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Morphological and physiological responses of two common bean cultivars to drought stress

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Abstract

Drought stress is the major problem in arid and semi-arid regions of the world. Investigating the response of different cultivars of crop plants to drought stress can help to select the tolerant cultivars ones for agriculture in arid lands. In this study, a greenhouse experiment was conducted as a factorial experiment based on completely randomized design with two common bean cultivars (*Phaseolus vulgaris* L.) including Derakhshan and Goli, and four moisture levels of 25, 50, 75 and 100% field capacity. When the true leaves emerged (10 days after planting), the seedlings were exposed to the different levels of drought stress until the end of the flowering stage. Based on the morphological (stem length, leaf and root area, dry weight of shoot and root, and the root/shoot ratio), physiological (leaf relative water content, membrane stability index, photosystem II photochemical efficiency, stomatal resistance, chlorophyll index, and water use efficiency), and biochemical (proline content, soluble protein content, and the activity of antioxidant enzymes including superoxide dismutase, polyphenol oxidase, and peroxidase) characteristics, both cultivars showed a relatively good tolerance to the low and intermediate drought stress, while both were sensitive to the highest level of drought condition (25% field capacity). The main characteristics which indicated the tolerance of common bean cultivars to drought stress was an increase in the root/shoot ratio, stomatal resistance, water use efficiency, proline content, and antioxidant enzyme activity. Regarding these characteristics, the responses of Derakhshan cultivar more associated with drought tolerance, compared with Goli cultivar.

Keywords: Antioxidant enzymes; Drought stress; Stress physiology; Water deficit; Water use efficiency

Introduction

One of the major problems in arid and semi-arid regions of the world is drought stress. The response of the various plants species and cultivars to drought stress is different (Zabet *et al.,* 2003). Drought stress affects plant growth and development through changes in plant structure, and changes in various physiological and biochemical processes (Jaleel *et al.,* 2009). Losing the turgor pressure is the first effect of drought conditions that influences cell expansion rate and its final size, resulting in a reduction of growth rate (Kumar and Purohit, 2001). Since water and mineral nutrients are taken up by the root, its growth and development may be affected by drought. (Abd Allah *et al.,* 2010). In soil water deficit conditions, guard cells lose their turgidity resulted in stomatal closure. Then, the rate of CO_2 diffusion through the stomata is limited and the photosynthetic rate declines (Sikuku *et al.,* 2010). Drought stress affects photosystem efficiency and decreases the electron transport rate and the effective quantum yield of photosystem II (PSII) photochemistry (Ahmed *et al.,* 2002; Zlatev and Yordanov, 2004).

Common bean (*Phaseolus vulgaris* L.) is one of the most important crops in the world, mainly cultured in arid and semi-arid regions. About 60% of the bean-cultivated lands around the world are under drought stress (Assefa *et al.,* 2015). Many common bean cultivars have been introduced after selection and/or breeding based on their productivity and tolerance to biotic and abiotic stresses such as drought stress (Ghanbari *et al.,* 2013). Some previous studies have investigated the effects of drought stress on different properties of a variety of cultivars of common bean plant. For instance, Cristina Lanna *et al.* (2016) have studied physiological responses of two Brazilian common bean genotypes to drought stress. Their results showed that the main physiological indicators of tolerance of common bean plants to water deficit were the robustness of the root system and osmotic adjustment. In another study, the evaluation of drought stress adaptation of common bean genotypes in Ethiopia showed that for all the investigated traits including plant height, chlorophyll content, yield data, and drought intensity, susceptibility, and tolerance indices, the different genotypic responses to drought stress were observed (Darkwa *et al.* 2016). Castaneda Saucedo et al. (2012) studied the alteration of some carbohydrates such as sucrose, glucose, fructose and starch in the leaves, pods and seeds of *P. vulgaris* under drought stress. They reported an increase in the concentrations of glucose and fructose and a decrease in starch and sucrose in mature leaves, under stress condition. In

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reproductive tissues, the sucrose and hexoses were increased and the starch was reduced.

In a study on two Iranian cultivars of common bean (Talash and Daneshkadeh), it was found that in terms of drought resistance in different growth and development stages including vegetative, flowering and pod filling stages, the tolerance and stress susceptibility indices there was a significant differences between two cultivars (Rafiiolhossaini *et al.,* 2016). Ghanbari et al. (2013) investigated the leaf responses of eight common bean cultivars to water deficit stress. Their results showed that water deficit induced a decrease in the relative water content, leaf wet weight, leaf dry weight, leaf area index, and plant leaf numbers. In another study investigating the effect of irrigation intervals on some physiological and morphological characteristics of red bean it was found that delay in irrigation decreased leaf relative water content, chlorophyll content, plant height, number of lateral branches and seed yield and increased electrolyte leakage, leaf prolin and soluble sugar content (Saeidi Aboueshaghi *et al.*, 2014).

Understanding drought tolerance and its mechanisms is inevitable for selection of the efficient genotypes for cultivating in drought-affected lands and applying a better management of agriculture in arid lands. Thus, this study was performed to evaluate and compare the tolerance of two common bean cultivars (mainly cultured in Iran) to different levels of drought stress, and to investigate their defense responses from different aspects of morphological, physiological and biochemical traits.

Materials and Method

Plant Cultivation and Experimental Design: A greenhouse experiment was conducted based on completely randomized design with three replicates in Plant Physiology Laboratory, Ferdowsi University of Mashhad, during May and June 2012. The experimental design was a 4*2 factorial (water stress vs bean cultivar) with two cultivars of common bean (Derakhshan and Goli) and four moisture levels of 100 (control), 75, 50 and 25% of field capacity [FC]). Each experimental unit formed a pot with 2 kg soil mixture composed of sand and farmyard manure in a ratio of 1: 3. This mixture was selected to prevent soil clogging and compression, supporting optimum plant growth. After emerging true leaves, three plants from the plants cultivated in each pot that were more robust were allowed to grow and others were removed. Before applying treatments, the pots were daily irrigated regarding field capacity. When the true leaves was emerged (10 days after planting), the seedlings were exposed to the different levels of drought stress until the end of the flowering stage, during which the pots were weighed daily to control the accuracy of the treatments and the time for irrigating, regarding field capacity for control pots. Pots were kept in a growth chamber under controlled conditions (at 25/14 ± 3 °C day/night, with light intensity about 600 µmol m^{-2} s^{-1}). At the end of the flowering stage (60 days after planting), plants were harvested.

Morphological trait Measurements: The roots with attached soil were carefully separated from the pots and placed on the immersed sieve, along with gently washing with tap water. For measuring the root surface area, they were stained with Magnesium permanganate for 5 minute. Surface-stained roots were scanned by a scanner connected to a computer followed by analyzing using ROOT EDGE software (Eshghizadeh et al. 2011). Leaf area was determined by the leaf area measurement device (ADC UK, Light Box model). For determining dry weight of shoots and roots, the samples were oven-dried for 48 hours at 72°C.

Physiological Measurements: Physiological measurements were carried out on one plant from three plants of each pot. Relative water content (RWC) of leaves was determined before harvesting (Bian and Jiang, 2009). Turgid weight was measured after soaking the leaf for 16 to 18 h in distilled water. After soaking, the leaves were quickly and carefully blotted dry with tissue paper prior to determination of leaf weight in turgid condition. Leaf dry weight was determined after drying the leaf sample for 72 h at 70°C. RWC was calculated from the following equation:

RWC = [(fresh weight – dry weight)/(turgid weight –dry weight)] × 100

To determine membrane stability index (MSI), leaf samples (0.1 g) of each plant were placed in the two series of tube containing 10 ml of distilled water and membrane stability index was obtained in the following equation (Shanahan *et al.,* 1990).

MSI = [1 - (C1/C2)] *100

C1 and C2: Electrical conductivity of water at 40°C and 100°C, respectively.

Photosystem photochemical efficiency (Fv/Fm) was measured using young leaves before harvesting by a portable chlorophyll fluorometer (OS5-FL modulated chlorophyll fluorometer) according to Maxwell and Johnson 2000. Stomatal resistance and chlorophyll index were measured by AP4 POROMETRE and chlorophyll meter (OPTI-SCIENCES CCM-200 model), respectively. Water use efficiency (WUE= CO_2 assimilation rate / transpiration rate) was calculated (Piper *et al.,* 2007) after measuring CO_2 Assimilation and transpiration rate from non-detached young and absolutely expanded leaves using a portable infrared gas analyzer (IRGA, LCA4, ADC Bio. Scientific Ltd., Herfordshire, UK).

Biochemical Measurements: Leaf proline was extracted and measured by the method of Bates *et al.*, (1973). A standard curve was prepared to calculate proline as micromole per gram fresh weight. Leaf soluble protein was measured according to the modified Lowry (1951) procedure. Protein concentration was calculated as mg per gram fresh weight after preparing a standard curve using bovine serum albumin.

Peroxidase (POX) activity was determined by the method of Holly (1972) after measuring the change in

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absorbance at 530 nm. Enzyme activity was calculated as changes per minute per mg of protein. Superoxide Dismutase (SOD) activity was defined with respect to its ability to inhibit the photochemical reduction of nitrobluetetrazolium (NBT) according to Giannopolitis and Ries (1997) method. Polyphenol Oxidase (PPO) activity was done according to Raymond *et al.,* (1993). The absorption changes at 430 nm per minute (Unit) was measured and enzyme activity was expressed as enzyme unit per mg protein.

Statistical Analysis: The data were analyzed by Mstat-C software and the significance of differences between means was checked with Duncan's Multiple Range Test ($P<0.05$) after a one-way analysis of variance (ANOVA).

Results

Morphological changes: Analysis of variance showed that drought stress significantly affected all morphological traits of common bean. The difference between the two cultivars was significant for these traits, except for shoot dry weight. For all morphological traits, the interaction of stress and cultivar were also significant (table 1). Mean's comparisons revealed that in both cultivars, leaf area (Fig. 1a), shoot dry weight (Fig. 1b), stem length (Fig. 1c), root area (Fig. 1d), and root dry weight (Fig. 1e) were significantly decreased under drought treatments compared to the control. In the highest level of drought stress (25% FC) the leaf area of Derakhshan and Goli was decreased by 49 % and 63 %, respectively. A severe decrease in shoot dry weight (65 and 63 % for Derakhshan and Goli, respectively) was observed under 25% FC. The decrease in the root area of Derakhshan was a drought level-depended response. The decrease in stem length under 25% FC was 55 and 48 % for Goli and Derakhshan, respectively. The root/shoot ratio of Goli was generally more than that of Derakhshan. However, the root/shoot ratio was significantly increased in both cultivars under drought stress in the drought level of 25 and 50% FC, compared to the control (Fig. 1f).

Physiological responses: Based on the analysis of variance, all physiological traits (including RWC, MSI, Fv/Fm, stomatal resistance, chlorophyll index, and WUE) were significantly affected by drought stress. Two cultivars showed a significant differences in term of physiological traits, except for chlorophyll index. ANOVA was also showed that the interaction between the drought and cultivar was significant for these traits, except for RWC and MSI (table 2).

Results of mean's comparisons showed that Goli had generally more RWC than that of Derakhshan. However, in both cultivars, RWC was affected by drought stress with a similar pattern, where it was significantly decreased under 50 and 25 % FC, compared to the control (Fig. 2a). In both cultivars, MSI was decreased in all drought treatments as compared to the control, except for Goli under 75% (Fig. 2b). However, there were significant differences between the two cultivars in all moisture levels, where MSI of Derakhshan was higher than that of Goli. Drought stress significantly decreased the Fv/Fm ratio relative to the control in both cultivars in a similar pattern (Fig. 2c). Although Fv/Fm ratio in control plants of Goli was more than that of Derakhshan, however, under the highest level of drought stress (25% FC), the result was inverse with a significant difference.

Stomatal resistance was significantly increased by drought stress of 50 and 25% FC in both cultivars compared to the control (Fig. 2d). Although under drought level of 25% FC (but not other levels) the stomatal resistance in Goli was significantly higher than that of Derakhshan, its increase relative to the control in later cultivar was higher (3.9 fold) than that of earlier one (3.8 fold). In both cultivars, Chlorophyll Index was significantly increased under 50 and 25% FC compared to the control (Fig. 2e). Under highest level of drought stress (25% FC), Derakhshan had significantly higher chlorophyll index than Goli cultivar at 25% FC so that it was increased by 63% in the first cultivar versus 42% in the second one. However, in other drought levels, the difference between the two cultivars was not significant. While WUE of Goli was significantly increased only under 25% FC of drought stress, it was significantly increased in all drought levels in Derakhshan, compared to the control. There was also a significant difference in WUE between the two cultivars under 25, 50, and 75% FC, where Derakhshan had higher WUE (Fig. 2f). While under 25% FC the WUE of Goli was increased by 42%, it was increased by 80% in that of Derakhshan.

Biochemical responses: Analysis of variance showed that drought stress significantly affected all biochemical traits (including proline content, soluble proteins, POX activity, SOD activity, and PPO activity) of common bean. The difference between the two cultivars was significant for these traits, except for soluble proteins. While the interaction between stress and cultivar was significant for proline content, POX activity, and PPO activity, it was not significant for soluble proteins, and SOD activity (table 3).

Results of mean's comparisons showed that in both cultivars the proline content was significantly increased under all drought levels compared to the control, except for Derakhshan cultivar under 75% FC (Fig. 3a). Under 25% FC, proline content was increased about 2-fold in both cultivars compared with controls (by 96 and 97% in Derakhshan and Goli, respectively). Mean's comparisons revealed that in both cultivars, only leaf soluble proteins were significantly affected by the most severe level of the drought stress (25% FC), where it was increased by 31 and 50% compared to the control in Goli and Derakhshan, respectively (Fig. 3b).

Results showed that with increasing drought stress, POX activity significantly increased in both cultivars compared to the control. In all drought levels, POX activity in Derakhshan was higher than that of Goli (Fig. 3c). The highest increase in POX activity was observed

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Table 1. Analysis of variance for some growth characteristics of Common bean cultivars under drought stress.

Source of variation	Degree of freedom	Mean of squares					
		Leaf area	Shoot dry weight	Stem length	Root area	Root dry weight	Root/Shoot
Drought	3	246861344.5**	0.642**	779.025**	310809072.3**	0.116636**	0.045390**
Cultivar	1	158736843.5**	0.001ns	7238.427**	64907425.7**	0.025350**	0.021420**
Drought×Cultivar	3	24353401.5**	0.012*	222.437**	18374403.1**	0.003029**	0.003067**
Error	16	1689586.566	0.003	3.197	1922683.9	0.0000165	0.000220

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

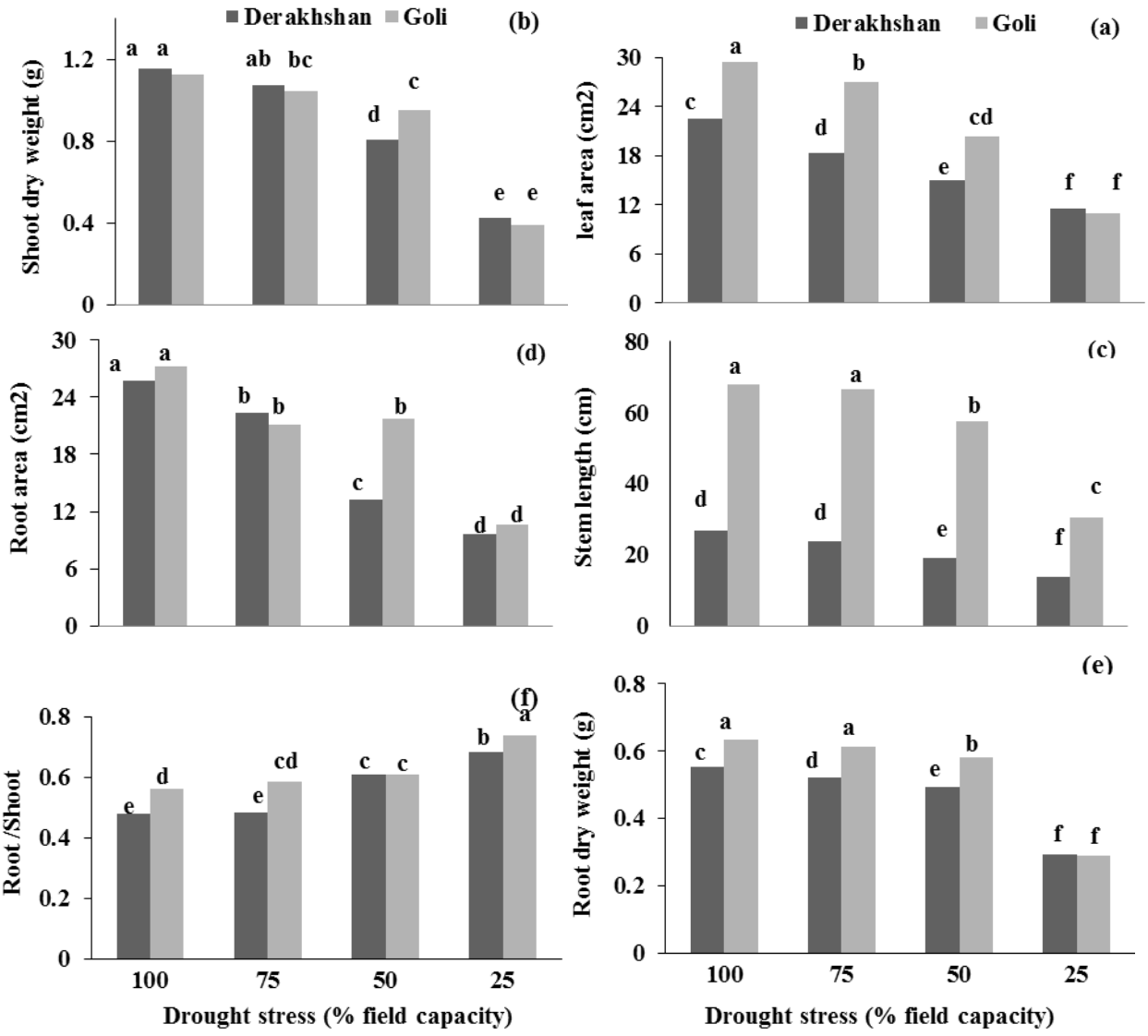

Fig. 1 Effects of drought stress on some growth characteristics of Common bean cultivars. a, Leaf area; b, Shoot dry weight; c, stem length; d, Root area; e, Root dry weight; f, Root/Shoot. Similar letters indicate that there is no significant difference among the means based on Duncan test ($p \leq 0.05$)

under 25% FC, where it was increased by 48% and 68% compared to the control in Goli and Derakhshan, respectively. Drought stress significantly affected the activity of SOD. Results indicated that under 50 and 25% FC, SOD activity was significantly decreased in both cultivars. However, there was no significant difference between the two cultivars in any level of drought condition (Fig. 3d). PPO activity was increased under drought stress conditions. In both cultivars, all drought levels increased PPO activity, except for Goli under 75% FC. In all treatment levels, a significant difference was observed between two cultivars, where

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Table 2. Analysis of variance for some physiological characteristics of Common bean cultivars under drought stress. RWC, Relative water content; MSI, Membrane stability index; Fv/Fm, PSII photochemical efficiency; WUE, water use efficiency.

Source of variation	Mean of squares						
	Degree of freedom	RWC	MSI	Fv/Fm	Stomatal resistance	Chlorophyll index	WUE
Drought	3	113.064**	193**	0.004657**	169.437**	43.998**	29.497**
Cultivar	1	136.804**	384**	0.000408**	13.202**	3.227ns	10.8**
Drought×Cultivar	3	0.184 ns	5 ns	0.000375**	1.545*	8.290**	2.524**
Error	16	1.876	2.625	0.000035	0.450	0.920	0.368

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

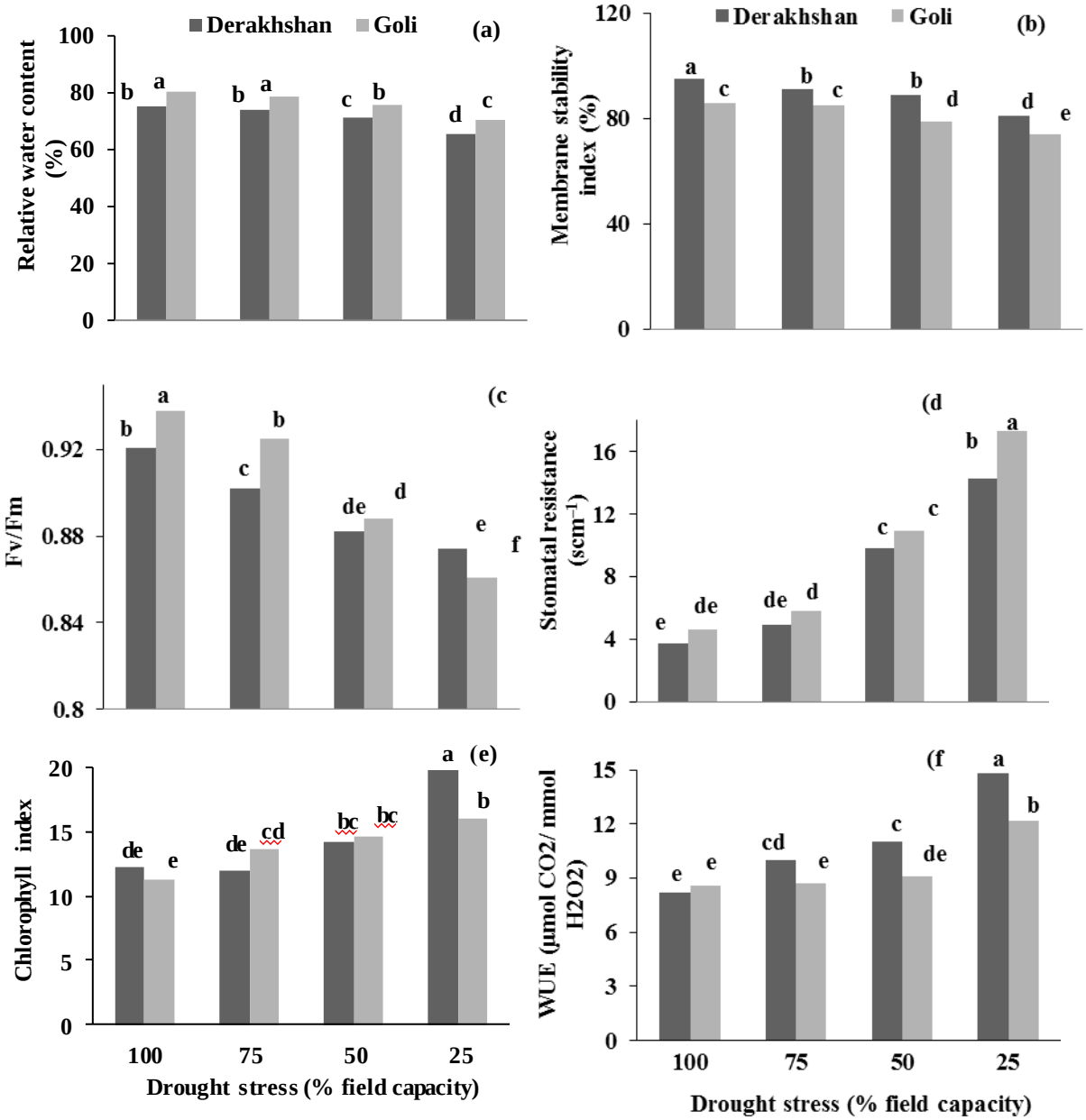

Fig. 2 Effects of drought stress on some physiological characteristics of Common bean cultivars. a, Relative water content; b, Membrane stability index; c, PSII photochemical efficiency; d, Stomatal resistance; e, Chlorophyll index; f, water use efficiency; Similar letters indicate that there is no significant difference among the means based on Duncan test ($p \leq 0.05$)

Derakhshan had higher PPO activity than another cultivar (Fig. 3e).

Discussion

Although both cultivars showed a relatively good tolerance to the low and intermediate drought stress, both were sensitive to the highest level of drought condition (Fig. 1-3 and tables 1-3). However, most growth-, physiological-, and biochemical-related results indicated that Derakhshan cultivar was relatively more

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Table 3. Analysis of variance for some biochemical characteristics of Common bean cultivars under drought stress; POX, peroxidase; SOD, Superoxide dismutase; PPO, polyphenol oxidase.

Source of variation	Mean of squares					
	Degree of freedom	Proline content	Soluble proteins	POX activity	SOD activity	PPO activity
Drought	3	0.581**	4.245**	172.379**	16039.622**	4.6**
Cultivar	1	0.634**	0.000 ns	121.050**	123.760*	16.335**
Drought×Cultivar	3	0.052**	0.450ns	14.043**	1.483ns	0.855**
Error	16	0.007	0.161	1.838	17.083	0.0120

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

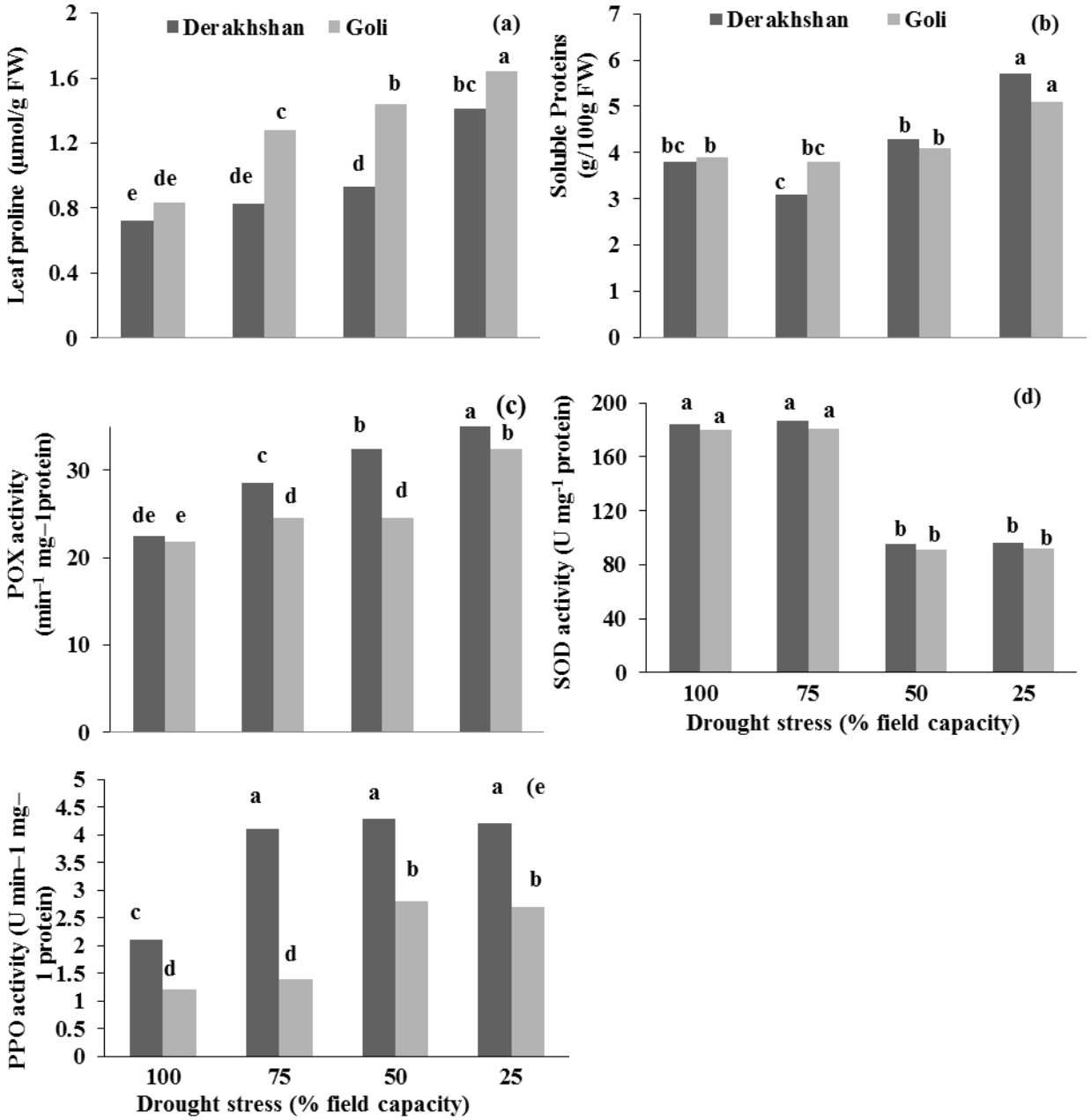

Fig. 3 Effects of drought stress on some biochemical characteristics of Common bean cultivars. a, Leaf Proline content; b, Leaf soluble proteins; c, peroxidase activity; d, Superoxide dismutase activity; e, polyphenol oxidase activity. Similar letters indicate that there is no significant difference among the means based on Duncan test ($p \leq 0.05$)

tolerant to the drought stress than Goli.

Drought stress is the main factor limiting growth and development in plants (Sikuku *et al.*, 2010). Reduction in shoot dry biomass under drought stress is the result of decreasing photosynthesis, increasing growth inhibitors and decreasing hormones (auxin and cytokinin) (Hayat and Ahmad, 2007). Furthermore, the reduction in shoot dry weight can be attributed to the reduced leaves

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number and/or leaf area (Sikuku *et al.,* 2010), that later was observed in this study.

Kusaka *et al.,* (2005) showed that the use of available water by root systems and osmotic adjustment could maintain plant cell turgor pressure for plant survival under drought stress. Drought tolerance can be increased by increasing root growth. Thus, under water deficit conditions, by a reduction in shoot growth coupled with continuing root growth and so the increase in the root/shoot ratio and the plant water status can be improved (Sikuku *et al.,* 2010). It seems that this is the main mechanism contributed to drought tolerance in Goli cultivar, while Derakhshan use this and other mechanisms such as increase in WUE to tolerate drought conditions.

Decreased MSI under drought stress has also been reported in other studies (Turkan *et al.,* 2005; Salehpour *et al.,* 2009). Increased ROS compounds under stress can induce lipid peroxidation, fatty acids oxidation in membrane, and thus decreased membrane resistance, led to the change in membrane permeability and increased electrolytes leakage (Eraslan *et al.,* 2007).

Several studies have been reported that drought stress can induce damages to the PSII oxygen-evolving complex and the PSII reaction centers, including the degradation of D1 protein, and the inhibition of the flow of electrons through PSII (Zlatev and Yordanov, 2004). Rahbarian *et al.,* (2011) showed that drought stress decreased Fv/Fm ratio in chickpea genotypes. The decrease in Fv/Fm ratio under drought stress may be due to Calvin cycle disturbances, delaying reduction of quinones, and damaging to thylakoid membrane electron transport chain (Terzi *et al.,* 2010; Zlatev and Yordanov, 2004).

RWC reflects water uptake by the roots and water loss by transpiration. Under water deficit condition RWC can be reduced (Anjum *et al.,* 2011) resulting in stomatal closure to prevent further water loss by transpiration. Simultaneously, the entry of CO_2 into the leaf could be limited resulting in a decrease in CO_2 assimilation. Photosynthesis can eventually be stopped by the decrease in intracellular CO_2 concentration (Reddy *et al.,* 2003; Kiani *et al.,* 2008). Irreversible damage to the photosystems, particularly to PSII, limits photosynthesis under drought stress. The damage to PSII is thought to be due to the light-induced oxidative stress rather than direct damage (Pastenes *et al.,* 2005).

In this work, drought stress induced accumulation of the proline content and soluble proteins in leaves of both cultivars. The accumulation of the compatible solutes such as proline in response to drought can lower the osmotic potential of the cell, resulting in water absorption into the cell to maintain its turgor and reduce the harmful effects of water deficit. (Yang and Miao, 2010; Najaphy *et al.,* 2010). Soluble proteins including antioxidant enzymes have an important role in plant protection under drought stress (Li *et al*., 2010).

Another result of the present study that indicated more tolerance of Derakhshan cultivar to the drought stress compared to Goli, was its more increased activity of antioxidative enzymes (POX and PPO) under drought stress that help to increase the defense capacity against oxidative damage (Anjum *et al.,* 2011). POX, as antioxidant enzyme, protects cells from the damaging influence of H_2O_2 and derived oxygen species (Pandey *et al.,* 2010). PPO has a role in drought resistance due to its involving in the Mehler reaction. Under water deficit conditions, when CO_2 assimilation reduces, any excess absorbed light energy can induce the formation of ROS. Mehler reaction can prevent over-reduction of components of linear electron transport (Thipyapong *et al.,* 2004).

In the present study, a marked decrease in SOD activity under drought stress was observed that can be attributed to the either decreased synthesis or increased degradation of the enzyme (Pandey *et al.,* 2010). There are similar reports about antioxidant enzyme activities under drought stress. For example, Krishnamurthy *et al.* (2000) reported that drought stress decreased catalase and SOD activity in black pepper plants. However, stress increased POX and PPO activity.

Conclusion

Under severe drought stress, a decrease in growth, RWC, MSI, and Fv/Fm of common bean cultivars was observed. However, the cultivars showed a relatively good tolerance to the low and intermediate drought stress. The main characteristics which indicated the tolerance of common bean cultivars to drought stress was an increase in the root/shoot ratio, stomatal resistance, WUE, proline content, and antioxidant enzyme activity. Based on these characteristics, the response of Derakhshan cultivar of common bean was more associated with drought tolerance, compared with Goli cultivar.

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