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Different mechanisms of photosynthetic response to drought stress in tomato and violet orychophragmus

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Abstract

Carbonic anhydrase (CA) catalyzes reversible hydration of CO_2 and it can compensate for the lack of H_2O and CO_2 in plants under stress conditions. Antioxidative enzymes play a key role in scavenging reactive oxygen species and in protecting plant cells against toxic effects. Tomato represents a stress-sensitive plant while violet orychophragmus belongs to adversity-resistant plants. In order to study the drought responses in tomato and violet orychophragmus plants, CA and antioxidative enzyme activities, photosynthetic capacity, and water potential were determined in plants under drought stress. We found that there were similar change trends in CA activity and drought tolerance in violet orychophragmus, and in antioxidative enzymes and drought tolerance in tomato plants. Basic mechanisms of drought resistance should be identified for understanding of breeding measures in plants under stress conditions.

Additional key words: chlorophyll fluorescence; gas exchange; *Orychophragmus violaceus*; *Solanum lycopersicum*.

Introduction

Carbonic anhydrase (CA, EC 4.2.1.1) catalyzes the reversible hydration of CO_2 ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$) and plays a direct role in photosynthesis. Plant CA can maintain adequate concentrations of CO_2 for Rubisco (Fett and Coleman 1994) and play a role in stomatal closure/opening (Hu *et al*. 2010). CAs have been also reported to play other functions, such as lipid synthesis, disease resistance, and tolerance to abiotic stresses (Kaul *et al*. 2011). Drought stress is one of the main abiotic streses limiting agricultural production and causing yield losses up to 80% in some arid regions (Cuellar-Ortiz *et al*. 2008). The complex responses of drought stress in plants consist of several pathways, such as transduction of signal, changes in expression of some specific genes, adjustment of metabolism *etc*. Identifying the traits of drought resistance is necessary for understanding the breeding measures of plants under stress conditions.

CA is a kind of ubiquitous zinc metalloenzyme in living organisms that catalyzes the reversible interconversion with very high catalysis rates reaching 10^6 s^{-1} (Khalifah 1980). None of enzyme family, except CA, have been described from catalytic, genic, cellular, and tissue aspects including almost all life forms. Plant CA facilitates CO_2 supply to Rubisco in C_3/C_4 plants, and facilitates CO_2 supply to phospho*enol*pyruvate carboxylase (PEPC) in C_4 plants (Tiwari *et al*. 2005). Higher plant chloroplastic CA content in higher plants, is sufficient while cytoplasmic CA content is inadequate; some cytoplasm CAs are induced by stress conditions (Badger 2003). Therefore it is more important to study the function and application of cytoplasm CAs in plant stress resistance. Plants need to adapt to the low CO_2 conditions resulting from stomatal closure under drought stress. It is important that the function of CA is to keep the high concentrations of CO_2

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Abbreviations: CA – carbonic anhydrase; CAT – catalase; C_i – intercellular CO_2 concentration; E – transpiration rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; FM – fresh mass; F_v/F_m – maximal photochemical efficiency of PSII; g_s – stomatal conductance; MDA – malondialdehyde; MiD – mild drought; MoD – moderate drought; $O_2^{\cdot-}$ – superoxide radical; OV – violet orychophragmus (*Orychophragmus violaceus*); PEPC – phospho*enol*pyruvate carboxylase; P_N – net photosynthetic rate; ROS – reactive oxygen species; RWC – relative water capacity; SeD – severe drought; SL – tomato (*Solanum lycopersicum*); SOD – superoxide dismutase; WUE – water-use efficiency; WW – well watered.
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around Rubisco and maintain the function of photosynthetic system under low CO_2 concentration caused by drought stress (Moroney *et al*. 2011). CA can quickly convert HCO_3^- into H_2O and CO_2 in order to compensate for the lack of H_2O and CO_2 under water stress. However, the role of plant CA in responding to abiotic stress has not been systematically examined.

At the same time, plants have developed a number of homeostatic antioxidant mechanisms to protect themselves against toxic reactive oxygen species (ROS) under conditions of drought stress. These mechanisms employ antioxidant enzymes, such as superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6), as well as nonenzymatic antioxidants. For example, in leaf cells, SOD catalyzes the dismutation of two molecules of superoxide radical ($O_2^{\cdot-}$) into oxygen and hydrogen peroxide (H_2O_2), and CAT reduces H_2O_2 to H_2O (Mittler 2002).

Tomato is one of vegetable crops with the largest cultivated area in the world and it is sensitive to environmental stress. The CA activity in tomato plants belongs to lower among several Solanaceae species we have studied. Violet orychophragmus (OV) is a plant species known for its tolerance to drought stress and barren soil. The CA activity in OV is almost the highest among the tested Cruciferae plants (Wu *et al*. 2006). In order to study the different stress/response mechanisms in tomato and OV plants under drought stress, our study investigated the activities of CA and antioxidative enzymes in these plants under the conditions of drought stress. The understanding of drought stress–response mechanisms could help improve plant drought resistance and contribute to higher crop productivity.

Materials and methods

Plant materials and stress treatments: Tomato (*Solanum lycopersicum* L. cv. 'Hezuo 908', SL) and violet orychophragmus (*Orychophragmus violaceus* L., wild variety of Jiao Hill in Zhenjiang, OV) plants were used as plant materials. The plants were grown in a greenhouse at 25 ± 5°C under a photoperiod of 16/8 h [day/night, 300–1,000 μmol(photon) m^{-2} s^{-1}] and 75 ± 5% of relative humidity. The seedlings were raised in a seedbed and were then transplanted into breeding ground filled with sterilized soil at Jiangsu University (32°12'N, 119°27'E).

Six-week-old plants were exposed to stress conditions for 14 d. Four water treatments were imposed: (*1*) well watered (WW), soil relative water capacity (RWC) of 75 ± 5%, (*2*) mild drought (MiD), RWC of 60 ± 5%, (*3*) moderate drought (MoD), RWC of 45 ± 5%, and (*4*) severe drought (SeD), RWC of 30 ± 5%. The tested plants were irrigated by drip irrigation and soil relative water capacity was determined at 10:00 h and 15:00 h according to soil gravimetric method.

CA and Rubisco activities: CA activity was determined according to electrochemical method using pH antimony microelectrode (Wu *et al*. 2006). Leaves (3–5 g) were grinded in a mortar. The assay procedure was carried out at 0–2°C. One unit of enzymatic activity (Willburg-Anderson, WA-U) was defined as $10(t_0/t_1 - 1)$. The duration of t_1 and t_0, when pH decreased for one unit, were calculated under conditions of enzyme activation according to the curve of time and potential value. The duration of t_0, when pH decreased for one unit, were calculated under conditions of enzyme inactivation (inhibitor added). Enzyme activity was expressed in WA-U, and unit was calculated per mg of proteins.

Rubisco activity was determined according to the method described by Lan and Mott (1991). Total protein was extracted according to the Bradford method (Bradford 1976). Rubisco activity was determined using a plant *Rubisco Activity Detection Kit* (*GenMed Scientifics*, Shanghai, China). The change of 3-phosphoglycerate transformed into NADH was measured by enzyme coupling method. Enzyme activity was calculated as μmol(CO_2) min^{-1} mg^{-1}(protein).

The test of expressions of *OvcCA* and *SlcCA*: Total RNA was extracted using the Trizol reagent extraction procedure from the treated plants. The detailed method of Northern blot was described by Sun *et al*. (2010). The fragment from the cDNA of *OvcCA* (violet orychophragmus cytoplasm CA gene, according to the *GenBank* databases under accession number AJ849375, NCBI) and *SlcCA* (tomato cytoplasm CA gene, according to the *GenBank* databases under accession number GU143061, NCBI) was used as the gene-specific probe, respectively.

SOD activity and CAT activity: SOD activity was assayed according to the classical method of Giannopolitis and Ries (1977) by measuring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT). One unit (U) of SOD activity was defined as the amount of enzyme used for the inhibition of NBT reduction to half of the control.

CAT activity was measured according to the classical specrtrophotometrical method of Aebi (1974) by decomposition of H_2O_2 at 240 nm (*UV-757CRT*, Hangzhou, China). One unit of CAT activity (per protein) was defined as mmol L^{-1} H_2O_2 degraded per min.

$O_2^{\cdot-}$ and H_2O_2 contents: The $O_2^{\cdot-}$ content was determined as follows: 2.0 g leaves were ground in grinding mortar with 10% (w/v) trichloroacetic acid. The grinding mixture was centrifuged for 10 min at 4,000 × *g*. The supernatant was supplemented by 10 mmol L^{-1} hydroxylamine chloride and kept at 25°C for 20 min. And then, the supernatant was replenished by 17 mmol L^{-1} ρ-aminobenzene

sulfonic acid and 7 mmol L^{-1} α-naphthylamine and kept at 25°C for 20 min again. Diethylether was added into the above mixture which was centrifuged at 1,500 × g for 5 min. At last, the supernatant was collected and the absorbance was determined spectrophotometrically at 530 nm (*UV-757CRT*, Hangzhou, China). $O_2^{\cdot-}$ content was calculated per leaf fresh mass (FM).

The H_2O_2 content was determined according to the method of Sairam and Srivastava (2002). The absorbance was determined at 415 nm (*UV-757CRT*, Hangzhou, China). At least three replicate measurements were used for each treatment. The H_2O_2 content was calculated per leaf FM.

Free proline and soluble sugars: Free proline content was quantified according to the method of Meloni *et al*. (2004). Sulfosalicylic acid was used to dissolve proline and acidic ninhydrin was used for staining. The absorbance was determined at 520 nm (*UV-757CRT*, Hangzhou, China). Free proline content was calculated per fresh leaf mass.

The soluble sugar content was determined according to the method of Zhao *et al*. (2002). Leaves (0.5 g) were ground in grinding mortar with 4 ml of ethanol (80%), and the grinding mixture was centrifuged for 10 min at 5,000 × *g*. The supernatant was supplemented by 0.5 g of acticarbon, and kept in water bath at 80°C for 30 min and then filtered. The 1 ml aliquots of the extract were supplemented by 5 ml of anthrone. The mixture was boiled for 10 min and cooled. Absorbance was determined at 630 nm (*UV-757CRT*, Hangzhou, China) and the sugar content was calculated from the standard curve. Soluble sugar content was calculated per leaf FM.

Plasma membrane injury and chlorophyll (Chl) content: The content of malondialdehyde (MDA) can be used to indicate a degree of plasma membrane damage in plants. The MDA content was measured according to the method of Sun *et al*. (2010). MDA content was calculated per fresh leaf mass.

The Chl content in the tested leaves was measured according to the method of Hemavathi *et al*. (2010). Chl *a* and Chl *b* absorbances were determined at 663 nm and 646 nm, respectively (*UV-757CRT*, Hangzhou, China). Acetone (80%) was as an extract solvent, and Chl content was calculated per leaf FM.

Photosynthetic parameters were measured with a portable photosynthetic system (*CIRAS-2*, *PP Systems*, Boston, USA). Tested plants were acclimated at temperature of 25°C and PPFD of 100 µmol m^{-2} s^{-1} for about 30 min, in order to open the stomata, and then adapted under PPFD of 800 µmol m^{-2} s^{-1} for about 15 min. At last, net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), leaf water-use efficiency (WUE), and intercellular CO_2 concentration (C_i) were measured at 25°C, PPFD of 800 µmol m^{-2} s^{-1}, O_2 concentration of 21%, ambient CO_2 concentration of 360 µmol mol^{-1}, and relative humidity of 75 ± 5%.

Photochemical quantum yield was measured by using a portable Chl fluorescence meter (*FMS2*, *Hansatech*, Norfolk, UK). Tested plants were adapted at temperature of 25°C and PPFD of 800 µmol m^{-2} s^{-1} for about 30 min, and then adapted in dark for 30 min after 3 s of far red light (PPFD of 3,000 µmol m^{-2} s^{-1}). Minimal fluorescence yield of the dark-adapted state (F_0), maximal fluorescence yield of the dark-adapted state (F_m), and maximal photochemical efficiency of PSII (F_v/F_m) were measured at 25°C. citace

Water potential and leaf water content: Simultaneously with the determination of photosynthetic capacity, water potential was measured in leaves by using a dew point microvoltmeter *PSYPRO* (*Water Potential System*, South Logan, Utah, USA) at 25°C and relative humidity of 75 ± 5%.

The leaf water content as a percentage of FM was calculated at the time of leaf sampled. Dry matter (DM) was calculated after fresh leaves were dried in a drying oven at 50°C for 48 h. The leaf water content [%] was calculated as 100 × (FM – DM)/FM.

Statistical analysis: At least three replicate measurements were used for each treatment. The values shown in the figures were mean values ± SD. Means were tested by least significant difference at $P \leq 0.05$.

Results

CA and Rubisco activities: Under the same stress treatment, tomato leaf CA activities increased slightly, meanwhile the CA activities in OV leaves increased apparently. Under SeD stress, the CA activity in OV was higher than that of the control, while the CA activity in tomato leaves was lower than that of the control (Fig. 1*A*). Rubisco activity changed significantly in all the tomato and OV plants under drought stress and the trends in both plant species were similar (Fig. 1*B*).

Expression of *OvcCA* and *SlcCA*: The expression level varied with the different soil water content, indicating that the expression of *OvcCA* in OV and the expression of *SlcCA* in tomato plants were induced by drought stress (Fig. 2*A*). However, compared to the CA expression level in OV plants, there was slight change of the CA expression level in tomato plants under the same degree of stress (Fig. 2*B*). Under SeD stress, the CA expression in OV was significantly higher than that of the control, while

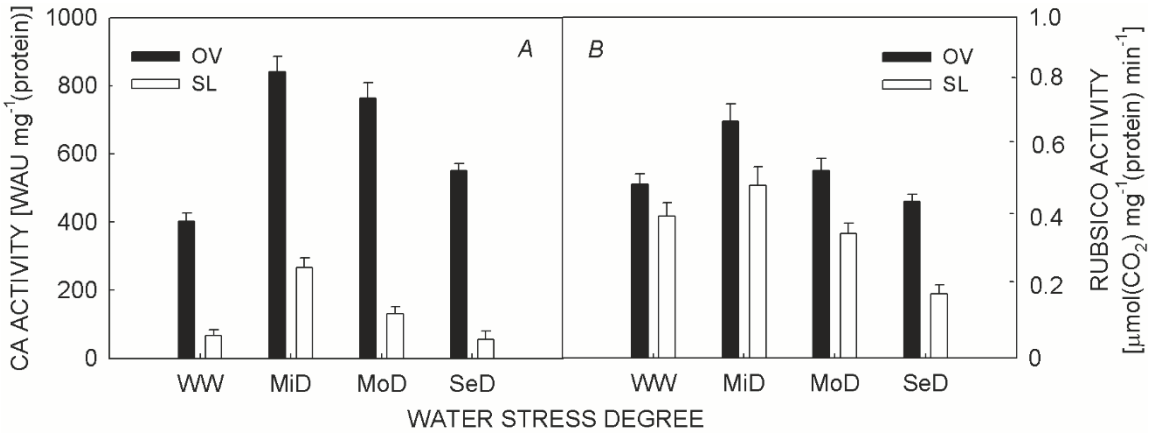

Fig. 1. The activities of carbonic anhydrase (CA) (*A*) and Rubisco (*B*) in leaves under conditions of drought stress for 7 d. OV – violet orychophragmus (*Orychophragmus violaceus*), SL – tomato (*Solanum lycopersicum*). WW – well watered, MiD – mild drought, MoD – moderate drought, SeD – severe drought.

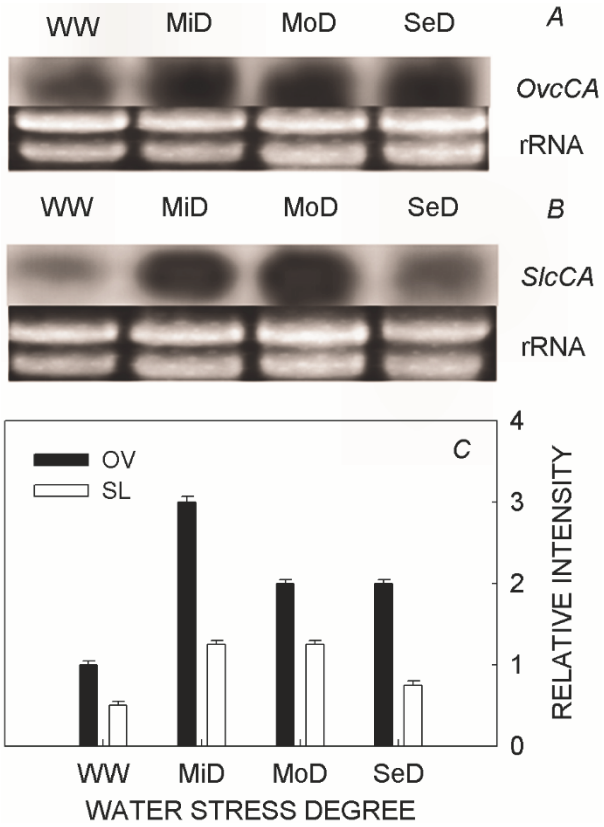

Fig. 2. The expression of *OvcCA* gene (*A*), the expression of *SlcCA* gene (*B*), and the relative intensity of carbonic anhydrase expression (*C*) in plants under conditions of drought stress. WW – well watered, MiD – mild drought, MoD – moderate drought, SeD – severe drought. OV – violet orychophragmus, SL – tomato.

the CA expression in tomato leaves was not significantly higher than the control.

SOD and CAT activity, $O_2\cdot^-$ and H_2O_2 content: Compared with the control, SOD activities in tomato plants increased significantly, while those in OV plants changed slightly under the conditions of drought stress. Under SeD stress, SOD activity in tomato plants was significantly higher than that of the control, while SOD activity in OV leaves was not significantly higher than the OV control (Fig. 3*A*).

Compared with the control, CAT activities in tomato plants increased significantly, while CAT activities in OV plants changed slightly under the conditions of drought stress. In tomato plants, CAT activity was the highest under MoD, while CAT activity under MiD was the highest in OV plants (Fig. 3*B*).

$O_2\cdot^-$and H_2O_2 contents in all tested plants constantly increased with the increase of drought stress severity. $O_2\cdot^-$ and H_2O_2 contents in OV plants increased significantly, while $O_2\cdot^-$ and H_2O_2 contents in tomato plants increased slightly under drought stress. $O_2\cdot^-$ and H_2O_2 contents in tomato plants were higher than those of the control from the beginning of MoD stress at 7 d. Meanwhile $O_2\cdot^-$ and H_2O_2 contents in OV plants were higher than the control from the beginning of MiD stress at 7 d (Fig. 3*C*,*D*).

Free proline and soluble sugar contents in all tested plants increased first and then decreased. Free proline and soluble sugar contents in the OV plants were significantly higher than those of the tomato plants under the same degree of drought stress (Fig. 4).

Plasma membrane injury and Chl content: Plasma membrane injury in the plants was involved under drought stress. MDA contents in tomato plants increased more than the MDA contents in OV plants under drought stress. MDA contents in tomato and OV plants were significantly higher at the beginning of MoD stress for 7 d compared with the control (Fig. 5*A*).

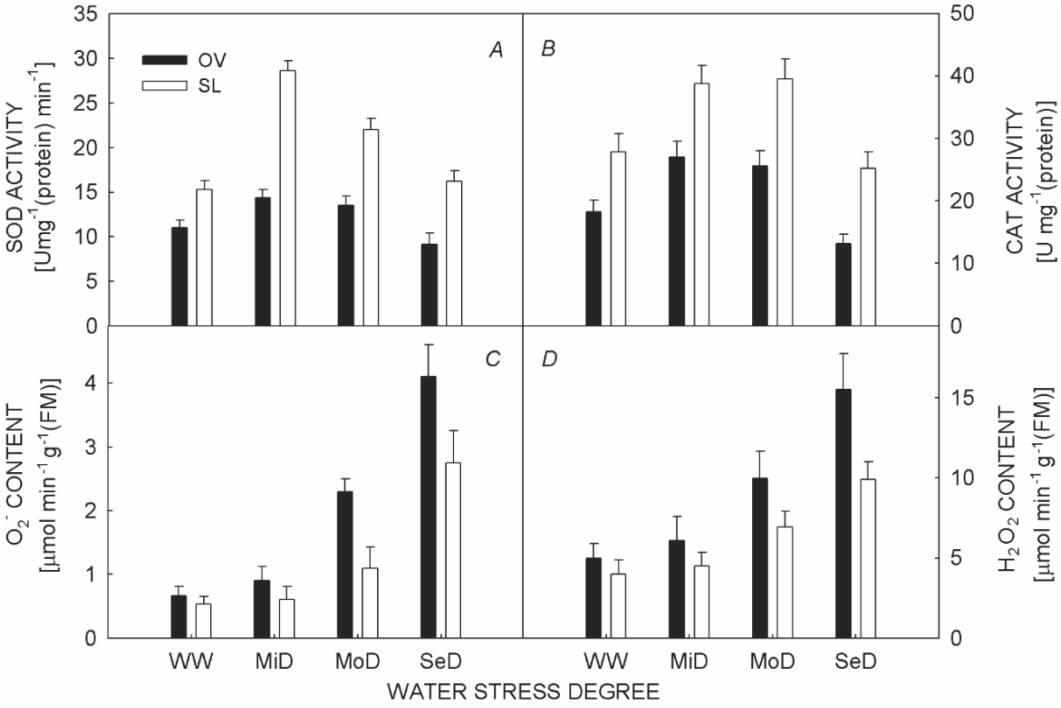

Fig. 3. The activities of superoxide dismutase (SOD) (*A*), catalase (CAT) (*B*), contents of $O_2^{\cdot-}$ (*C*), and H_2O_2 (*D*) in the leaves under the conditions of drought stress for 7 d. WW – well watered, MiD – mild drought, MoD – moderate drought, SeD – severe drought. FM – fresh mass, OV – violet orychophragmus, SL – tomato.

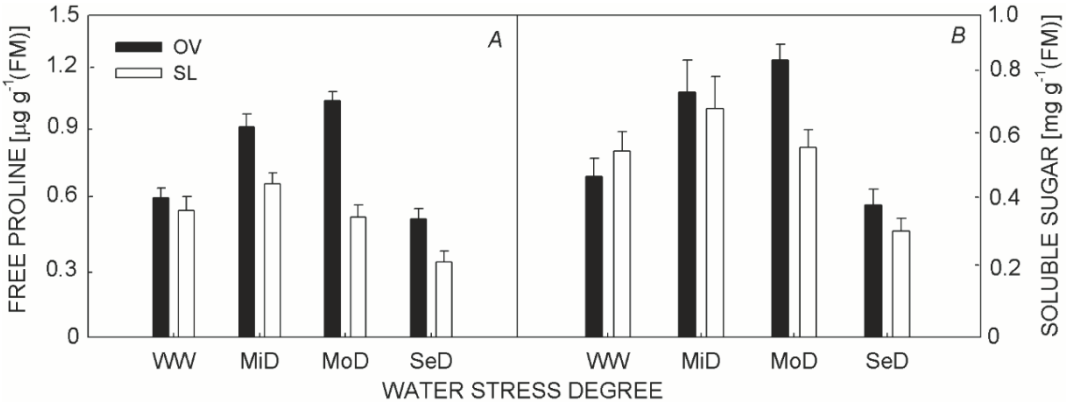

Fig. 4. The free proline (*A*) and soluble sugar (*B*) contents in the leaves under the conditions of drought stress for 7 d. WW – well watered, MiD – mild drought, MoD – moderate drought, SeD – severe drought. FM – fresh mass, OV – violet orychophragmus, SL – tomato.

The Chl content was significantly higher in the OV plants than that of tomato plants when they were subjected to drought stress (Fig. 5*B*).

Photosynthetic parameters: In OV leaves, g_s first increased and then decreased along with advancing drought stress, while g_s in tomato leaves decreased (Fig. 6*A*). *E* decreased obviously under the conditions of drought stress both in OV and tomato plants (Fig. 6*B*). C_i increased in all plants under drought stress, and C_i in tomato plants increased significantly compared to C_i in OV plants (Fig. 6*C*). WUE first increased and then decreased along with the advancing stress in both OV and tomato plants (Fig. 6*D*).

Photosynthetic capacity, water potential, and leaf water content: P_N of tested plants decreased under drought stress, and the decrement of P_N was more significant in tomato plants than that in OV plants with the extension of stress degree (Fig. 7*A*). F_v/F_m in tomato and OV plants decreased under drought stress (Fig. 7*B*). Water potential in tomato and OV plants decreased significantly under drought stress (Fig. 7*C*). The average leaf water content in tomato and OV plants decreased significantly under drought stress, but OV showed greater ability to preserve the higher leaf water content than that of the tomato plants (Fig. 7*D*).

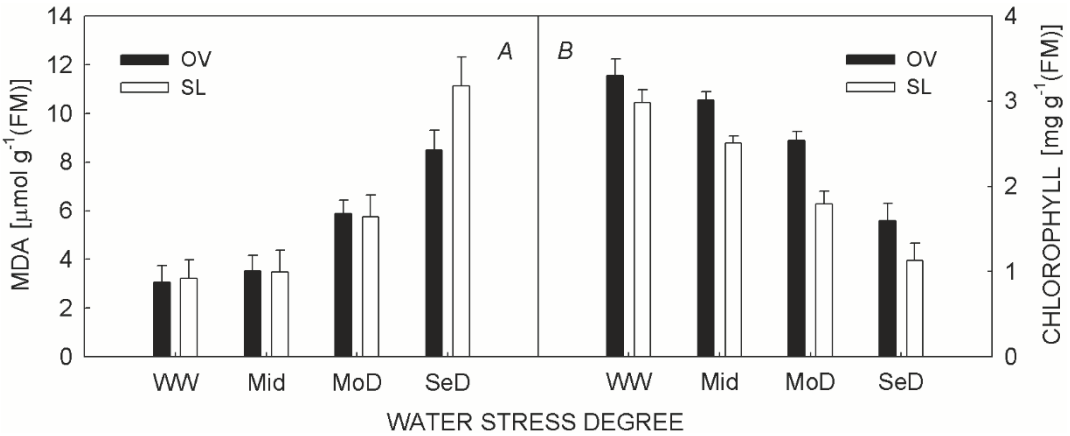

Fig. 5. The changes of leaf plasma membrane injury (*A*) and chlorophyll content (*B*) in the leaves under the conditions of drought stress for 7 d. WW – well watered, MiD – mild drought, MoD – moderate drought, SeD – severe drought. FM – fresh mass, OV – violet orychophragmus, SL – tomato.

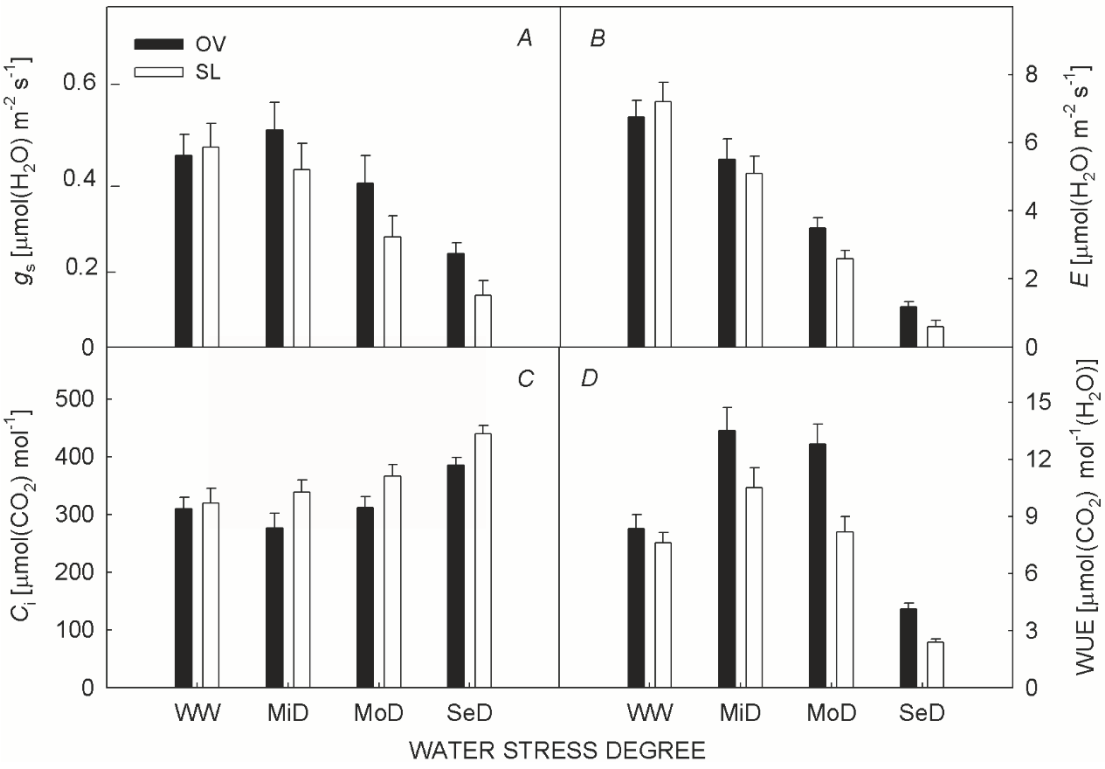

Fig. 6. The changes of g_s (*A*), *E* (*B*), C_i (*C*), and WUE (*D*) in the leaves under the conditions of drought stress for 7 d. g_s – stomatal conductance, *E* – transpiration rate, C_i – intercellular CO_2 concentration, WUE – water-use efficiency, OV – violet orychophragmus, SL – tomato. WW – well watered, MiD – mild drought, MoD – moderate drought, SeD – severe drought.

Discussion

Environmental drought causes leaf stomata closure in order to maintain relatively high water potential in plants. At the same time, too much salt in soil reduces the soil solution permeability for plant roots and leads to difficulties in root water uptake and results in physiological drought. The physiological drought causes leaf stomatal closure in order to avoid further water loss and maintain relatively high water potential in leaves. However, the closed stomata seriously hinder CO_2 entry into the mesophyll cells, thus reduce photosynthesis (Sage and Coleman 2001). Some plants have gradually evolved a kind of carbon-accumulation mechanism in order to increase the carbon assimilation by Rubisco and CA plays an important role in this process (Moroney *et al*. 2011).

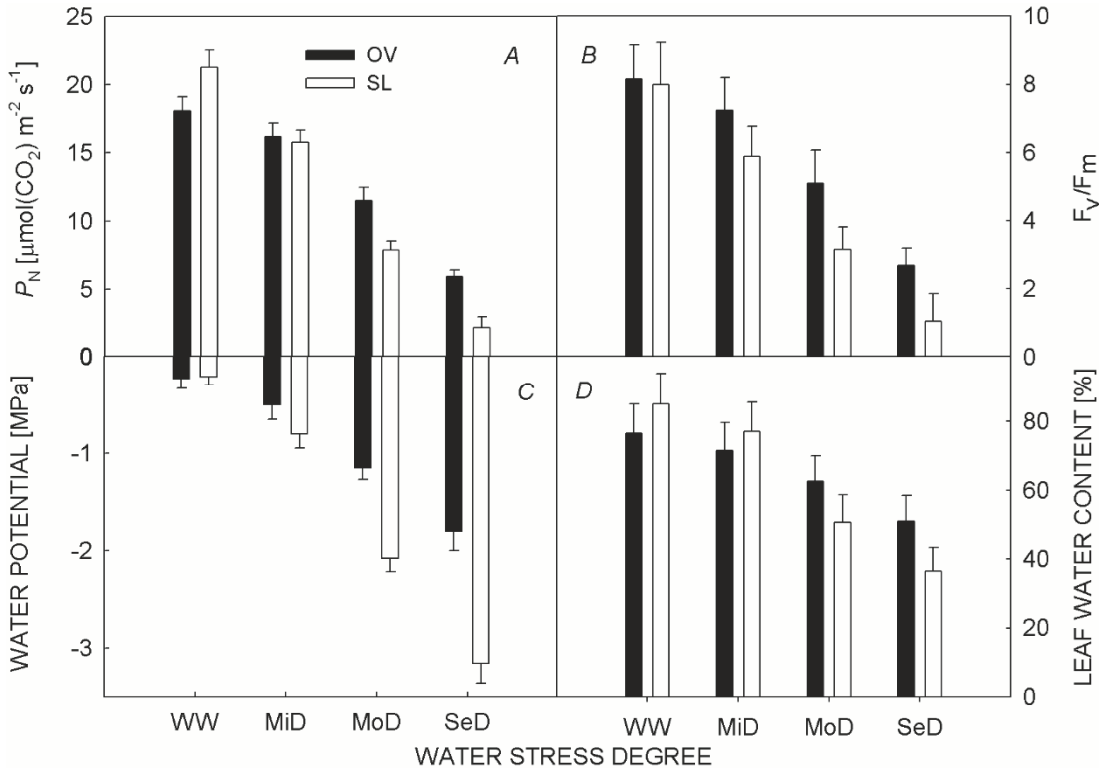

Fig. 7. The changes of net photosynthetic rate (P_N) (*A*), maximal photochemical efficiency of PSII (F_v/F_m) (*B*), water potential (*C*), and leaf water content (*D*) in the leaves under the conditions of drought stress for 7 d. WW – well watered, MiD – mild drought, MoD – moderate drought, SeD – severe drought. OV – violet orychophragmus, SL – tomato.

A study of Li *et al*. (2010) suggested that the expression of CA was related to environmental stress and the CA activity was upregulated by salt and osmotic stresses. This study showed that CA expression and activity was regulated by drought stress and the change of CA activity in OV was more obvious than that in the tomato plants (Figs. 1,2).

Under conditions of stress, the enzymatic activity of Rubisco is limited by the low concentration and diffusion rate of CO_2 (Reinfelder 2011). Under stress conditions, the capacity of CA for efficient delivery of CO_2 to Rubisco would restrict photorespiration, and therefore would improve the crop yield potential (Surridge 2002). Compared to activities of antioxidant enzymes, activities of CA and Rubisco in OV were enhanced more than those in the tomato plants under drought stress. It suggests that CA and Rubisco enzymes in OV photosythesis are more resistant to drought stress than the enzymes in the tomato plants (Fig. 1). This study showed that the OV and tomato plants maintained the higher CA enzyme activity under mild drought stress, retained higher photosynthetic capacity, higher WUE, and higher water potential without cellular damage. Under severe drought stress, the regulation capacity of CA was out of control and it resulted in damage of photosynthetic apparatus in the tested plants. However, CA activities and photosynthetic capacity in OV was still higher than that in the tomato plants (Figs. 1,6,7).

At the same time, the protective function of antioxidative enzymes were lost, and it could result in plasma membrane injury in the plants under severe drought stress. Drought stress stimulated ROS production, damaged membrane integrity, and some enzyme functions were reduced or lost. This study suggested that activities of antioxidative enzymes were regulated by drought stress and enhanced activity of antioxidative enzymes prevented ROS from damaging cellular structures. The changes of SOD and CAT activity in the tomato plants were more pronounced than those in the OV plants (Fig. 3).

Osmotic adjustment is thought to be one of the adaptive reactions in order to resist drought stress in plants. However, some crops, such as tomato, accumulate small amounts of osmotically active substances under water stress (Reddy *et al*. 2004). We found that the accumulation of soluble sugars and free proline was not outstanding in the tomato plants, but it was more substantial in the OV plants (Fig. 4). We suggest that osmotic adjustment might be important for OV resistance to water stress.

Conclusion: There was the similar trend in changes of CA activity, Rubisco activity, water-use efficiency, and photosynthetic efficiency in the OV plants. There was another trend between antioxidative enzymes, membrane integrity protection, and photosynthetic efficiency in the tomato plants. The OV and tomato plants utilize different stress/tolerance mechanisms under the conditions of

drought stress. The transfer of key enzymes from stress-resistant plants to stress-sensitive plants could improve photosynthetic capacity and increase crop yield. The results indicated that different resistance as breeding measures should be taken into account according to different stress responses in different plants. Our work provides the theoretical basis for cultivation and breeding of plants drought resistance in future studies.

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