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RESEARCH ARTICLE

The Role of Uniconazole in Improving Physiological and Biochemical Attributes of Bean (*Phaseolus vulgaris* L.) Subjected to Drought Stress

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Abstract

Adverse environmental conditions such as drought are among the major factors limiting the growth and productivity of land plants. Plant growth regulators are defined as naturally occurring or synthetic compounds that affect the development or metabolism of higher plants, mostly in abiotic stress conditions. Two varieties of common bean, Talash (Sensitive) and Dehghan (Tolerant) were used to examine the influences of uniconazole on the photosynthetic traits, antioxidant activities, and morphological parameters of bean leaves and root under drought stress conditions. Results showed that drought stress considerably depressed the plant growth. However, the drought-stressed plants treated with uniconazole showed significantly higher biomass than in plants without uniconazole. Uniconazole treatments on Dehghan showed superior results to those on Talash. The leaves of uniconazole plants exhibited an increased leaf greenness content, photosynthetic rate, transpiration rate, and stomatal conductance but lower lipid peroxidation content and relative electrical conductivity compared to those of drought-stressed plants. Soluble sugar, soluble protein content, and the activities of superoxide dismutase, peroxidase, and catalase in the leaves were increased by uniconazole in drought stress and under well-watered conditions. Finally, the findings indicated that uniconazole can effectively alleviate the adverse effects caused by drought stress; these unwanted changes are partially attributable to the modifications in morphology and physiological characteristics resulting in yield increase in drought bean.

Key words : Bean, drought stress, biochemical traits, uniconazole

Introduction

Common bean (*Phaseolus vulgaris* L.) is cultivated worldwide as a valuable oil seed and protein-rich crop. However, its nutritional value and plant performance are influenced by environmental stresses. Drought is considered to be a major limiting factor of abiotic stress that affects the growth and development of plants (Farooq et al. 2009). Moreover, this abiotic stress impairs numerous metabolic, photosynthetic, physiological structure, and crop productivity in plants. Effective improvement of crop drought resistance has become a concern in agricultural production and research (Passioura 2007). Responses to drought are multidimensional and interconnected. Drought stress induces several morphological and physiological reactions and exhibit drought resistance mechanisms (Farooq et al. 2009; Jaleel et al. 2009). Drought-tolerance mechanisms are involved in producing lengthened roots to maintain root growth (Hossain et al. 2014), inhibiting chloroplast activity, and stomatal closing and most importantly, decreasing the production of reactive oxygen species (ROS) and requiring elevated levels of antioxidants for stress compensation (Chakhchar et al. 2016). More importantly, crop damages due to unseasonal and severe water events amount to billions of dollars in yield losses (Laurentius and Julia 2015). Plant growth regulators are defined as naturally occurring or synthetic compounds that affect the development or metabolism of higher plants, mostly at low dosages (Rademacher 2015). Uniconazole, a triazole compound, is a well-known plant growth retardant that operates by inhibiting gibberellin biosynthesis and reducing stem elongation in higher plants (Izumi et al. 1985). Uniconazole offers potential

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advantages and serves various functions, such as decreasing plant height in sea marigold (Carver et al. 2014), increasing root length, and root volume in soybean (Wan et al. 2013) and increasing root dry weight, chlorophyll content, and net photosynthetic rate in plantlets and duckweed (Liu et al. 2015). Uniconazole has also been used to induce environmental stress tolerance to abiotic stress in certain plants. Uniconazole spray instigated drought tolerance in apple seedlings (Todoroki et al. 2009), improved rapeseed seedling growth in relation to physiological changes under waterlogging stress (Qiu et al. 2005), and induced thermotolerance by enhancing antioxidant activity and consequently reducing stress-related oxidative damage to cell membranes (Upadhyaya et al. 1990). Uniconazole also increased the contents of proline and soluble sugars and the activities of superoxide dismutase (SOD) and peroxidase (POX) but reduced the malondialdehyde (MDA) content or electrical conductivity of soybean leaves under water stress conditions (Zhang et al. 2007).

Although the alleviation of environmental stress by uniconazole has received attention, information on the capability of uniconazole to protect bean plants from drought stress. Therefore, experiments were conducted to determine the effects of uniconazole spray on bean under drought stress conditions. The main goals are to understand (1) photosynthesis rate (P_n), transpiration rate (T_r), stomatal conductance (G_s), and chlorophyll content; (2) soluble sugar and protein contents; (3) activities of antioxidant systems, such as SOD, POX, and CAT which may be valuable in scavenging free radicals that damage membranes during drought stress; (4) relative electrical conductivity and MDA content; and (5) morphological changes in the root and shoot.

Materials and Methods

Planting and uniconazole sprays

Two experiments (2016-2017 and 2017-2018) were carried out in a glasshouse at the Faculty of Agriculture, Tarbiat Modares University, Iran in order to investigate the effect of drought stress and uniconazole on some morphological and physiological traits of two varieties of common bean (*Phaseolus vulgaris* L.). The seeds of bean (Talash and Dehghan termed as drought sensitive and drought tolerant) which were provided by Seed and Plant Certification and Registration Institute, Karaj, Iran) were surface sterilized by 10% chlorox solution within 1 min, washed thoroughly, and then imbibed in distilled water. Seeds were planted in pots (30 × 30 cm) containing a mixture of sandy loam soil with 0.8% organic matter content (11 kg) and then grown in a glasshouse, under condition of 14 h light/10h dark with 300 µmol $m^{-2}s^{-1}$ irradiance, 25±2°C/15±2°C day/night temperature and 60±5% relative humidity. Fifteen seeds were planted in each pot and at the first trifoliate leaf stage (V1) the number of plants were reduced to seven seedlings per pot. The water content of pots was maintained at 80% of the soil field capacity (FC) by manual watering until the water treatments were initiated.

The experimental design was a randomized as a complete block arrangement in 2×4 factorial with three replications. Six weeks after sowing (at the V6 stage), drought stress treatment was initiated (under conditions as indicated above) and half of the pots were objected to drought stress until full maturity by withholding water supply until the soil water potential reduced to 65% FC. After that, the plants were irrigated. Irrigation water needed prior to irrigation was estimated base on the soil water content (θ_i) usingby TDR method (Time Domain Reflectometry, model 4593, soil moisture equipment, Santa Barbara), and effective rooting depth (D, 0.3 m here) according to the following equation (1) (Cuenca1989):

$$V_w:(\theta_{F.C}-\theta_i)\times D\times A \quad (1)$$

Where, $\theta_{F.C}$ is the volume of soil moisture at FC and A is the pot area (m). Data on volumetric water content were collected daily prior to set the experiment to calibrates before to seed sowing and during the growing season to calculated the time of irrigation. The amount of water irrigation applied was according to the soil deficit to bring it back to FC. The remaining plants were irrigated with tap water. Throughout the growth period, each pot received 50 ml of B&D nutrient solution. After two weeks growth, all plants were sprayed with 50 $mg.L^{-1}$ uniconazole (0.8 mM). The treatments included: 1) unsprayed plants exposed to well-watered conditions (control); 2) uniconazole sprayed plants exposed to well-watered conditions; 3) unsprayed plants exposed to drought stress conditions; and 4) uniconazole-sprayed plants exposed to drought stress conditions. Seven days after foliar application, greenness content, photosynthesis rate, transpiration rate, and stomatal conductance in the third leaf from the top were measured. Greenness content was measured using a portable chlorophyll meter (Minolta SPAD-502, Japan) following the method of Turner and Jund (1991).

Gas exchange parameters (The photosynthesis rate, transpiration rate, and stomatal conductance) were measured on fully developed leaves with an open gas-exchange system Li-6400 XT (Li-Cor, Lincoln, NE, USA) equipped. The measurements were recorded between 11 and 12 am when the photo synthetically active radiation above the canopy was 300 $\mu mol.m^{-2}.s^{-1}$. Air temperature and air relative humidity were maintained at 25°C and 40-45%, respectively. Light intensity (PPFD) and CO_2 inside the chamber were 1000 µmol m^{-2} s^{-1} (with 10% blue light to maximize stomatal aperture) and 370 µmol mol^{-1}, respectively. A total of four leaves from each treatment were adopted to measure these traits.

At the end of the experiment, entire experimental plots were hand-harvested at seed maturity for morphological traits, yield, and yield components determination. For this purpose, plants were uprooted and separated in the roots and shoots. The roots were gently washed with water to remove soil. Afterwards, they were dried at 105°C in a hot air oven for 72 h to calculated dry weight. Number of pods was recorded on an entire experimental plot basis and this was used to determine the number of pods per plant. Number of

seeds was counted for 10 plants. Seed yield (g/plant) was recorded on an entire experimental plot basis with subsamples oven-dried at 80°C to a constant weight to allow correction of plot data to absolute dry weight. All the measurements were performed 80 days after sowing.

Biochemical measurements

A total of three leaves (third leaf from top) from each treatment were sampled between 9:30 and 10:30 am, seven days after uniconazole spray. *Relative* electrical conductivity was measured by using an EC215 conductivity meter (HANNA Instruments, Portugal) following the method in Leul and Zhou (1999). Soluble sugars were evaluated using anthrone method described by Fales (1951). For the physiological study, samples were then washed, frozen in liquid nitrogen, and then stored at -80°C pending biochemical analysis. Measurements were taken for MDA content, activities of antioxidant enzymes (SOD, POX, and CAT) as well as protein and sugar solution content in leaves.

Enzyme activity assay

Tissue samples (0.2 g) were homogenized in a mortar and pestle with 3 ml ice-cold extraction buffer (50 mM potassium phosphate, pH 7). The homogenate was centrifuged at 18,000 g for 30 min at 4°C and then the supernatant was filtered through filter paper. The supernatant fraction was used as a crude extract for assays of enzyme activity and protein content. All operations were carried out at 4°C. Enzyme activities were measured at 25 °C using a spectrophotometer model Varian Cary Win UV 6000i, Australia.

Superoxide dismutase (EC 1.15.1.1) activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) according to the method cited by Giannopolitis and Ries (1977). The reaction mixture contained 200 µl 1 µM riboflavin, 200 µl 12 mM L methionine, 200 µl 50 mM Na2CO3 (pH 10.2), and 200 µl 75 µM NBT in 2 ml 50 mM potassium phosphate buffer (pH 7), with 200 µl crude enzyme extract in a final volume of 3 ml (with some modification). Glass test tubes containing the mixture were illuminated with a fluorescent lamp (120 W); identical tubes that were not illuminated served as blanks. After illumination for 15 min, absorbance was measured at 560 nm. A blank was run in the same way but without the enzyme. One unit of SOD was defined as the amount of enzyme activity that was able to inhibit photo reduction of NBT by 50% to purple formazan. The SOD activity of the extract was expressed as SOD units per milligram of protein.

Catalase (EC 1.11.1.6) activity was estimated by the method cited by Cakmak and Horst (1991). The reaction mixture contained 100 µl crude enzyme extract, 500 µl 10 mM H2O2, and 1.9 ml 50 mM potassium phosphate buffer. The decrease in absorbance at 240 nm was recorded for 1 min. Catalase activity of the extract was expressed as CAT units per milligram of protein.

Peroxidase (EC 1.11.1.7) activity was determined by the oxidation of guaiacol in the presence of H2O2. The increase in absorbance at 470 nm was recorded for 1 min (Ghanati et al. 2002). The reaction mixture contained 100 µl crude enzyme, 500 µl 5 mM H2O2, 500 µl guaiacol 28 mM, and 1.9 ml 50 mM potassium phosphate buffer (pH 7). Peroxidase activity of the extract was expressed as POX units per milligram protein. Total protein content was estimated by the method cited by Bradford (1976), using bovine serum albumin as the standard.

Malondialdehyde assay

The level of lipid peroxidation was analyzed in terms of malondialdehyde (MDA) contents reacting to thiobarbituric acid (TBA) reactive substance using the method cited by Heath and Packer (1969). Samples were homogenized in an aqueous solution of TBA (10% w/v) and then a 1 ml aliquot of an appropriately diluted sample was added to a test tube with an equal volume of thiobarbituric acid (TCA) solution containing 25% (w/v) TCA then mixtures were heated at boiling water (95°C) for 25 min. The amount of MDA was determined from the absorbance of the supernatant at 532 and 600 nm. MDA content was determined using the extinction coefficient of MDA (ε=155 $\mu M^{-1}cm^{-1}$).

Statistical Analysis

The present data are the means value of two independent experiments. The pooled data were analyzed using SAS (SAS release 9.0, 2002) because the results followed a similar trend and the variances were homogenous. All data were subjected to two-way analysis of variance (ANOVA). When an F-test indicated statistical significance at $P \leq 0.01$ or $P \leq 0.05$, the protected least significant difference (protected LSD) was used to separate the means of significant interaction effects were separated by slicing method. Experiments were repeated twice and the pooled data were analyzed using SAS because the results followed a similar trend and the variances were homogenous.

Results

Effect of uniconazole on photosynthetic characteristics under drought stress

Table 1 shows the changes in the leaf greenness contents, photosynthetic rate, and stomatal conductance of bean. Under well-watered conditions, the leaf greenness contents of uniconazole-treated plants were 4.4% higher in Talash and 8.8% higher in Dehghan compared with those of untreated plants (Table 1). Drought stress significantly decreased the leaf chlorophyll contents in Talash and Dehghan. However, the leaf greenness contents of the uniconazole-treated drought-stressed plants also significantly increased compared with those of the non-treated stressed plants by 7.3 and 6.9%, respectively, in Talash and Dehghan. Drought stress significantly decreased the leaf photosynthetic rates of Talash and Dehghan.

Table 1. Mean comparisons of treatment combinations of varieties, drought stress, and uniconazole application the leaf greenness, Pn, Gs, Tr, SSC and SPC for common bean.

Treatment		Traits					
Variety treatment		Leaf Greenness(SPAD)	*Pn* (μmol $m^{-2}s^{-1}$)	*Gs* (mmol $m^{-2}s^{-1}$)	*Tr* (mmol $m^{-2}s^{-1}$)	SSC ($mg.g^{-1}FW$)	SPC ($mg.g^{-1}FW$)
Talash	Control	38.5±0.6b	12.7±0.7b	182.4±8.1b	7.5±0.5a	51.2±0.1b	12.8±0.3b
	Uniconazole	40.3±0.7a	16.1±0.5a	201.6±6.6a	7.1±1.3a	55.7±0.2a	15.1±0.2a
	Drought Stress	35.1±0.3c	7.9±0.3d	128.7±3.4d	1.8±0.9c	41.4±0.3d	10.5±0.1d
	Drought+Uniconazole	37.9±0.4b	11.3±0.2c	175.4±5.1c	3.1±0.8b	45.8±0.1c	11.5±0.1c
Dehghan	Control	38.9±0.4b	14.9±1.3b	225.6±2.7b	7.8±0.4b	51.2±0.4b	13.1±1.1b
	Uniconazole	41.8±0.1a	17.1±1.5a	281.4±25.5a	9.3±0.2a	63.1±0.2a	14.2±0.1a
	Drought Stress	34.9±0.2b	10.1±0.9d	132.4±5.3d	6.1±0.3d	42.2±0.2d	11.1±0.3d
	Drought+Uniconazole	37.5±0.3b	11.8±1.4c	185.1±7.2c	6.8±0.7c	44.3±0.9c	12.7±0.1c

Control: spraying of water on well-watered plants; Uniconazole, foliar spraying of uniconazole under well-watered plants; Drought Stress, foliar spraying of water under drought stress; foliar spraying of uniconazole under drought stress. *Pn*: photosynthesis rate; *Gs*: stomatal conductance; *Tr*: transpiration rate; SSC: sugar solution content; SPC: solution protein content. Vertical bars represent ± SEM. Means with the same letter are not significantly different from each other ($P \leq 0.05$ ANOVA followed by LSD test).

However, the photosynthetic rate of the uniconazole-treated drought-stressed plants was higher than that of the non-treated stressed plants. Under well-watered conditions, the photosynthetic rate of uniconazole-treated plants was 21.11% higher in Talash and 12.8% higher in Dehghan compared with those of untreated plants (Table 1). The stomatal conductance of uniconazole-treated Talash and Dehghan were significantly higher than those of the control plants in the absence (Table 1) and presence of water stress.

Effect of uniconazole on the transpiration rate

Table 1 reveals the changes in leaf transpiration rate in bean. Drought stress significantly decreased the leaf transpiration rate in Talash and Dehghan. However, the transpiration rate of the uniconazole-treated, drought-stressed plants were higher than that of the non-treated stressed plants (Table 1). Under drought conditions, the photosynthetic rate of uniconazole-treated plants was 41.9% higher in Talash and 10.0% higher in Dehghan varieties compared with those of untreated plants (Table 1).

Effect of uniconazole on the soluble sugar and soluble protein content under drought stress

The contents of soluble sugar and soluble protein decreased under drought stress in the leaves of Talash and Dehghan (Table 1). Under well-watered conditions, the soluble sugar contents in the uniconazole-treated plants of Talash and Dehghan were 8.0 and 18.8% higher, respectively, than those of the untreated plants (Table 1). Meanwhile, the soluble protein contents of uniconazole-treated plants under well-watered conditions were 15.2 and 7.7% higher, respectively, in Talash and Dehghan than that in the untreated plants (Table 1). When plants were exposed to drought stress, the contents of soluble sugar and soluble protein in Talash and Dehghan treated with uniconazole exceeded those of the untreated plants (Table 1). Moreover, under well-watered and drought-stressed conditions, the leaf soluble sugar content in Dehghan was higher than that in Talash; this result further clarified the superior resistance to drought stress of Dehghan compared with Talash (Table 1).

Effect of uniconazole on SOD, POX, and CAT activities under drought stress

Under well-watered conditions, uniconazole significantly increased the activities of SOD and POX by 3.7 and 2.7%, respectively, in Talash and 61.0 and 18.6%, respectively, in Dehghan (Table 2). Drought stress markedly decreased the SOD and POX in the bean cultivars than in the unsprayed controls. However, uniconazole significantly raised the SOD, POX, and CAT activities under drought stress conditions (Table 2). Moreover, when the plants were exposed to drought stress, the SOD activities of the drought-stressed plants were increased by 8.5% in Talash and 16.5% in Dehghan compared with those of unsprayed controls, whereas the decrements were only 12.1 and 7.7%, respectively, for the drought stress +uniconazole treatments compared with those of uniconazole-treated in well-watered plants (Table 2). In Talash and Dehghan, the POX activities of drought-stressed plants were increased by 50.8 and 43.8%, respectively, in the uniconazole-sprayed treatments, whereas the reductions were only 41.7 and 29.8%, respectively, for drought stressed, uniconazole-treated plants compared with those of the uniconazole-treated in well-watered plants (Table 2). Similarly, the CAT activities of the drought-stressed plants were decreased by 56.2 and 23.2%, respectively, compared with those in the unsprayed controls in Talash and Dehghan, whereas the reductions were only 10.9 and 9.2%, respectively, in the CAT activities of drought stress+uniconazole compared with those of uniconazole-treated in well-watered plants (Table 2).

Effect of uniconazole on MDA content and relative electrical conductivity under drought stress

Drought stress increased MDA content and relative electrical conductivity in both bean cultivars sprayed with uniconazole

Table 2. Mean comparisons of treatment combinations of varieties, drought stress, and uniconazole application on the SOD, POX, CAT, MAD, and REC for common bean.

Treatment		Traits				
Variety		SOD (U g^{-1}FW)	POX (U g^{-1}FW)	CAT (U g^{-1}FW)	MDA (nmol kg^{-1}FW)	REC (%)
Talash	Control	1658.1±71.1b	1.52±0.5b	6.45±0.2ab	7.61±0.2b	24.71±0.5c
	Uniconazole	1722.2±63.2a	2.13±0.4a	6.91±0.6a	6.12±0.3c	23.92±0.4d
	Drought Stress	1382.4±75.2d	0.61±0.5d	2.82±0.5c	9.11±0.2a	26.75±0.3a
	Drought+Uniconazole	1512.3±61.2c	1.24±0.2c	6.15±0.5b	8.21±0.2b	27.44±0.6b
Dehghan	Control	1762.2±82.4b	1.88±0.3b	6.21±0.2ab	5.58±0.1c	24.12±0.2b
	Uniconazole	1812.4±63.1a	2.31±0.2a	6.95±0.5a	4.72±0.1d	21.45±0.1d
	Drought Stress	1395.2±72.4d	0.91±0.3d	4.78±0.3c	10.81±0.5a	27.63±0.1a
	Drought+Uniconazole	1672.0±52.1c	1.62±0.2c	6.31±0.2b	8.16±0.1b	25.61±0.1b

Control: spraying of water on well-watered plants; Uniconazole, foliar spraying of uniconazole under well-watered plants; drought stress, foliar spraying of water under drought stress; foliar spraying of uniconazole under drought stress. SOD: superoxid dismutase; POX: peroxidase; CAT: catalas; MDA: malondialdehyde; REC: relative electrical conductivity. Vertical bars represent ± SEM. Means with the same letter are not significantly different from each other ($P \leq 0.05$ ANOVA followed by LSD test).

Table 3. Mean comparisons of treatment combinations of varieties, drought stress, and uniconazole application on the plant height, stem diameter, root length, stem dry weight, and root dry weight for common bean.

Treatment		Traits							
Variety		Plant Height (cm)	Stem diameter (mm)	Root length (cm)	Stem dry weight (g)	Root dry weight (g)	Seed /plant	Pod / plant	Yield / plant (g)
Talash	Control	51.8±1.4a	5.1±0.2a	38.3±0.3d	0.3±0.0b	0.3±0.0a	31.8±0.7a	11.8±0.7a	5.5±0.2b
	Uniconazole	49.8±2.0b	5.6±0.6a	41.4±0.5a	0.4±0.0a	0.3±0.0a	32.5±1.1a	12.5±1.1a	6.0±0.4a
	Drought Stress	45.9±1.0c	4.3±0.3b	39.0±0.2c	0.2±0.0c	0.2±0.0b	22.1±0.9c	7.3±0.9c	4.8±0.9c
	Drought+Uniconazole	42.6±0.7d	4.9±0.2b	40.0±0.0b	0.1±0.0d	0.2±0.0b	23.9±1. 6b	9.9±1. 6b	5.0±0.5c
Dehghan	Control	55.6±0.9a	6.1±0.4b	43.5±0.5d	0.3±0.0b	0.4±0.0a	34.6±0.3a	14.6±0.3a	7.0±0.1b
	Uniconazole	52.3±1.1b	7.0±0.3a	48.0±0.2a	0.4±0.0a	0.4±0.0a	34.8±0.5a	14.8±0.5a	8.0±0.8a
	Drought Stress	50.6±0.9c	5.5±0.2c	44.5±0.0c	0.2±0.0c	0.2±0.0b	27.3±0.8c	10.1±0.8b	6.2±0.2c
	Drought+Uniconazole	47.8±1.4d	6.4±0.1b	46.0±0.3b	0.3±0.0b	0.4±0.0a	28.9±0.2b	11.9±0.2b	6.5±0.4b

Control: spraying of water on well-watered plants; Uniconazole, foliar spraying of uniconazole under well-watered plants; drought stress, foliar spraying of water under drought stress; foliar spraying of uniconazole under drought stress. Vertical bars represent ± SEM. Means with the same letter are not significantly different from each other ($P \leq 0.05$ ANOVA followed by LSD test).

compared with the unsprayed control (Table 2). Uniconazole decreased the MDA content and the relative electrical conductivity in both bean cultivars within each of the water conditions (Table 2). Uniconazole spray significantly decreased the MDA content under well-watered conditions and drought stress by 19.5 and 9.8%, respectively, in Talash and 15.4 and 24.5%, respectively, in Dehghan relative to those of the control (Table 2). Moreover, uniconazole significantly reduced the relative electrical conductivity under well-watered conditions and drought stress by 3.1 and 2.5%, respectively, in Talash and 11.0 and 7.3%, respectively, in Dehghan (Table 2). By contrast, the MDA content and relative electrical conductivity significantly increased by 56.2 and 48.3%, and 7.6 and 12.70%, respectively, in unsprayed controls under drought stress compared with unsprayed controls in well-watered conditions for Talash and Dehghan, respectively (Table 2).

Effect of uniconazole on morphological parameters under drought stress

Plant height, stem diameter, root length, stem dry weight, and root dry weight are important indices for judging plant growth in drought stress. In the experiment, uniconazole treatments reduced plant height relative to those of the controls in drought stress and well-watered conditions. Conversely, uniconazole significantly increased the stem diameter, root length, and stem and root dry weight under each water condition except for the stem dry weight in Talash (Table 3). Uniconazole application clearly alleviated the drought-induced inhibition of growth in Talash and Dehghan varieties (Table 3).

Uniconazole significantly reduced plant height by 7.1% in Talash and 5.5% in Dehghan under drought stress. Furthermore, uniconazole significantly decreased plant height by 3.8% in Talash and 5.9% in Dehghan under well-watered conditions (Table 3). Uniconazole also significantly increased stem diameter and root length by 12.2 and 2.5%, respectively, in drought stress and 8.9 and 7.3%, respectively, in well-watered conditions relative to those of the controls in Talash. Similarly, uniconazole significantly increased the stem diameter and root length by 4.6 and 5.4%, respectively, in drought stress and 12.8 and 9.3%, respectively, in well-watered conditions

in Dehghan compared with the controls. Stem dry weight and root dry weight were significantly increased by 33.3 and 33.3%, respectively, in Talash and 33.3 and 50.0%, respectively, in Dehghan under well-watered plants relative to those of the drought stress (Table 3).

Effect of uniconazole on yield under drought stress

There were no significant drought stress and drought stress +uniconazole interaction effects on seed yield per plant (Table 3). Drought stress resulted in a significant yield reduction (12.7 and 11.4%) in Talash and Dehghan, repectively, due to variations in seed number of per plant (30.5 and 21.0%) and pod number of per plant (38.1 and 30%), with respect to the control. However, uniconazole increased seed yield per plant by 9 and 7.1% in drought stress and 8.3 and 12.5% in well-watered conditions relative to those of the controls in Talash and Dehghan, repectively.

Discussion

Drought stress is a vital limiting factor at the initial phase of plant growth and establishment (Jaleel et al. 2009). In terms of drought stress responses in morphological characteristics, plant growth and dry matter (shoot and root) were remarkably reduced (Harb et al. 2010). Uniconazole can induce various morphologies in plants. In particularly, rooting is stimulated; hence, the treatment is an ideal candidate for modifying bean seedling growth and development during environmental stresses (Wan et al. 2013; Zhang et al. 2007). In this study, results showed that the morphological characteristics, including plant height, stem diameter, root length, stem dry weight, and root dry weight decreased after drought stress (Table 3). By contrast, the plants treated with uniconazole revealed improved morphological characteristics, excluding the plant height; this result indicated the increased tolerance of the bean seedling to water stress. Under well-watered conditions, the morphological characteristics were enhanced after the uniconazole treatment (Table 3). This observation is similar to that of Leul and Zhou (1998) who found that following waterlogging stress, uniconazole-treated plants showed significantly improved growth, including plant height, stem width, root and shoot length, and total dry weight. We speculate that uniconazole is a kind of growth-retarding regulator, which can reduce plant height and increase stem, which was one reason for the bean improving plant growth under drought.

Drought stress reduced the rates of net photosynthesis because of the metabolic limitations, namely, oxidative damage to chloroplasts and the stomatal closure (Farooq et al. 2014). The stomatal closure guarantees that photosynthesis proceeds well and also helps avoid excessive water loss (Wang et al. 2006). When plants are subjected to drought conditions, they implement functional measures to reduce water loss by transpiration and protect the appropriate water content in tissues. As recently reported, a drought-tolerant chickpea genotype presented low transpiration; this adaptation may lead to a low photosynthesis rate, which can render the chickpea capable of accessing water available in soil during drought (Saglam et al. 2014). By contrast, Lawson and Blatt (2014) believed that raised stomatal conductance and transpiration rate increase the CO2 influx into mesophyll cells; as a result, carbon fixation enhances and leads to an augmented photosynthesis rate. In our study, drought stress significantly decreased the leaf stomatal conductance in Talash and Dehghan (Table 1). However, when uniconazole was applied, the stomatal conductance and transpiration rate were enhanced to maintain an increased photosynthesis rate for improving drought tolerance. Uniconazole also significantly increased the chlorophyll content of seedling leaves (Table 1); this effect is a vital cause for the promotion of photosynthesis in plants. Chlorophyll content can be regarded as an indicator of biochemical modification and quantifier of drought stress intensity (Leufen et al. 2016). Our results are similar to those of the previous studies (Zhang et al. 2007, 2012) where the photosynthetic pigments in uniconazole-treated crops were maintained and resulted in a high photosynthetic rate, transpiration rate, and stomatal conductance under drought stress.

The accumulation of soluble sugar and soluble protein content increases plant resistance to drought (Ghaderi et al. 2015). Couee et al. (2005) reported that soluble sugar is useful to plants because some defenses and signals function not only in sensing and controlling photosynthetic activity but also in ROS scavenging with a consequent reduction of oxidative damage. Qiu et al. (2005) showed that rapeseed film coating with suitable uniconazole concentration significantly increases soluble sugar concentration to improve rapeseed seedling growth during waterlogging. In the current study, the soluble sugar and soluble protein content significantly increased in the leaves of all individuals of the two varieties subjected to uniconazole treatment under well-watered and drought stress conditions (Table 1). This result is partly consistent with those of Zhou et al. (2016). The enhanced levels of soluble sugar and soluble protein content in the uniconazole-treated plants exposed to drought stress may contribute to the buildup of osmolytes and thus help in alleviating stress.

Various environmental stresses often lead to the increased generation of ROS; in this regard, SOD, POX, and CAT are proposed to play important roles in the stress tolerance of plants (Chakhchar et al. 2016). SOD activity plays a key role in cellular defense mechanisms against superoxide (O^{2-}) and hydrogen peroxide (H_2O_2), which may cause severe damage to membranes, protein, and DNA (Golldack et al. 2014). CAT and POX are in charge of eliminating H_2O_2 by reducing H_2O_2 to H_2O in plants (Yu et al. 2017). Increased SOD, POX, and CAT activities can help reduce the damage to plants under drought stress (Jaleelet al. 2009). Yan et al. (2011) showed that soybean seed treatment with niconazole powder increases SOD and POX activities in soybean seedling roots and leaves; and this effect was beneficial for

improving soybean seedling growth and resisting the lodging of corn under shading in relay strip intercropping systems. Our data are consistent with those of Li et al. (1998) or Leul and Zhou (1999), who suggested that the increased tolerance to drought and waterlogging stress induced by uniconazole in resistant cultivars of maize and winter rapeseed seedling, respectively, are due to augmented SOD, POX, and CAT activities. In the present study, the SOD, POX, and CAT activities were enhanced by uniconazole application not only under drought stress but also in well-watered conditions (Table 2). This result indicated that drought stress damaged the protective enzyme system and thus accelerating leaf senescence. Uniconazole enhanced the efficiency of the antioxidant system and mitigated the damage of drought stress to seedlings.

MDA is described as an indicator of the damage extent of membrane lipid peroxidation, which indicates the magnitude of oxidative stress (Piotrowska-Niczyporuk and Bajguz 2014). The relative electrical conductivity is also considered as an index of stress intensity. For example, under drought stress, with increasing MDA content and relative electrical conductivity, the extent of peroxidation of plasma-lemma increased under drought stress to induce leaf cell-membrane damage (Wang et al. 2012). Ren et al. (2018) showed that the reduction of MDA content by exogenous 6-benzyladenine in summer maize was one of causes in the increased tolerance of the plant to water stress.

Zhou et al. (2016) also reported that although the drought-treated plants showed increased relative electrical conductivity, triadimefon-treated plants exhibited decreased relative electric conductivity compared with plants not treated with triadimefon. In this study, drought stress significantly increased the leaf MDA content and relative electrical conductivity in the two varieties tested (Table 2). While, uniconazole decreased MDA content, which indicated that exogenous spraying uniconazole effectively countered oxidative stress and alleviated drought stress on the cell membrane system, which resulted in a relatively stable biological membrane. These results were conductive to keep normal physiological function, restoring photosynthetic properties of drought bean plants and then resulted in the improvement of photosynthesis and seed yield of bean.

Conclusion

Drought stress down-regulated the activities of antioxidative enzymes, accelerating leaf senescence, decreases in plant photosynthesis, and resulted in yield loss. Exogenously applied uniconazole effectively alleviated drought stress damages on osmotic adjustment substances and antioxidative system of bean seedlings by increasing soluble sugar, soluble protein, and antioxidant enzymes, which was mainly attributed to the delay of leaf senescence and the improvement of photosynthetic performance and thus resulting in the increase in grain yield of drought bean.

Compliance with Ethical Standards

The authors declare no conflict of interest.

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