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UNICONAZOLE MITIGATES DISADVANTAGEOUS EFFECTS OF DROUGHT STRESS ON *CANNABIS SATIVA* L. SEEDLINGS

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Abstract

Uniconazole, *S*-(+)-uniconazole plays a primary role in plants experiencing different adverse stresses. Its role in hemp (*Cannabis sativa* L.) drought tolerance is unknown at this time. We assessed the physiological indices of plants under drought stress to elucidate the functions of exogenous uniconazole on the growth of industrial hemp. The results showed that, exogenous uniconazole significantly inhibited plant height, and reduced leaf area. Uniconazole supplementation increased leaf relative water content (RWC) but decreased the proline (Pro) contents. Uniconazole application reduced oxidative damage and accumulations of superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA). Uniconazole increased antioxidant enzymes (superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)) activities and promoted the antioxidant defense system to scavenge excessive reactive oxygen species (ROS). The uniconazole-treated plants maintained leaf cell structure integrity and relieved the damage of photosynthetic system subjected to drought stress. Uniconazole enhanced chlorophyll (Chl) content and P_n. Thus, these results indicated that uniconazole had a positive effect in improving the drought tolerance of industrial hemp seedlings.

Key words: Uniconazole, Drought stress, *Cannabis sativa* L., Morphological structure, Photosynthesis, Antioxidant systems.

Introduction

Water shortage has become increasingly serious problem due to global warming (Adams *et al*., 2009). Drought stress is one of many abiotic stressors that affects the morphological structure, photosynthetic capacity, and physiological behaviour of plants (Wang *et al*., 2012; Reddy *et al.*, 2004), and exerts a unfavourable impact on plant growth and yield (Aliche *et al*., 2018). The sequence of morphologic, biochemical, physiological, and molecular changes affecting plant growth and productivity are caused by drought or water deficit. Drought brings about a series of physiological processes damage including reduced plant height and lessened leaf area (Anjum *et al*., 2011), lower leaf relative water content (Meher *et al*., 2018), inhibited photosynthetic system Ⅱ (PSⅡ) activity (Ma *et al*., 2006) and photosynthesis (Pompelli *et al*., 2010), cellular damage (Dong & Zhang, 2001) and reactive oxygen species overproduction. There are some modifications, such as osmotic change (Subbarao *et al*., 2000) and antioxidant defense (Guo *et al*., 2006), to counteract these effects in drought stress plants. Proline, a major osmoprotectant, its content reflects plant stress resistance to some extent and is considered the protective mechanism of plants (Szabados & Savouré, 2010). Antioxidant defense systems include non-enzymatic and enzymatic antioxidants.

Different methods are adopted, to relieve disadvantageous actions of drought on plants. Varieties with strong drought resistance can improve the plant's toleration to drought stress (Li *et al*., 2017). Besides, several researchers have discovered that exogenous compound application can promote plants' resistibility. Applied substances can increase antioxidant enzyme activities, reduce ROS levels, alleviate oxidative damage, improve the photosynthetic rate and regulate hormone levels under drought stress (Boogar *et al*., 2014; Zhang *et al*., 2007).

Uniconazole, one plant growth retardant with high efficiency and low toxicity, can reduce the level of endogenous gibberellins (GA) by inhibiting the biosynthesis of GA (Saito *et al*., 2006), and has a strong growth regulation function that improves plants' resistance under stresses (Todoroki *et al*., 2008). Uniconazole application is important to alleviate harmful effects of waterlogging (Leul & Zhou, 1998), phytotoxicity (Upadhyaya *et al*., 1991), chilling (Chucheep *et al*., 2005), water deficit (Zhang *et al*., 2007), drought (Feng *et al*., 2020), salt (Al-Rumaih, 2007), melt (He *et al*., 2017) and lodging (Ahmad *et al*., 2018).

Hemp (*Cannabis sativa* L.), one of the most ancient annual herbs and the first domesticated crops, has a history of cultivation for thousands of years in China. Industrial hemp allowed for cultivation in China has to have a delta-9-tetrahydrocannabinol (THC) concentration of <0.3%, and it is widely used for various applications more than 25,000 commodities, including the production of seeds and fibre (Abot *et al*., 2013; House *et al*., 2010). Drought stress limits hemp growth, delays the maturation of fibre and seed, and decreases crop productivity and quality (Abot *et al*., 2013; Amaducci *et al*., 2008). Few studies of industrial hemp are reported subjected to drought stress. Gao *et al*., (2018) investigated the molecular responses of hemp to drought stress by RNA sequencing technology and revealed the important biological processes and pathways under stress. A thorough comprehension of the physiological responses of industrial hemp to stress is required to obtain good quality and stable yields under drought stress. However, the

effects of uniconazole on hemp subjected to soil moisture deficit have not been researched to date.

The total target of the study was to clarify a putative involvement of uniconazole in hemp in response to drought stress. Therefore, to accomplish this goal we investigated key growth parameters of uniconazole-treated and -untreated industrial hemp seedlings including RWC, Pro content, Chl content, fluorescence parameters, photosynthetic indices, leaf structure, oxidative damage, and activities of the antioxidant enzyme.

Materials and Methods

Plant material and treatment: Industrial hemp seeds, 'Huoma No. 1', were obtained from the Daqing branch of the Heilongjiang Academy of Sciences. The surface of seeds was sterilized with 75% ethyl alcohol for 2 min and rinsed with water three times. The seeds were immersed in distilled water and 0.4 mg L^{-1} of uniconazole (Jiang *et al*., 2020) for 10 h, respectively, and washed surface using distilled water. Three seed soaking treatments included (1) distilled water, (2) distilled water under drought condition and (3) 0.4 mg L^{-1} of uniconazole under drought condition. The soaked seeds were planted in pots (20 cm diameter and 16 cm height) which including a 2.5 kg mixture of 2:1 (v:v) grass peat (pH 6.3) and sand. The grass peat contained 20.4% of organic matter content, 46% maximum field capacity (FC), available P (13.7 mg kg^{-1}), ammonium nitrogen (315.3 mg kg^{-1}) and available K (206.0 mg kg^{-1}). The pots were watered and placed in a greenhouse (light/dark cycle as 14/10 h, 25/20℃) under 70% relative humidity. A system of weighing was used to manage the soils water content (SWC) and 70% FC was maintained with the proper quantity of water until trifoliate stage. The seedlings of (1) grew normally at a FC of 70%, and the seedlings of (2) and (3) were subjected to drought stress at the three-leaf stage that expressed as CK, drought (D), uniconazole + drought (S + D). The completely unfolded third pair of leaves from base were harvested after 0 (normal water supply, SWC=32%), 2 (SWC=28%), 4 (SWC=23%), 6 (SWC=18%) and 8 (SWC=14%) days of drought stress, then 4 days after rehydration. Immediately after excision leaves were frozen and preserved at -80℃ until analyses. Twenty leaves were collected for one biological repeat.

Plant height and leaf area: The plant height of industrial hemp seedlings was measured with a metric ruler. The leaf area of the fully expanded third leaves from the base was determined with an LI-3100C area meter using a protocol provided by the manufacturer (LI-COR, Inc., USA).

Determination of relative water content and proline content: To survey RWC, sheared fresh leaves from each treatment were weighed immediately (FW). All leaves were placed in distilled water. After 12 h of dark, the leaves were removed from the water and weighed for the turgid weight (TW) after wiping surface water from the leaves with absorbent papers. Then, the leaves were put in a drier (80℃) to a constant weight and weighed for the dry weight (DW).

Pro content was measured using procedures developed by Bates *et al*., (1973). Leaves (0.3 g) of industrial hemp were homogenized with sulfosalicylic acid, then boiled. Subsequently, the mixture of filtrate (2 mL), glacial acetic acid (2 mL), and acid ninhydrin (2 mL) was put into a test tube. The reactants were incubated in boiled water for 40 min. Then, toluene (4 mL) was mixed fully with the reaction mixture after cooling. After layering, the absorbance was read from the upper solution at 520 nm. Pro-level was calculated taking a calibration curve.

Determination of Chl content: The contents of photosynthesis pigments (total Chl, Chl a, Chl b, and carotenoids (Car)) were measured according to the methods of Arnon (1949) and Lichtenthaler & Wellburn (1983) with some modification. Fresh leaves (0.1 g) were cut and put into the mixture of ethanol and acetone (v:v=1:1, 10 mL), kept in dark for 24 h. Then, filtrates were obtained to determine its absorbance at 663, 645, and 470 nm.

Measurement of photosynthetic indices and Chl fluorescence parameters: Photosynthetic indices, which contained net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), stomatal conductance (G_s), and transpiration rate (T_r), were determined using an LI-6400 XT portable photosynthesis system (LI-COR, Inc., Lincoln, NE, USA) with light 1,000 μmol m^{-2} s^{-1}.

Chl fluorescence parameters were determined by a Chl fluorescence analyzer (OS5p, OPTI-sciences, USA) between 9:00 and 12:00. The relative electron transfer rate (ETR) of PSⅡ under light adaptation and maximum photochemical efficiency (F_v/F_m) after 20 min dark adaptation were performed on the wholly developed leaves of the control and drought stress.

Observation of the leaf structure: The leaf structure was observed through the glycol methacrylate (GMA) semi-thin section method according to Shi *et al*., (2015). Cannabis leaves treated with drought stress for 6 days (CK, drought, uniconazole + drought) were selected as experimental materials. The leaves were fixated in a formalin-acetic alcohol mixture (FAA) stationary solution for 24 h and successively dehydrated by 50% ethanol, 100% ethanol, isoamyl alcohol, and n-butanol twice (4 h each time). After dehydration, the leaf sample was penetrated with GMA three times (1 day for the first and second times and 2 days for the third time). The material and an appropriate amount of GMA were placed into a capsule and polymerized in a 60℃ incubator for 24 h. Approximately 2 μm slices were made with a microtome (Leica Ultralcut R, Germany). The slices were dyed (Feder & O'Brien, 1968) and sealed with neutral gum after drying. A biological microscope (Leica DM4000B, Germany) and a microscopic imaging system (Leica DFC550) were used to observe and photograph the slices.

Measurement of superoxide anion production rate, hydrogen peroxide content, and lipid peroxidation: Superoxide anion was assayed according to Elstner & Heupel (1976). To collect supernatant, leaves (0.3 g) were homogenized in 100 mM pH 7.0 pre-cooling phosphate

buffer (5 mL) and centrifuged at 4℃ for 15 min. Then, supernatant (2 mL) mixing with phosphate buffer (0.5 mL, pH 7.8, 50 mM) and hydroxylamine hydrochloride (0.1 mL,10 mM) were held at 25℃. 20 min later, sulfanilic acid solution (58 mM) and α-naphthylamine solution (7 mM) mixed with the reaction mixture were reacted at 30℃. After 30 min, an equal volume of trichloromethane was put in, then centrifuged. The absorbance was recorded at 530 nm at the upper aqueous phase.

Hydrogen peroxide content was determined as follows (Sergiev, 1997). Briefly, leaves (0.5 g) were homogenized with trichloroacetic acid (TCA, 0.1% (w:v)) by an ice bath, and centrifuged. Subsequently, the supernatant, phosphate buffer (pH 7.0, 0.1 M), and KI (1 M) were blended and incubated in darkness at room temperature for 1 h. The absorbance was recorded at 390 nm.

MDA content was tested with the method described by Heath & Packer (1968) and Dionisio-Sese & Tobita (1998). To detect MDA content, leaves (0.5 g) were ground in pre-cooling phosphate buffer (5 mL, pH 7.8), and centrifuged. Then, thiobarbituric acid (TBA, 0.6%) was added into the supernatant and boiled. 15 min later, the cooled mixture was centrifuged again. The absorbance was determined at 600, 532, and 450 nm using supernatant.

Histochemical localization of superoxide anion and hydrogen peroxide: The fully developed and healthy third leaves from the base were selected for histochemical localization after 6 days of drought stress (CK, drought, uniconazole + drought). The histochemical localization of $O_2^{\cdot-}$ and H_2O_2 was detected by staining methods following Bournonville & Díaz-Ricci (2011) and Daudi & O'Brien (2012), respectively. The dyed leaves were soaked in absolute ethanol and glacial acetic acid (v:v=3:1) mixture, and placed in a 65℃ hot water bath. The leaves were taken out then photographed.

Enzyme extraction and determination: Industrial hemp leaves (0.5 g) were homogenized in ice-cold phosphate buffer (50 mM, pH 7.8), and centrifuged at 4℃. 20 min later, the supernatant was prepared for enzyme activity determination as crude enzyme extract.

SOD activity was analyzed following by Giannopolitis & Ries (1977). A SOD reaction solution containing 15:3:3:3:3:2.5 (v:v) of phosphate buffer (pH 7.8), methionine (Met), blue tetrazoline (NBT), EDTA-Na_2, riboflavin (FD), and distilled water was prepared to measure SOD activity. The mixture, including enzyme extract (20 μL) and SOD reaction solution (3 mL), and the controls without the enzyme extract were placed in 4000 lux. The blank without the enzyme extract was put in dark and compared at 560 nm.

POD activity was estimated as follows (Hernández *et al*., 2000). A POD reaction solution containing phosphate buffer (0.1 M, pH 6.0), guaiacol, and 30% H_2O_2 was used to measure POD activity. The reaction was initiated when enzymic extract (20 μL) was put into POD reacting solution (3 mL), and recorded the absorbance at 470 nm once every 30 s.

CAT activity was assayed following Beers & Sizer (1952). A 0.1 M H_2O_2 solution and 0.1 M pH 7.0 phosphate buffer (1:4 (v:v)) were mixed as the CAT reaction solution. Then, enzyme extract (100 μL) was mixed with CAT reaction solution, and read the absorbance at 240 nm once every minute.

Statistical analysis: Data were evaluated through ANOVA with SPSS version 13.0, and values are presented as mean ± SE. Duncan's test ($p<0.05$) was applied to detect the differences between means. All the figures were drawn by *OriginPro 9.1* software.

Results

Plant height and leaf area: Plant height and leaf area increased with the prolongation of days (Table 1). However, plant height decreased by 5.8% subjected to drought stress for 8 days, in contrast to that of control. Leaf area of drought-treated leaves was 24.9% lower than that of control after 8 days of stress. Similarly, exogenous uniconazole application significantly decreased the plant height by 13.4%, and reduced the leaf area by 7.4%, relative to untreated plants.

Table 1. Effect of uniconazole on plant height and leaf area under drought stress in industrial hemp leaves.

Indicators	Treatments	Times	CK	D	S+D
Plant height (cm)	Drought stress	0 d	18.59 ± 0.84a	18.59 ± 0.84a	15.76 ± 0.22b
		2 d	20.63 ± 0.78a	19.01 ± 0.88b	16.22 ± 0.81c
		4 d	20.88 ± 0.40a	19.50 ± 0.28b	17.30 ± 0.48c
		6 d	21.43 ± 0.54a	19.60 ± 0.51b	17.35 ± 0.49c
		8 d	21.84 ± 1.15a	20.57 ± 0.55b	17.82 ± 0.70c
	Recovery to natural environment	4 d	22.82 ± 1.03a	21.26 ± 0.50b	20.78 ± 0.62c
Leaf area (cm^2)	Drought stress	0 d	7.00 ± 0.69a	7.00 ± 0.24a	5.11 ± 0.24b
		2 d	7.45 ± 0.87a	6.80 ± 0.81ab	5.68 ± 0.70b
		4 d	9.87 ± 0.40a	8.06 ± 0.43b	7.35 ± 0.37c
		6 d	10.59 ± 0.32a	8.81 ± 0.44b	7.68 ± 0.23c
		8 d	12.49 ± 0.17a	9.38 ± 0.18b	8.69 ± 0.12c
	Recovery to natural environment	4 d	15.09 ± 0.50a	11.11 ± 0.23b	9.70 ± 0.33c

Data are represented as the means ± SE (n = 4) of treatments. CK, D and S+D refer to treatments under normal water supply, drought stress and uniconazole + drought stress, respectively

RWC and Pro content: Leaf RWC was markedly changed by uniconazole under drought stress and recovery (Fig. 1A). The RWC showed no clear difference in the control. RWC decreased with the prolongation of the drought stress treatment. RWC changed dramatically after 8 days of drought stress, it decreased by 31.7% compared with that of the control. Uniconazole application increased leaf RWC by 13.4% contrasted with that of control when drought stress continued for 8 days. The RWC of leaves increased after rehydration and was improved by uniconazole treatment.

Pro is one of the osmolytes that reflect the stress resistance of plants. The Pro contents increased rapidly by 1.47-, 11.01- and 35.32-fold compared with the controls when drought stress lasted 4, 6, and 8 days, respectively. Pro contents of uniconazole-treated plants were decreased by 34.8, 38.2, and 37.0% against those of non-uniconazole-treated plants after 4, 6, and 8 days of drought, respectively (Fig. 1B). Pro contents decreased significantly after rehydration.

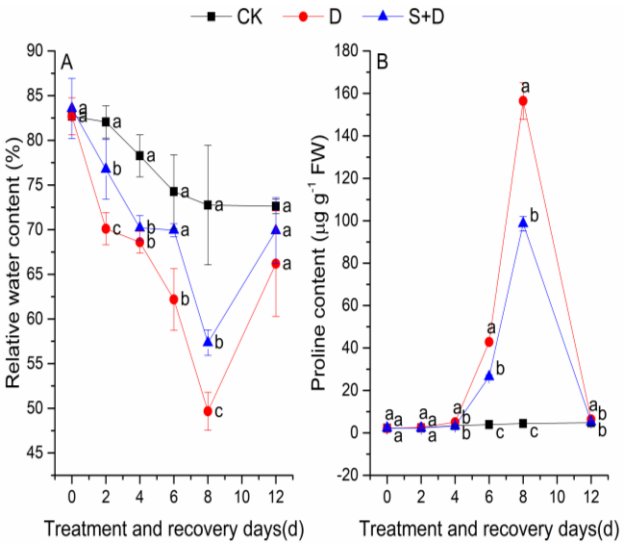

Fig. 1. Effect of uniconazole on leaf RWC (A) and Pro content (B) under drought stress in industrial hemp leaves. From day 2 to 8 indicates the plants are drought stress; on day 12, the plants recover to the normal water condition (recovery is 4 days). Data are represented as the means ± SE (n = 4) of treatments. CK, D and S+D refer to treatments under normal water supply, drought stress and uniconazole + drought stress, respectively.

Chl content: Drought stress inhibited Chl content compared with those in the non-drought-treated plants. From days 2 to 8, the Chl a content decreased by 9.2, 25.0, 25.8, and 32.6%, respectively (Fig. 2A). The Chl b content was significantly reduced by 28.4, 32.8, and 46.8% from days 4 to 8, respectively (Fig. 2B). From days 2 to 8, the total Chl content and Car content were 7.0-36.1% and 8.9-25.6% lower than the contrast, respectively (Figs. 2C, 2D). Uniconazole treatments effectively relieved the decrease in Chl content under stress conditions. The Chl a, Chl b, total Chl, and Car contents dramatically increased by 32.6, 46.8, 36.1, and 25.6%, respectively, in plants treated with uniconazole after 8 days of stress contrasted with those without uniconazole. The Chl contents were restored after rehydration.

Photosynthesis and Chl fluorescence parameters: A lessening of P_n, G_s, and T_r was measured in industrial hemp leaves under drought stress, whereas C_i increased. P_n, G_s, and T_r were 76.1, 85.8, and 84.4% lower than the controls after 8 days of stress, respectively (Figs. 3A, 3C, 3D). C_i markedly increased by 14.1% in comparison with the contrast, when drought stress was treated for 8 days (Fig. 3B). Uniconazole-treated plants showed a significant increase in P_n (51.47%), G_s (71.0%), and T_r (71.8%) compared with the non-uniconazole-treated plants for 8 days drought stress. The P_n, G_s, and T_r of the leaves were inversed after rehydration (Figs. 3A, 3C, 3D). Uniconazole application significantly decreased C_i by 16.5% compared with the non-uniconazole-treated plants after 6 days of drought stress (Fig. 3B).

Chl fluorescence is an important tool for identifying drought stress in plants (Banks, 2018). The relative ETR and F_v/F_m of PS Ⅱ were measured. ETR of drought-treated plants decreased significantly by 40.9-51.4% compared with that of control from days 2 to 8. Exogenous uniconazole application significantly increased ETR by 42.6, 55.9, and 90.8% compared with non-uniconazole-treated plants when drought stress continued for 2, 4, and 6 days, respectively (Fig. 3E). A similar change in F_v/F_m was observed, it was 20.2% lower than control after 8 days of stress. The F_v/F_m in the uniconazole-treated plants increased by 7.2, 11.7, 10.9, and 12.1% against that in the non-uniconazole-treated plants when drought stress continued for 2, 4, 6, and 8 days, respectively (Fig. 3F). ETR and F_v/F_m were restored after 4 days of rehydration.

Leaf structure: The leaf structure was examined using the GMA semi-thin section method. The compact palisade tissue of the leaves, the small gap between cells, and the evenly arranged spongy tissue with good regularity and distribution were observed under normal water treatment. The structure of the leaves was considerably affected under drought stress, the density of the palisade tissue cells decreased, the arrangement of spongy tissue cells was disordered, the leaves became thicker and the internal space of the leaves increased. Uniconazole application alleviated the damage in the leaf cell structure. The blue dots in the cells are starch grains that decreased under drought stress, but uniconazole treatment inhibited this phenomenon (Fig. 4).

Reactive oxygen metabolism: Histochemical staining was expressed by the localization of $O_2^{\cdot-}$ and H_2O_2. $O_2^{\cdot-}$ and H_2O_2 accumulation were indicated as deep blue spots and dark brown pieces, respectively, which were raised in the drought-treated plants contrasted with those in control. Exogenous uniconazole supplementation dramatically reduced spots number compared with drought-treated plants alone, showing that the accumulation of $O_2^{\cdot-}$ and H_2O_2 was inhibited (Fig. 5).

Oxidative stress, such as $O_2^{\cdot-}$ production rate, H_2O_2, and MDA contents increased dramatically under drought stress (Fig. 6). $O_2^{\cdot-}$ and H_2O_2 reached their maximum values that increased by 2.4- and 2.0-fold, respectively, when drought stress continued for 8 days, in contrast to those of the controls (Figs. 6A, 6B). MDA content was 49.2, 46.0, and 49.5% higher than control subjected to drought stress for 4, 6, and 8 days, respectively (Fig. 6C). Uniconazole supplementation reduced $O_2^{\cdot-}$, H_2O_2 and MDA compared with the seedling facing drought stress only. Uniconazole treatment relieved the oxidative damage of drought stress and improved drought resistance in industrial hemp. These indicators and contents were restored after rehydration.

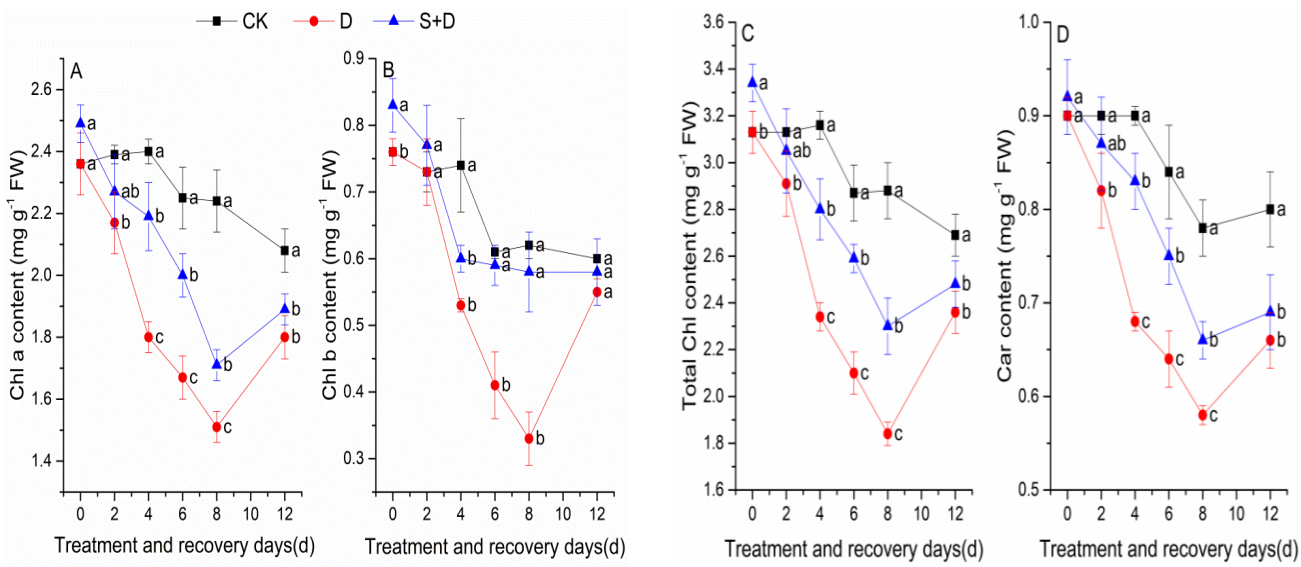

Fig. 2. Effect of uniconazole on Chl a (A), Chl b (B), total Chl (C) and Car (D) contents under drought stress in industrial hemp leaves. From day 2 to 8 indicates the plants are drought stress; on day 12, the plants recover to the normal water condition (recovery is 4 days). Data are represented as the means ± SE (n = 4) of treatments. CK, D and S+D refer to treatments under normal water supply, drought stress and uniconazole + drought stress, respectively.

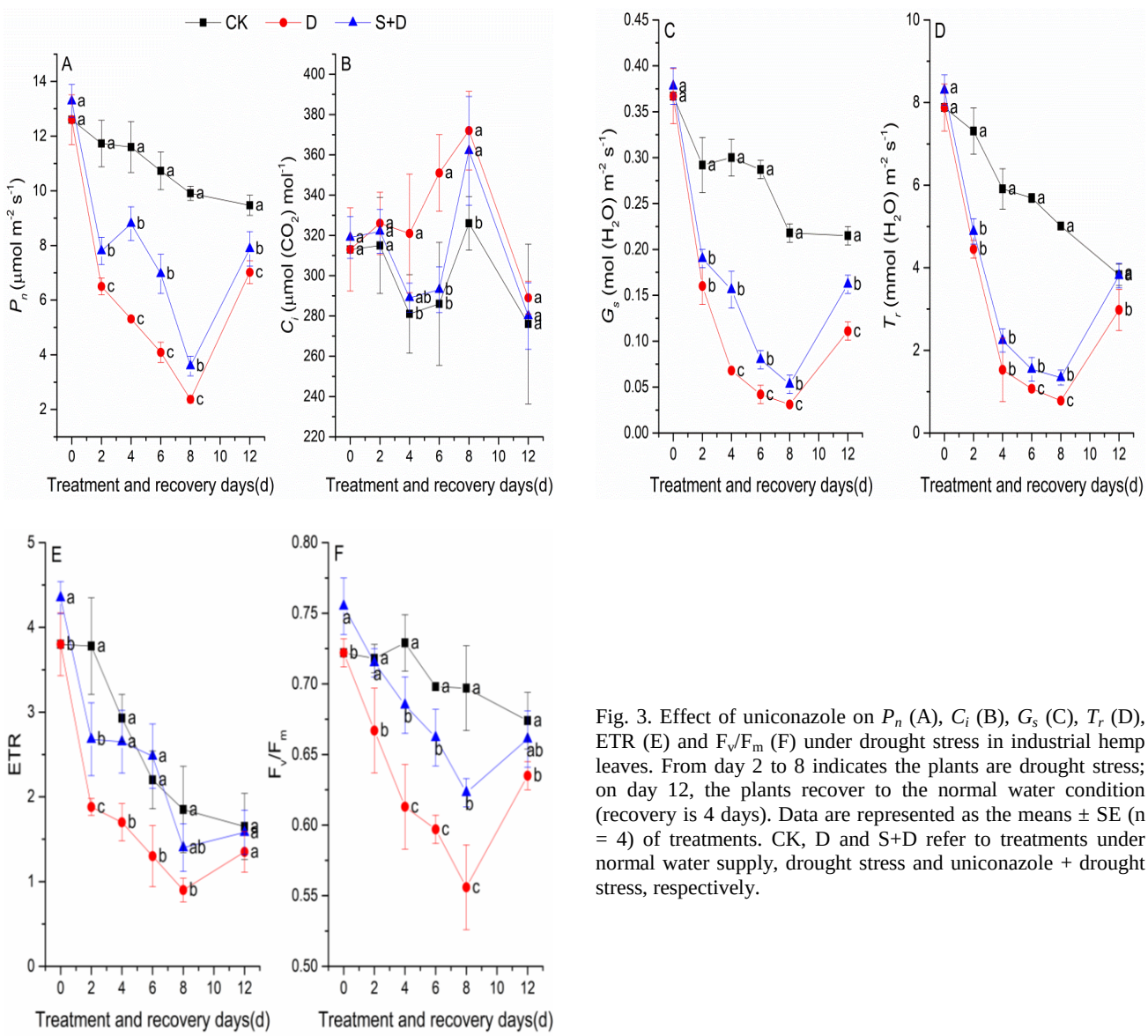

Fig. 3. Effect of uniconazole on P_n (A), C_i (B), G_s (C), T_r (D), ETR (E) and F_v/F_m (F) under drought stress in industrial hemp leaves. From day 2 to 8 indicates the plants are drought stress; on day 12, the plants recover to the normal water condition (recovery is 4 days). Data are represented as the means ± SE (n = 4) of treatments. CK, D and S+D refer to treatments under normal water supply, drought stress and uniconazole + drought stress, respectively.

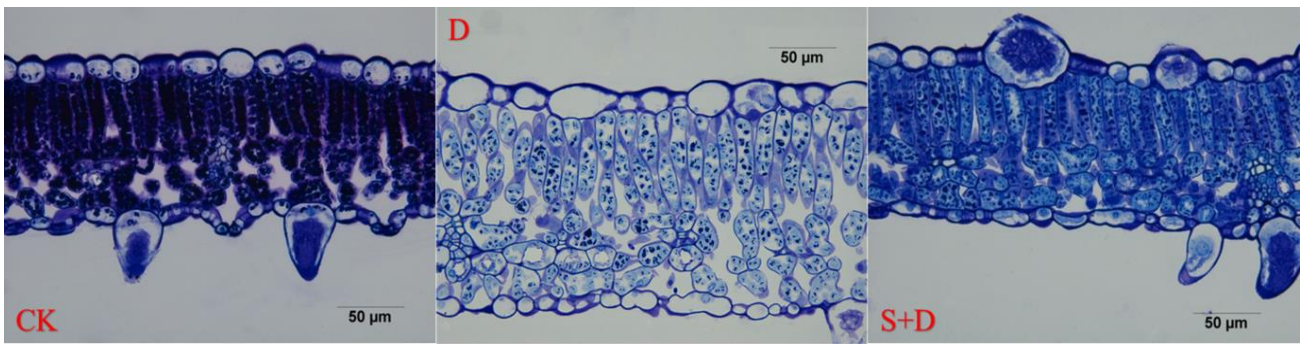

Fig. 4. Leaf structure under CK, D and S+D in industrial hemp leaves. CK, D and S+D indicate the treatments under normal water supply, drought stress and uniconazole + drought stress for 6 days, respectively.

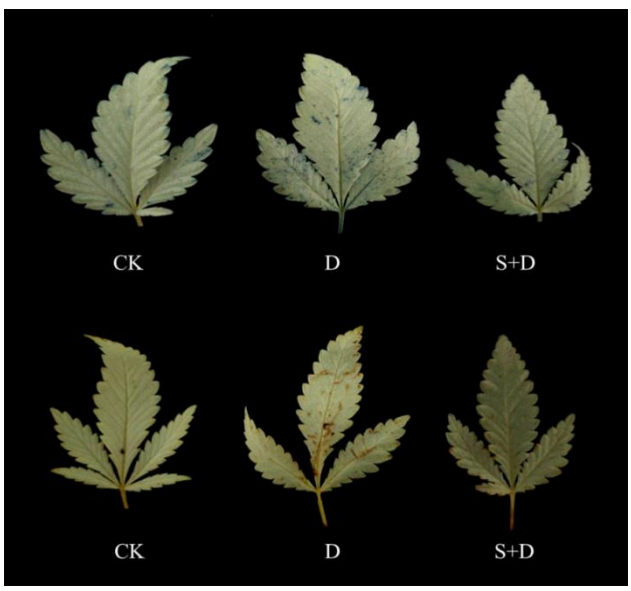

Fig. 5. Histochemical localisation of $O_2^{\cdot-}$ and H_2O_2 in industrial hemp leaves. CK, D and S+D indicate the treatment under normal water supply, drought stress and uniconazole + drought stress for 6 days, respectively.

Activities of antioxidant enzyme: Antioxidant enzymes (SOD, POD, and CAT) activities were assayed to further confirm the influence of uniconazole on drought stress in hemp plants. After 2 to 8 days of drought stress, SOD activity increased initially, and decreased, then increased. SOD activity increased notably by 9.1 and 24.4% contrasted with the controls, after 2 and 8 days of drought stress, respectively. When stress lasted 8 days, SOD activity of uniconazole-treated plants had a sharp rise with 4.8%, in comparison with that of non-uniconazole-treated plants (Fig. 7A). POD activity increased dramatically by 29.2, 58.1, and 66.5% subjected to drought stress for 4, 6, and 8 days, respectively, in contrast with those of the controls. POD activity of uniconazole-treated plants was 5.9% higher than that of the plants exposed to drought stress for 8 days (Fig. 7B). CAT activity was 26.4 and 46.2% greater than the control after 6 and 8 days drought stress, respectively. The uniconazole-treated plants showed a significant increase of 13.1 and 13.4%, respectively, in CAT activity contrasted with the non-uniconazole-treated plants when drought stress continued for 6 and 8 days (Fig. 7C). SOD, POD, and CAT activities still kept high levels after rehydration.

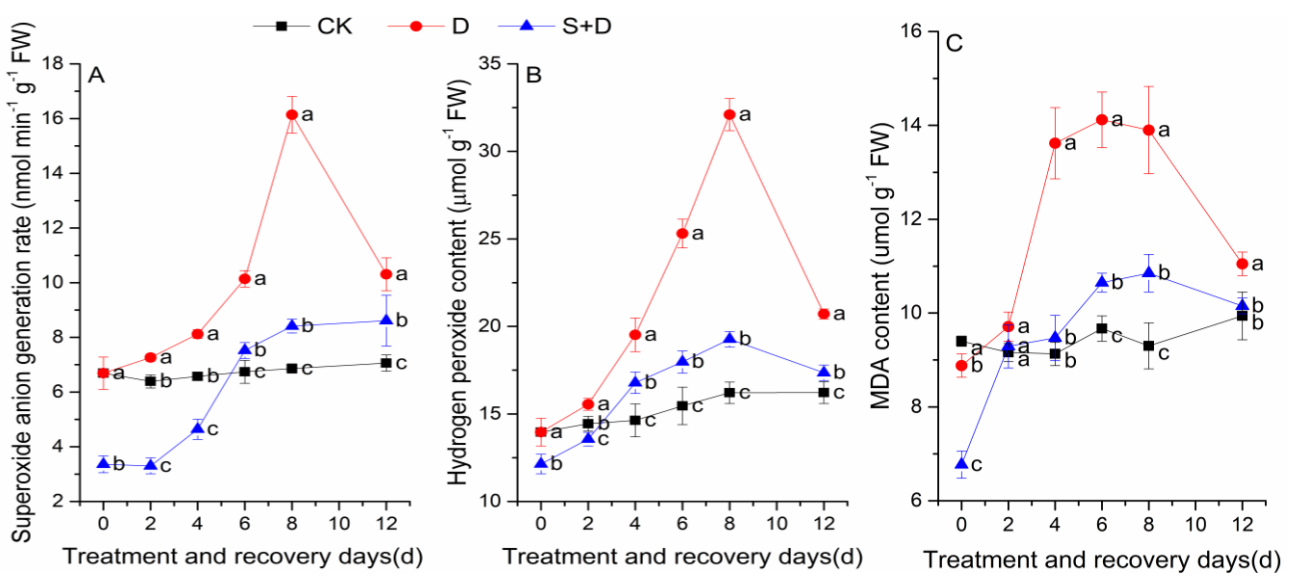

Fig. 6. Effect of uniconazole on $O_2^{\cdot-}$ production rate (A), H_2O_2 content (B) and MDA content (C) under drought stress in industrial hemp leaves. From day 2 to 8 indicates the plants are drought stress; on day 12, the plants recover to the normal water condition (recovery is 4 days). Data are represented as the means ± SE (n = 4) of treatments. CK, D and S+D refer to treatments under normal water supply, drought stress and uniconazole + drought stress, respectively.

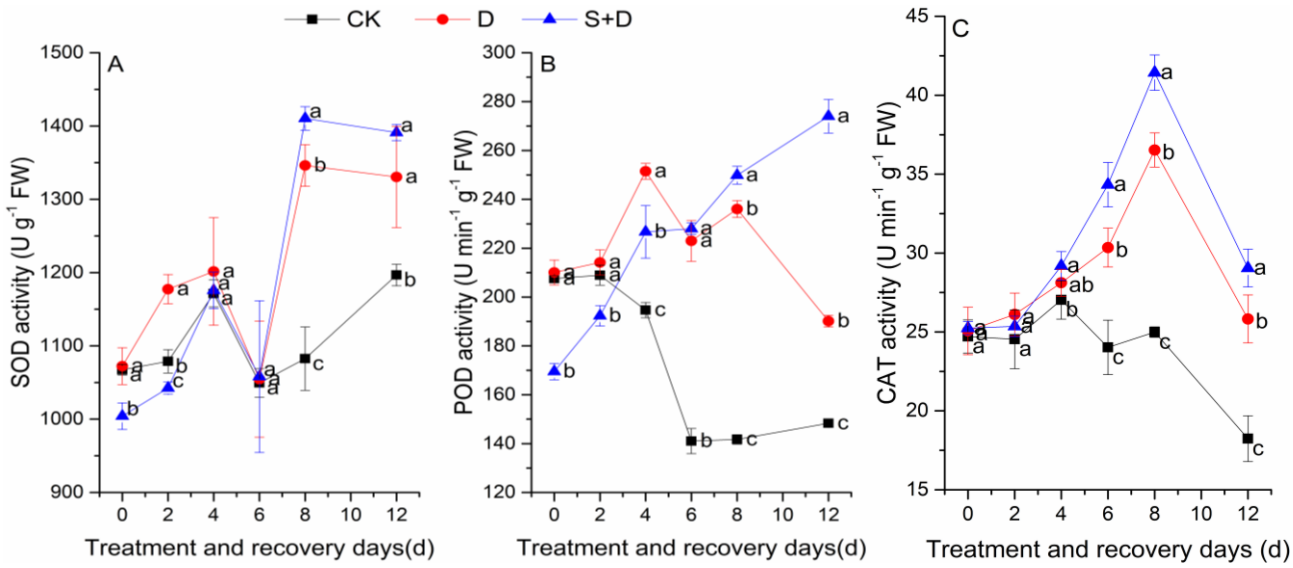

Fig. 7. Effect of uniconazole on SOD activity (A), POD activity (B) and CAT activity (C) under drought stress in industrial hemp leaves. From day 2 to 8 indicates the plants are drought stress; on day 12, the plants recover to the normal water condition (recovery is 4 days). Data are represented as the means ± SE (n = 4) of treatments. CK, D and S+D refer to treatments under normal water supply, drought stress and uniconazole + drought stress, respectively.

Discussion

The plant height and leaf area reduced with the prolongation of drought stress (Zhang *et al*., 2018). Plant height and leaf area of industrial hemp subjected to drought stress also significantly decreased contrasted with those of normal water supply plants in our results (Table 1). Uniconazole, one of the plant growth retardants, has been confirmed that can protect plants from injuries by inhibiting plant height and decreasing leaf area effectively (Schluttenhofer *et al*., 2011; Zhang *et al*., 2007; Fletcher *et al*., 2010). An analogical result was found in this study since uniconazole supplementation lessened plant height and leaf area, compared with drought stress only in the experiment (Table 1).

Drought affects the water absorption capability of plants, and the main effect is water content decrease. RWC can directly reflect plants' drought resistance as one of the simplest agricultural parameters (Hasanuzzaman *et al*., 2018). In our experiments, the leaf RWC decreased under drought stress as was reported in other researches (Huseynova, 2012; Hasanuzzaman *et al*., 2018). Plants treated with triazole showed higher tolerance to drought since they can maintain high water content and minimize water losses due to the long roots and small leaves (Fletcher *et al*., 2010). Similarly, hemp seeds soaked with uniconazole exhibited increased leaf RWC under drought stress (Fig. 1A).

Accumulations of proline (Pro), betaine, and glycol in plants can lessen the effects of environmental stresses. Pro, one of the major osmolytes, plays a critical function not just in plant development but in responses to stress. Hu *et al*., (2019) indicated that Pro content increased significantly with a salt concentration in hemp. Anjum *et al*., (2012) found that Pro content continued to increase with the progression of drought in pepper plants to diminish the effects of drought stress. Our research also revealed that the Pro content increased rapidly in drought-treated hemp plants, especially drought stress for 8 days (Fig. 1B). Interestingly, Pro content of uniconazole-treated plants considerably decreased in contrast to that of non-uniconazole-treated ones under drought stress (Fig. 1B), which was comparable to the reports of Upadhyaya *et al*., (1991). Hasanuzzaman *et al*., (2018) also discovered that silicon reduced Pro content compared with drought stress alone in rapeseed seedlings, which mainly due to the increase in Pro content triggered by cells stress-induced damage (Upadhyaya *et al*., 1991; Bhaskaran *et al*., 1985) rather than as a deliberate protective mechanism response to drought stress (Greenway & Munns, 1980).

Chl and Car are often used as indicators gauging plant performance under environmental stress. A lessening of Chl content subjected to drought stress (Meher *et al*., 2018) is largely due to chloroplast structure damage and Chl degradation by chlorophyllase and peroxidase as well as phenolic compounds (Salehi *et al*., 2016). In our paper, Chl contents of industrial hemp seedlings significantly decreased under drought stress (Fig. 2). Drought caused damage to the synthesis of photosynthetic pigments. Plants treated with triazole can increase the Chl contents in leaves (Kishorekumar *et al*., 2007) under drought stress (Rezayian *et al*., 2018) (Fig. 2). Our results similarly found that leaf Chl contents decreased with increasing drought stress, and uniconazole application improved the leaf Chl contents.

Drought stress causes ROS accumulation and membrane lipid peroxidation, damages the chloroplast structure, which may inhibit photosynthetic capacity (Cui *et al*., 2017). A decrease in P_n, G_s, and T_r, whereas an increase in C_i concentration was noted in hemp leaves under drought stress (Figs. 3A, 3B, 3C, 3D), similar to a study by Cui *et al*., (2017). This result may suggest that the lessening of P_n is due to non-stomatal restriction when drought stress continued for 8 days. One can argue that drought stress damages the chloroplast structure in hemp leaves (Fig. 4),

resulting in photosynthetic capacity decrease and CO_2 assimilation reduction. Uniconazole application in plants has been shown to affect the leaf conductance and hormone levels, maintain membrane stability, improve relative chlorophyll concentration, increase leaf photosynthetic rates and enhance plant resistance to adversity stress (Leul & Zhou, 1998; Steffens & Zimmerman, 1992). In our study, the uniconazole-treated plants exhibited significantly improved photosynthesis compared with the non-uniconazole-treated plants against drought stress, which was comparable to studies of Zhang *et al*., (2007). Uniconazole application significantly increased P_n, G_s, and T_r, but decreased C_i (Fig. 3). Uniconazole application could also alleviate the damage to the chloroplast structure (Fig. 4) and improve the leaf RWC (Fig. 1C), thus enhancing P_n, G_s, and T_r. Consequently, these effects increased the utilization of CO_2 and reduced C_i in hemp seedling leaves.

PSⅡ converts light energy to chemical energy in higher plants and is a major part of photosynthetic damage during stress (Sharkey & Zhang, 2010). The absorption, transfer, dissipation, distribution, and utilization of light energy are reflected directly by Chl fluorescence, which can be used as an indicator of plant stress resistance under disadvantages (Ow *et al*., 2011). Our results demonstrated that ETR and F_v/F_m decreased significantly in the drought-treated plants (Figs. 3E, 3F). These responses are analogous to those obtained by Su *et al*., (2007), Colom & Vazzana, (2003) and Pilon *et al*., (2018). Drought stress damages photosynthesis organ, and the initial part is associated with PSⅡ. The primary photochemistry activity of PSⅡ is inhibited, the active centre of PSⅡ is damaged, the photosynthetic electron transport is limited by drought stress and the ability of chloroplast membrane complexes to transform light energy into chemical energy is altered, causing a reduction in chloroplast quantum yield (Zai *et al*., 2012). The F_v/F_m of the uniconazole-treated plants was greater than that of non-uniconazole-treated plants subjected to drought stress (Zhang *et al*., 2007). Our results illustrated that uniconazole supplementation noticeably increased ETR and F_v/F_m in comparison with non-uniconazole-treated plants under drought stress (Figs. 3E, 3F). Uniconazole application is beneficial for the photosynthetic pigments of plant leaves to convert the captured light energy into chemical energy at high speed and efficiency, thus providing sufficient energy for carbon assimilation and improving photosynthetic efficiency.

The thickness and tightness of the palisade and spongy tissues in the plant leaves are two of the indicators of plant resistance to drought stress (Shields, 1950). Previous studies had suggested that leaf structure arranged loosely with larger intercellular gap and lower starch grains under adversity stress (Fan *et al*., 2019). Similar studies were found here, the density of palisade tissue cells decreased, the arrangement of spongy tissue cells was disordered, the leaves became thicker, the internal space of leaves increased and the starch grains decreased subjected to drought stress (Fig. 4B). Furthermore, an increase in leaf thickness contributes to the reduction of transpiration and water loss to resist drought stress damage (Dong & Zhang, 2001; Chartzoulakis *et al*., 2002). The uniconazole-treated plants displayed a less affected leaf cell structure and more starch grains under drought stress (Fig. 4C). The abundant chloroplasts in mesophyll cells under uniconazole treatment can improve the photosynthetic capability and consequently sustain the production of organic substances (i.e. starch).

ROS as $O_2^{\cdot-}$, H_2O_2 and $HO^{\cdot}$ may accumulate in plants under different stresses (Zhang *et al*., 2018). Drought stress affects plant survival as a most limiting stress factor (Talbi *et al*., 2015). Plants exhibit oxidative stress responses to ROS overproduction, which results in cell membrane damage and cellular macromolecules peroxidation under drought stress; meanwhile, the increase of MDA content is an index of membrane injury (Zhou *et al*., 2015; Gill & Tuteja, 2010). In our study, histochemical staining localized the $O_2^{\cdot-}$ and H_2O_2 in hemp leaves. The cumulation of $O_2^{\cdot-}$ and H_2O_2 increased in drought-treated plants compared with that in control (Fig. 5), which was close to the studies of Shaw *et al*., (2016). The superoxide anion production rate, hydrogen peroxide content and MDA increased rapidly under drought stress (Fig. 6), which corresponds to several preceding reports (Liu *et al*., 2009; Jiao *et al*., 2016). Uniconazole application reduced accumulations of $O_2^{\cdot-}$, H_2O_2 and MDA causing by water stress (Zhang *et al*., 2007), heat stress (Zhou & Leul, 1999), sulphur dioxide stress (Upadhyaya *et al*., 1991) and waterlogging stress (Leul & Zhou, 1998) since the uniconazole-treated plants were capable of maintaining high enzymatic and non-enzymatic antioxidant systems that can eliminate ROS effectively and defend the cell membrane from damage (Zhang *et al*., 2018). Similarly, we found that uniconazole application markedly lowered the accumulation of $O_2^{\cdot-}$, H_2O_2 and MDA under drought stress (Figs. 5, 6).

Antioxidant enzymes are ROS scavengers that can protect plants from the damage of ROS overproduction (ÖZdemir *et al*., 2004). SOD and CAT as the first defense antioxidants can inhibit the generation of reactive species or free radicals in cells; SOD and CAT scavenge superoxide radicals to hydrogen peroxides (H_2O_2) and decompose H_2O_2 to O_2 and H_2O, respectively (Ighodaro & Akinloye, 2018; Li *et al*., 2011). POD is a stress-related enzyme (Li *et al*., 2011) that can also detoxify H_2O_2 to H_2O (Boogar *et al*., 2014). Antioxidant enzyme activities, as SOD, POD, and CAT, apparently heightened when drought stress continued for seven days (Zhang *et al*., 2018). Ghobadi *et al*., (2013) also revealed that CAT activity strengthened with drought severity, whereas POD activity showed no significant change. Similarly, we confirmed that antioxidant enzymes (SOD, POD, and CAT) improved with the aggravation of drought stress to relieve the oxidative stress damage (Fig. 7). Triazole compounds can improve antioxidant enzyme activities in oxidative stressed plants (Fletcher *et al*., 2010). Uniconazole supplementation enhances the activity of SOD and POD to reduce oxidative injury under water stress (Zhang *et al*., 2007) and heat stress (Zhou & Leul, 1999). Similarly, we found that uniconazole-treated plants indicated a dramatic increment in SOD, POD, and CAT activities in the leaves of industrial hemp contrasted with non-uniconazole-treated plants subjected to drought stress (Fig. 7). Uniconazole played a crucial role in maintaining antioxidant enzyme activities of drought-treated plants. It promoted the antioxidant defense system to scavenge excessive ROS for enhancing the drought tolerance of plants.

Conclusion

Our research provided evidence that exogenous uniconazole alleviated drought-induced damage in industrial hemp seedlings by lessening plant height and leaf area, increasing leaf relative water content, and decreasing the proline contents. Uniconazole application maintained the integrity of the leaf cell structure, lowered the damage to membranes, and reduced drought stress damage on the photosynthetic system. Exogenous uniconazole enhanced antioxidant enzyme activities and promoted the antioxidant defense system to scavenge excessive reactive oxygen species. Therefore, our studies demonstrated that uniconazole application could have an important practical significance in protecting industrial hemp against soil moisture deficit.

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