 1992
- Fischer RA, and Maurer R (1978) Drought resistance in spring wheat cultivars. Part 1: grain yield response. *Aust J Agric Res.* 29: 897–912
- Golabadi M, Arzani A, Mirmohammadi Maibody SAM (2006) Assessment of drought tolerance in segregating populations in durum wheat. *Afr J Agr Res* 1: 162-171
- Guttieri MJ, Stark JC, Brien K, Souza E (2001) Relative sensitivity of spring wheat grain yield and quality parameters to moisture deficit. *Crop Sci* 41: 327-335
- Karkanis A, Bilalis D, Efthimiadou A (2011). Architectural plasticity, photosynthesis and growth responses of velvetleaf (*Abutilon theophrasti* Medicus) plants to water stress in a semi-arid environment. *Aust J Crop Sci* 5(4): 369-374
- Kaya Y, Palta C, Taner S (2002) Additive main effects and multiplicative interactions analysis of yield performances in bread wheat genotypes across environments. *Turk J Agric For.* 26: 275- 279
- Kirigwi, FM, Van Ginkel M, Trethowan R, Seaes RG, Rajaram S, Paulsen GM, (2004) Evaluation of selection strategies for wheat adaptation across water regimes. *Euphytica* 135: 361-371
- Knowles PF, Ashri A (1995) Safflower: *Carthamus tinctorius* (Compositae). In: Smartt J, Simmonds NW (eds), *Evolution of Crop Plants*, Longman, UK, pp 47–50



- Li D, Mundel HH (1996) Safflower: *Carthamus tinctorius* L. promoting the conservation and use of underutilized and neglected crops 7. Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany/International Plant Genetic Resources Institute (IPGRI), Rome, Italy
- McWilliam JR (1989). The dimensions of drought. In: Baker FWG (eds) Drought Resistance in Cereals, CAB International, Wallingford, Oxon, UK. pp 1–11.
- Mitra J (2001). Genetics and genetic improvement of drought resistance in crop plants. *Curr Sci* 80: 758-762
- Mundel HH, Bergman JW (2009) Safflower. In: Vollmann J, Rajcan I (eds) Oil crop, Hand book of plant breeding. Springer, PP 423-448.
- Pourdad SS (2008) Study of drought resistance indices in spring safflower. *Acta Agronomica Hungarica*, 56: 203–212
- Ramirez P, Kelly JD (1998). Traits related to drought resistance in common bean. *Euphytica* 99: 127-136.
- Reynolds M, Dreccer F, Trethowan R (2007) Drought-adaptive traits derived from wheat wild relatives and landraces. *J Exp Bot* 58: 177–186.
- Rizza F, Badeck FW, Cattivelli L, Lidestri O, Fonzo ND, Stanca AM (2004). Use of a water stress index to identify barley genotypes adapted to rainfed and irrigated conditions. *Crop Sci* 44: 2127-2137
- Rosielle AA, Hamblin J (1981) Theoretical aspects of selection for yield in stress and non-stress environment, *Crop Sci* 21: 943–946
- Sabzalian MR, Saeidi G, Mirlohi A (2008) Oil content and fatty acid composition in seeds of three safflower specie. *J Am Oil Chem Soc* 85: 717–721
- Sabzalian MR, Mirlohi A, Saeidi G, Rabbani MT (2009) Genetic variation among populations of wild safflower, *Carthamus oxyacanthus* using agro-morphological traits and ISSR markers. *Genet Resour Crop Evol* 54: 415–420
- Sabzalian MR, Saeidi G, Mirlohi A, Hatami B (2010) Wild safflower species (*Carthamus oxyacanthus*): a possible source of resistance to the safflower fly (*acanthiophilus helianthi*). *Crop Protection* 29: 550–555
- Talame V, Sanguineti MC, Chiapparino E, Bahri H, Ben Salem M, Forster BP, Ellis RP, Rhouma S, Zoumarou W, Waugh R, Tuberosa R (2004) Identification of *Hordeum spontaneum* QTL alleles improving field performance of barley grown under rainfed conditions. *Ann Appl Biol* 144: 309–319
- Thomas H, Dalton SJ, Evans C, Chorlton KH, Thomas ID (1996). Evaluating drought resistance in germplasm of meadow fescue. *Euphytica* 92: 401-411.
- Walker WR, Skogerboe GV (1987) Surface irrigation: Theory and practice. Prentice-Hall, Englewood Cliffs.
- Weiss EA (2000) Safflower. In: Oilseed crops. Blackwell, pp 93–129