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ARTICLE

Leaf anatomical characteristics in safflower genotypes as affected by drought stress

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ABSTRACT Safflower (*Carthamus tinctorius* L.) is a drought-tolerant species that grows in arid and semi-arid environments exposing to long periods of water deficit stress. Structure and functions of the plant organs including leaf anatomy are affected by drought stress. The objective of this study was to evaluate the effects of drought stress on some leaf anatomical traits including leaf thickness, upper and lower epidermal thickness, xylem width, metaxylem diameter, and vascular bundle width and their relationship with grain yield using 20 safflower genotypes under field conditions. A randomized complete block design was used in each of the non-stress and drought stress field conditions. Analysis of variance showed that drought stress significantly reduced all variables measured except vascular bundle width. The results also revealed the positive and significant correlations between grain yield with leaf thickness (r = 0.53**) and xylem width (r = 0.51**) under drought stress conditions. Due to this fact, the leaves thickness and xylem width could be considered key structural features of leaves that manage the ability of a safflower genotype to tolerate water deficit stress. Therefore these traits could be used as criteria to select tolerant genotype that were more tolerant to drought.
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KEY WORDS

anatomy
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Drought is a major abiotic stress limiting growth and production of crop plants and its impact will be increasing due to the global climate change. Plant strategies to cope with drought normally involve stress avoidance and tolerance mechanisms that employed with a variety of physiological and botanical features. Therefore, a range of traits has been suggested that could be used as selection criteria to indirectly select for improving yield under stress conditions. A better understanding of the effects of drought on plants is vital to improve crop management practices, to guide breeding efforts in agriculture, and to predict the fate of natural vegetation under periods of climate change (Chaves et al. 2003).

Safflower (*Carthamus tinctorius* L.) is an important oilseed crop grown in arid and semi-arid regions of the world due to its drought tolerance (Weiss 2000). In general drought stress causes decrease grain yield.

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 deficit stress conditions for efficient evaluation of large populations (Ramirez and Kelly 1998).

The leaf is the most adaptable organ in its response to environmental conditions (Kumarparida et al. 2004; Marchi et al. 2008), also leaf structures represent more effects than stems and/or roots (Ennajeh et al. 2010). Plants respond to water deficit using mechanisms of avoidance by improved leaf traits and by reducing water loss through reduced epidermal (stomatal and cuticular) conductance, reduced radiation absorption and reduced evaporative surface (Price et al. 2002). Although some literature is available on the effects of drought stress on anatomical traits in a limited number of trees, including olive (Bosabalidis and Kofidis 2002; Ennajeh et al. 2010), and *Acacia auriculiformis* (Liu et al. 2004), there is no such literature in the field crops. It is acknowledged that there are differences in the way annual field crops and perennial tress respond to drought stress, anatomically. Therefore, further studies into deeper understanding of anatomical characteristics related to drought tolerance in annual field crops are necessary. The objective of the present study is to assess the effects of drought stress on several anatomical leaf characteristics in order to reveal the origin of the variability in drought-tolerance using 20 safflower cultivars differing in drought tolerance.

Materials and Methods

Twenty safflower genotypes comprising with both native and exotic origins were used in this study. Plant materials were grown in two separate experiments under stress and non-stress irrigation regimes 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 soil at this

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Table 1. Combined analysis of variance for grain yield and anatomical characteristics in safflower genotypes grown under two environments (drought and non-stress field conditions).

Source of variation	df	Mean square						
		Upper epidermal (μm)	Lower epidermal (μm)	Xylem width (μm)	Metaxylem diameter (μm)	Vascular bundle width (μm)	Leaf thickness (μm)	Grain yield (gr per plant)
Environment (E)	1	348.6**	140.4**	29992.5**	2194.5**	9526.61^{ns}	657002.8**	1741.8**
Block (E)	2	1.11^{ns}	2.42^{ns}	462.8^{ns}	10.11^{ns}	1034.3*	1789.9^{ns}	4.26^{ns}
Genotype (G)	19	3.87**	4.17**	448.85*	16.57**	605.2*	2356.8**	18.69**
G×E	19	3.11*	2.62^{ns}	183.2^{ns}	11.85**	239.2ns	1345.7*	6.07^{ns}
Residual	38	1.55	1.84	212.7	4	305.2	703.2	3.96
CV (%)		17.43	23.42	20.71	17.69	15.88	12.13	14.93

ns: non-significant; * significant at P < 0.05 and ** significant at P < 0.01.

site is silty clay loam, typic Haplargids of the arid tropic with pH = 7.3-7.8, electrical conductivity (EC_e) = 1.2-1.4 dS m^{-1} and 0.9% organic matter and mean annual precipitations and mean annual

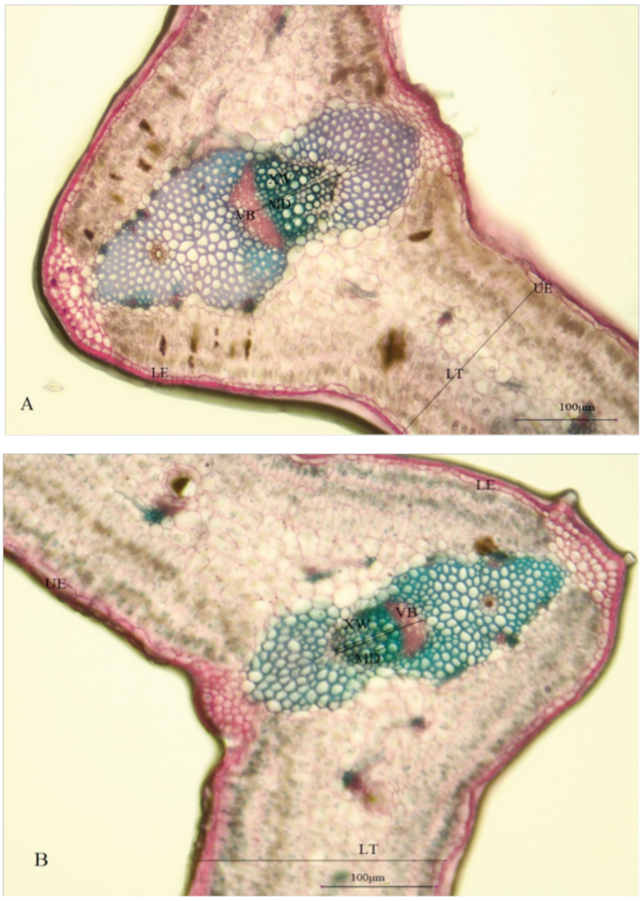

Figure 1. Cross-sections of the leaves of safflower plant. A. non-stress (control), B. drought stress, UE: Upper epidermal, LE: Lower epidermal, LT: Leaf thickness, XW: Xylem width, MD: Metaxylem diameter, VB: Vascular bundle width. Scale bar 100 μm.

Each plot consisted of three 4m long rows spaced 30 cm apart. Plants were grown under full irrigation until branching growth stage when drought stress was initiated. Soil samples were taken from a soil depth of 0-60 cm from the plots and soil water content was determined. The soil-moisture regimes were established by irrigating after 50% (non-stress) and 80% (drought stress) soil moisture depletions of soil available water (SAW) from the root zone. SAW was calculated as difference between field capacity (FC) and permanent wilting point (WP) and the product multiplied by root depth. The volumetric soil water contents (%) were equal to 0.03 MPa for FC and 1.5 MPa for WP. The amount of irrigation was calculated on the basis of 60-cm rooting depth.

Leaf anatomy was studied using three fresh leaves in each plot. Leaf samples were fixed in glycerin alcohol solution (30% glycerin and 70% alcohol). One square centimeter (1 cm^2) segments were taken from the centre of leaves after the samples were bleached in sodium hypochlorite (10%) for 15-20 minutes and thorough washing in water. Then a double staining procedure was performed using methyl green and carmine alum and ultimately was observed under light microscope (Nikon Eclipss v. E600). In this study some anatomical traits include leaf thickness, xylem width, upper and lower epidermal thickness, metaxylem diameter and vascular banding width (Fig. 1) were assessed in this study.

Statistical analysis

The data were examined for homogeneity and normality of residuals using Kolmogorov-Smirnov and Bartlett's tests, respectively. Afterward the data from two experimental conditions subjected by combined analysis of variance (ANOVA) using GLM procedure of SAS statistical program. The differences among genotype means were compared using the Fisher's least-significant difference (LSD) test.

Results and Discussion

Results of combined ANOVA indicated a significant influence of drought stress on the studied anatomical leaf characteristics

Table 2. Means of anatomical traits in 20 safflower genotypes grown under non-stress (control) and drought field conditions.

Genotype	Origin	Leaf thickness (μm)		Upper epidermal (μm)		Lower epidermal (μm)	
		control	drought	control	drought	control	drought
C444	Iran	331a-d	174a	9.5b-e	5a-d	7bc	5ab
C111	Iran	2435g	117bc	11ab	6.5ab	8b	7.5a
C4110	Iran	256.5gf	147ab	8c-f	6abc	65bc	5ab
LRV-55-295	Iran	355.5a	133ab	8.5b-e	3.5d	7bc	4.5b
56-58/41	Iran	305.5a-f	140.5ab	9.5b-e	5.5a-d	7.5bc	4.5b
N/27	Iran	270efg	128.5ab	10a-d	5a-d	5.5bc	4.5b
IL	Iran	353.5ab	130ab	10.5abc	5.5a-d	7bc	3.5b
Kordestan1	Iran	248.5g	135ab	9.5b-e	3.5d	6.5bc	3b
Kordestan2	Iran	295.5c-g	118b	10.5abc	5.5a-d	8b	4.5b
Kordestan5	Iran	282d-g	162ab	7.5def	7a	7bc	5.5ab
Kermanshah47	Iran	351abc	151ab	9.5b-e	6abc	7.5bc	4b
Kermanshah60	Iran	325a-e	132ab	5.5f	4.5bcd	5c	4.5b
Darab4	Iran	275efg	62c	7ef	4.5bcd	6.5bc	3.5b
Esfahan4	Iran	334a-d	137ab	10a-d	6abc	6bc	4.5b
301055	Turkey	306a-f	133ab	9b-e	4.5bcd	7.5bc	4b
Dincer	Turkey	270.5efg	134.5ab	9b-e	4cd	12.5a	4.5b
Syrian	Syria	308.5a-f	135ab	8c-f	4cd	8b	5.5ab
Kino-76	Mexico	298b-g	159.5ab	9b-e	6bcd	8b	4.5b
PI-258417	Portugal	356a	147.5ab	12.5a	5a-d	6bc	4b
Gila	USA	288.5d-g	109bc	11ab	4cd	5.5bc	3b
LSD 0.05		55.56	55.43	2.77	2.44	2.71	2.47
LSD 0.01		75.95	75.77	3.79	3.33	3.7	4.06

Different letters in the same column show significant differences at $P \leq 0.01$.

(Table 1). The results also showed that safflower genotypes varied significantly for all the characteristics under both moisture regimes (Table 1).

Means of grain yield per plant, leaf thickness, upper and lower epidermal thickness, xylem width and metaxylem diameter (Table 2 and 3, Fig. 1) reduced significantly under drought stress conditions. The high sensitivity of leaf expansion to water deficit stress together with its potential influence on yield suggest that the response of leaf thickness to water status might provide an effective criterion of drought resistance for using in plant breeding programs. The results of the present study are in agreement with those of Liu et al. (2004), who reported the reduction in leaf and mesophyll thickness of *Acacia auriculiformis* under water deficit condition. Under drought stress leaf thickness had significant positive relationship ($r = 0.53^{**}$) with grain yield.

In this investigation the means of upper and lower epidermal thickness (Table 2) reduced under drought stress conditions. The small size and the integral walls of the epidermal cells contribute to the significant resistance against cell collapsing due to arid conditions. Small epidermal cells have been found to be more resistant to collapse than large ones (Bosabalidis and Kofidis 2002).

Xylem width and metaxylem diameter decreased in drought stress conditions due to decline in vascular bundles width (Table 3) that is showing an adaptive mechanism to water loss. The xylem is not only the main transport pathway of water, nutrients and hormonal signals from the roots to the transpiring organs , but also the modifications of the vessel size have an important role in the adaptation to undesirable environmental conditions (Comstock and Sperry 2000). Therefore, a genotype possessing higher xylem width and metaxylem diameter is benefit to the breeding programs aiming at drought tolerance improvement. The results also showed a positive and significant relationship between xylem width with grain yield ($r = 0.51^{**}$) in drought stress conditions. Xylem vulnerability to cavitation was reported to be positively associated with drought tolerance (Marchi et al. 2008; Zimmermann and Milburn 1982).

Mean comparisons of the safflower genotypes for the anatomical traits indicated the superiority of Kermanshah 47 under normal and Kordestan 5 under drought stress conditions. These genotypes also had the greatest seed yield under the respected conditions. These results suggest that the effects of drought stress upon leaf cell development lead to enhanced ability to avoid drought stress-induced by decrease xylem width and metaxylem diameter at the expense of reduced water transport capacity and efficiency. On the other hand this ability leads to lowering of yield loss due to the drought stress.

Water restriction is one of the abiotic stresses that have a seriously impacts on growing crops. Structure and functions

Table 3. Means of anatomical traits and seed yield in 20 safflower genotypes grown under non-stress (control) and drought field conditions.

Genotype	Xylem width (µm)		Metaxylem diameter (µm)		Vascular bundle width (µm)		Grain yield (g per plant)	
	control	drought	control	drought	control	drought	control	drought
C444	95abc	55a-d	18b-e	12a	132a-d	110abc	16.09def	5.73e
C111	81bcd	56.5a-d	13f	7.5a-d	104cde	106abc	17.17c-f	6.11e
C4110	78cd	64abc	16def	6.5a-d	106cde	114ab	17.74c-f	6.24e
LRV-55-295	80a-d	53a-d	15ef	3.5cde	119a-e	102abc	18.81cd	9.01a-e
56-58/41	88a-d	46.5a-d	13f	4.5b-e	120a-e	96abc	18.44cde	7.36cde
N/27	99abc	62abc	20abc	6.5b-e	132a-d	100abc	16.17def	7.02abc
IL	96abc	54a-d	18b-e	6b-e	134abc	98abc	23.01ab	11.51abc
Kordestan1	103ab	52a-d	14ef	2.5e	133abc	73c	18.11cde	11.67abc
Kordestan2	79cd	27.5cd	15ef	9ab	100de	77bc	16.31c-f	9.16a-e
Kordestan5	106a	59a-d	17b-e	8abc	119de	105abc	19.18bcd	12.56a
Kermanshah47	107a	65ab	17cde	3de	112b-e	90bc	26.08a	11.92ab
Kermanshah60	87a-d	33.5bcd	16c-f	4.5b-e	119b-e	83.5bc	14.28f	10.01a-e
Darab4	67d	43.5a-d	9g	6.5b-e	98e	91.5abc	18.43cde	7.05d-e
Esfahan4	94abc	76a	19a-d	7.5a-e	152a	131a	16.55c-f	10.01a-e
301055	104a	66.5ab	17cde	4cde	143ab	115ab	18.85bcd	8.39a-e
Dincer	97abc	45a-d	21ab	5.5b-e	132a-d	100abc	17.83c-f	6.34de
Syrian	69.5d	40a-d	15def	4.5b-e	102cde	90bc	20.23bc	10.59a-d
Kino-76	84a-d	47a-d	16c-f	5bcd	118b-e	93abc	14.76ef	8.17b-e
PI-258417	79cd	51.5a-d	17cde	8abc	103cde	101abc	16.14def	8.60a-e
Gila	93abc	23cd	22a	7a-e	135abc	103abc	15.92def	5.87e
LSD 0.05	22.81	36.64	3.76	4.57	33.36	39.51	2.57	4.34
LSD 0.01	31.19	50.08	5.14	6.25	45.6	54.01	5.95	5.93

Different letters in the same column show significant differences at $P \leq 0.01$.

of all plant organs are affected by water deficit stress and modification in leaf anatomy have been also greatly reported (Bosabalidis and Kofidis 2002; Ennajeh et al. 2010; Pereyra et al. 2012).

Superior performance of a native accession of safflower (Kordestan 5) under drought stress conditions for the anatomical traits including leaf thickness, xylem width and metaxylem diameter and their association with seed yield revealed that these anatomical traits play a major role in drought tolerance in safflower. However, more studies with more cultivars are needed to confirm these results. Such studies should also encompass the root system, in order to obtain a more complete picture of the drought resistance strategies of safflower genotypes.

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References

Bosabalidis AM, Kofidis G (2002) Comparative effects of drought stress on leaf anatomy of two olive cultivars. Plant Sci 163:375-379.

Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought: from genes to the whole plant. Func Plant Biol 30:239-264.

Comstock JP, Sperry JS (2000) Theoretical considerations of optimal conduit length for water transport in vascular plants. New Phytol 148:195-218.

Ennajeh M, Vadel AM, Cochard H, Khemira H (2010) Comparative impacts of water stress on the leaf anatomy of a drought-resistant and a drought-sensitive olive cultivar. J Hort Sci Biotech 85:289-294.

Kumarparida A, Das AB, Mittra B (2004) Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora*. Trees 18:167-174.

Liu LX, Xu SM, Woo KC (2004) Deficit irrigation effects on photosynthesis and the xanthophylls cycle in the tropical tree species *Acacia auriculiformis* in north Australia. New Zealand J Bot 42:949-957.

Marchi S, Tognetti R, Minnocci A, Borghi M, Sebastiani L (2008) Variation in mesophyll anatomy and photosynthetic capacity during leaf development in a deciduous mesophyte fruit tree (*Prunus persica*) and an evergreen sclerophyllous Mediterranean shrub (*Olea europaea* L.). Trees 22:559-571.

Pereyra MA, García P, Colabelli MN, Barassi CA, Creus CM (2012) A better water status in wheat seedlings induced by *Azospirillum* under osmotic stress is related to morphological changes in xylem vessels of the coleoptiles. Appl Soil Ecol 53:94-97.

Price AH, Cairns JE, Horton P, Jones HG, Griffiths H (2002) Linking drought-resistance mechanisms to drought avoidance in upland rice using a QTL approach: progress and new opportunities to integrate stomatal and mesophyll responses. J Exp Bot 53:989-1004.

Ramirez P, Kelly JD (1998) Traits related to drought resistance in common bean. Euphytica 99:127-136.

Weiss EA (2000) Safflower. Oilseed Crops. Blackwell Science, Oxford, 93-129.

Zimmermann MH, Milburn JA (1982) Transport and storage of water. In Pirson A, Zimmermann MH, eds., Encyclopedia of Plant Physiology. Springer Verlag, New York.