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Full Length Research Paper

Effects on physiological and biochemical characteristics of medicinal plant pigweed by drought stresses

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The physiological and biochemical responses of pigweed under artificially simulated drought stresses were examined to provide a theoretical basis for medicinal plant cultivation and effectively increasing agricultural production in arid areas in this study. The results showed that the relative water content in leaves of the control, mild-stressed, moderately-stressed and severely-stressed plants were 94.07, 87.01, 76.35 and 64.03%, respectively. Under moderate drought stress, the relative water content (RWC) and free water content (FWC) in leaves were decreased, while the bound water content (BWC) was increased. The activities of superoxide dismutase (SOD) and peoxidase (POD) in leaves reached the highest level among the four treatments. Membrane permeability, malondialdehyde (MDA) content and the O_2 production rate in leaves declined, whereas, soluble sugars, proline, K^+ and Ca^{2+} in leaves accumulated rapidly, indicating that pigweed has the ability to adapt to drought stress by regulating the internal osmolarity and protecting the membrane. Under severe drought stress, however, the O_2 production rate and the MDA content increased remarkably, causing membrane damage and increasing membrane permeability of leaf cells. The activities of SOD and POD were initially increased as compared to those under the moderate drought stress and then declined; ascorbic acid (ASA) content was also decreased. These results suggest that severe drought stress could cause some damage on the pigweed.

Key words: Pigweed, drought stress, osmoregulatory molecules, membrane lipid peroxidation, protective enzyme.

INTRODUCTION

Drought is one of the most severe constrains to crop production (Toumi et. al., 2008) and the bottleneck of agriculture development in various regions (Cao et al., 2003). A number of well-observed physiological and biochemical changes occur in a drought-stressed plant. Pigweed, also referred to as Lambsquarters, whose leaves are used not only as vegetable for human and feeding material for animals, but also as traditional Chinese medicine (such as clearing heat, dieresis, insecticidal, diarrhea, Eczema, etc) with strong growing capability and drought resistance, especially on alkaline land, pigweed can be found at various types of soils all over world (Yao et al., 2010). Pigweed is an ideal model for studying physiological processes and molecular mechanisms of plant responses to drought stress. So, pigweed is used as a model plant to research the drought tolerant mechanism of plant (Sun et al., 2010). Sun Cunhua had reported the influence of simulated drought on the drought resistance capability of pigweed (Sun et al., 1999), the impact of drought stress on photosynthesis (Sun et al., 2007), drought-induced protein of pigweed (Sun et al., 2009a, b) and other prominent physiological and biochemical responses of pigweed to drought stress. This thesis is to explore the physiological and biochemical reactions of pigweed to drought stress through simulated drought realized by manual control of moisture, hoping to provide a scientific evidence for understanding the mechanism of the drought-resistance in field crops and to provide a reference for the cultivation of drought-tolerant medicinal plants drought.

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MATERIALS AND METHODS

The seed of pigweed (*Chenopodium album* L.) harvested in the autumn of 2008 at Xuzhou is planted in pots under natural conditions. The pot is 25 cm in diameter and 23 cm high. The culture media in the pot was yellow fluvo-aquic soil obtained from the Yellow River sediments, which was characterized by a slightly high level of calcium carbonates and soluble salts as detected by alkaline reactions. Each culture pot contained 12 kg of culture media with a soil moisture content of 12.46% and a maximal moisture content of 29.8% after saturated watering. Sufficient base fertilizer was added. Well-stacked pigweed seeds were selected and sown at the end of March. When seedling emerged, 5 pigweed plants were kept in each culture pot and fostered under natural conditions of rooftop of the number 4 teaching building in Xuzhou Normal University.

Exposure to drought stress

When reaching 6 leaves (in mid-May), drought stress was imposed by irrigation while weighing method was used to control soil moisture to make the soil moisture meet the experimental requirements. 12 pots were selected randomly and divided into 4 groups (15 plants per group): including control: watered every morning to keep the 80% of field moisture capacity (well watered) for 20 days and then drought stressed 0 day before the measurement, mild stress: watered every morning to keep the 80% of field moisture capacity for 15 days and then drought stressed 5 days before the measurement, moderate stress watered every morning to keep the 80% of field moisture capacity for 10 days and then water stressed 10 days before the measurement and severe stress: watered every morning to keep the 80% of field moisture capacity for 5 days and then water stressed 15 days before the measurement, each group contains three pots. All pots were moved into houses to avoid influence of natural precipitation in the experiments.

On June 21st (namely the third day after the last watering of control group), the third to eighth functional leaves were collected for analytic determination. The soil moisture content is measured with soils 5 to 7 cm under soil surface. Each parameter is measured three times and the average is calculated, then the average of three pots is used. In 2009, this experiment is repeated and some experimental parameters modified.

Measuring method

Relative water content (RWC) was calculated with the leaf disc floating method according to Petsas (Petsas and Grammati, 2009) using the formula:

RWC = (FM–DM) ×100/(SM–DM), where FM is fresh mass, DM is dry mass and SM is saturated mass. 8 to 10 discs about 1 cm in diameter, were punched from 8 to 10 leaves (one leaf per plant, randomly sampled among 50 plants of each treatment, making sure to avoid the main leaf vein.), placed in a tiny sealed plastic bag and was immediately transferred to the laboratory (in about 1 to 2 min). FM was measured and then discs floated on distilled water in covered Petri dishes for 18 h to become fully turgid (Kyparissis and Manetas, 1993). Saturated water mass was measured and discs were then oven dried at 80℃ for about 24 h and finally, their DM was measured and also free water content (FWC) and bound water content (BWC) was measured with Zhang Zhiliang's refractometer method (Zhang, 2009). Leaf soluble sugar content and free proline: Plants were immediately defoliated following measurement of bulk water content for chemical and biochemical determinations. Measurements were made in physiologically comparable leaves to those used for the determination. Leaf total soluble sugars and proline were quantified in potassium phosphate buffer (50 mM, pH D 7:5) extracts of fresh leaves (0.1 g). These extracts were filtered through four cheese cloth layers and centrifuged at 15,500 rpm for 15 min at 4℃. The supernatant was collected and stored at 4℃ for total soluble sugars and praline determinations. Total soluble sugars were analyzed with the anthrone reagent in a Bausch and Lomb Spectrophotometer (Yemm and Willis, 1954). Free proline was estimated by spectrophotometric analysis at 515 nm of the ninhydrine reaction (Irigoyen et al., 1992). The measurement of K^+ and Ca^{2+} were realized as follows: 0.25 g oven dried and grounded sample is put into the test tube with stopper, then 20 ml 1 mol·L-1HCl was added to soak the sample for 24 h. During this period, filtrate after agitation and filtration was obtained. The content of K^+ is measured with colorimetric method and Ca^{2+} with methyl thymol blue colorimetric method (calcium kit was purchased from Nanjing Jiancheng Bio-engineering Institute). The detection of relative permeability of cell membrane. The method described by Zhou et al. (2006) was adopted and modified. 1 g of pigweed leaves were loaded to a 30 ml test tube, in which 10 ml double distilled water was added so that the leaves were completely submerged. The air was extracted by using syringe and the test tubes were placed in air for 30 min (with vibrations every 4 min). The electrodes of the DDS-11A Conductivity Detector were interpolated into the test tube and the conductivity of the exudates was detected, while the temperature was measured at the same time. The test tubes were then placed in boiling water bath for 10 min to deactivate tissues and cooled to the same temperature. The conductivity of the exudates was detected 3 times and averaged.

$$\text{Relative membrane permeability (\%)} = \frac{\text{L1 (the conductance value before leaf was killed)}}{\text{L2 (the conductance value after leaf was killed)}} \times 100$$

The generating rate of superoxide radical $O_2^{\cdot-}$ with the method of Luo Guanghua (Luo et al.,1990), malondialdehyde (MDA) content with method of Zhang Chenlie (Zhang et al., 1990), superoxide dismutase (SOD) activity with NBT method (Zhang, 2009), peoxidase (POD) activity with guaiacol method (Bai et al., 1998) and ascorbic acid (ASA) content with Li Zhongguang method (Li et al., 2003).

RESULTS AND ANALYSIS

Influences of drought stress on the water content of pigweed

When physiological parameters were measured (on June 21st), the soil water content of four groups, namely control group, mild group, moderate group and severe group, was 23.33, 16.92, 10.82 and 7.73%, respectively and the corresponding leaf water content was 94.07, 87.01, 76.35 and 64.03% (Figure 1), which conforms to the water stress degree of Hsiao criteria (Hisao,1973). The results indicated that as the soil drought stress increased, the relative water content of leaves decreased.

From Figure 1 we could also conclude that with the drought aggravated, the free water content (FWC) decreased while relative water content (BWC) relative increased. Changes of above indicators of pigweed

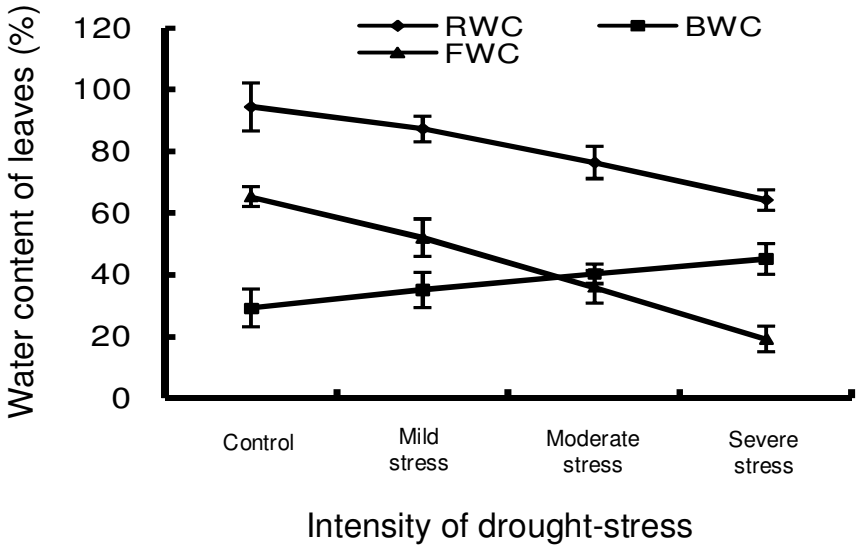

Figure 1. Effect of drought stress on water status in the leaves of pigweed.

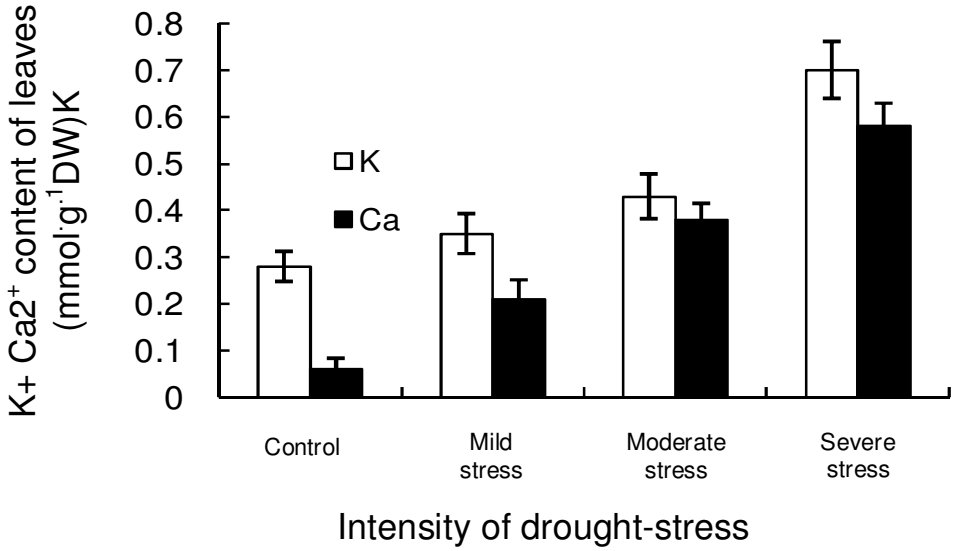

Figure 2. Effects of drought stress on content of K^+ and Ca^{2+} in leaves of pigweed.

leaves indicated a kind of adaptive response of pigweed to drought stress.

Influences of drought stress on osmoregulating materials of pigweed leaf

Influences of drought stress on Abio-Ion of pigweed Leaf

As the drought stress aggravated, the content of K^+ and Ca^{2+} increased remarkably. The content of K^+ of mild, moderate and severe stress is 1.25, 1.54 and 2.50 times of that of control group, respectively and the corresponding data for Ca^{2+} is 3.50, 6.30 and 9.67. The increased amplitude of Ca^{2+} was far larger than that of K^+ under different drought stresses (Figure 2).

Influences of drought stress on soluble sugar and proline contents of pigweed leaf

As Figure 3 indicated, there was a remarkable increase in contents of soluble sugar and proline under the drought stress. However, when the stress was more severe, the soluble sugar and proline contents increased more.

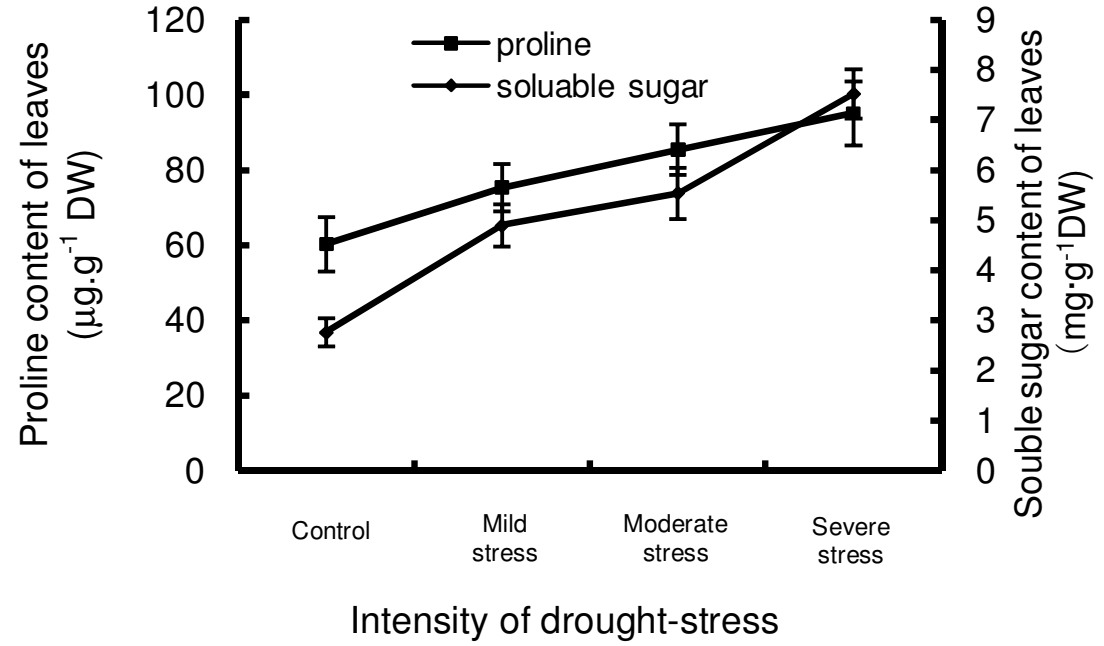

Figure 3. Effect of drought stress on organic solutes in leaves of pigweed.

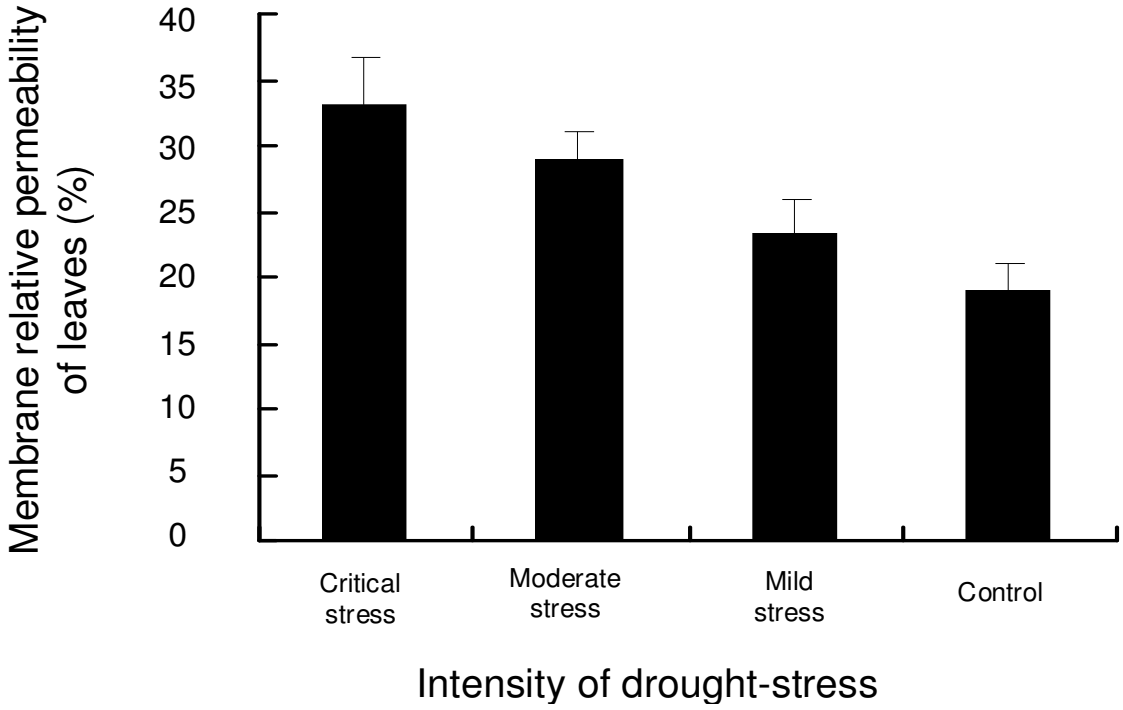

Figure 4. Effect of drought stress on relative membrane permeability in leave of pigwee.

Relative analysis indicated, there was a remarkable negative correlation between the increase of soluble sugar and proline contents and the decrease of relative leaf water content (−0.99304 and −0.96009, respectively for r value) yet a remarkable positive correlation with the increase of BWC (0.98582 and 0.99381, respectively for r value), which reflects the adaptation of pigweed to drought stress.

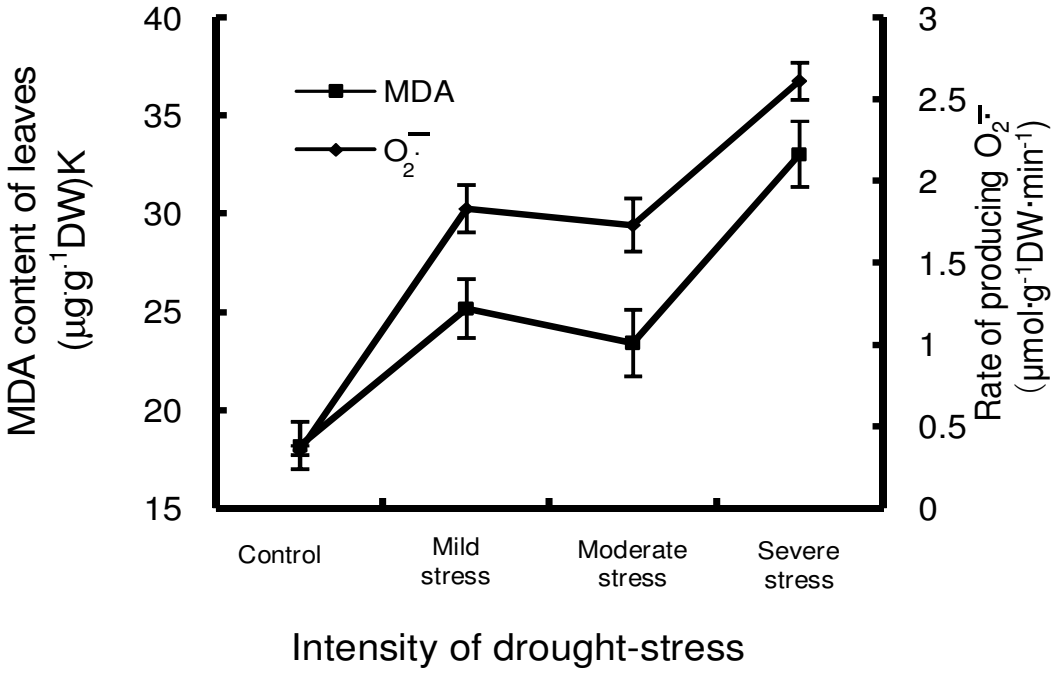

Figure 5. Effects of drought stress on the producing rate of $O_2^{\cdot}$ and MDA content in leaves of pigweed.

Influences on the membrane lipid peroxidation and protective enzyme system of pigweed leaf by drought stress

Influences of drought stress on membrane lipid peroxidation of pigweed leaf

From Figure 4, we can see that the relative plasma membrane permeability increased under mild drought stress; however, compared with that of mild stress, the relative plasma membrane permeability under moderate stress decreases. Nevertheless, the relative plasma membrane permeability under severe stress increased remarkably again, while changes of the generating rate of $O_2^{\cdot-}$ and contents of the product of membrane lipid peroxidation (MDA) assume the alike trend with that of relative plasma membrane permeability (Figure 5), namely, the generating rate of $O_2^{\cdot-}$ and contents of MDA increased under mild drought stress; however, compared with that of mild stress, the generating rate of $O_2^{\cdot-}$ and contents of MDA under moderate stress decreased and the generating rate of $O_2^{\cdot-}$ and contents of MDA under severe stress increased remarkably again. Statistical analysis indicated that under drought stress, there was a positive correlation between plasma membrane permeability and $O_2^{\cdot-}$ generating rate and MDA content (0.96860 and 0.84186, respectively for r value), while at the same time there was also a remarkable positive correlation between $O_2^{\cdot-}$ generating rate and MDA content (0.94948 for r value). This result indicates that the increase of plasma membrane permeability was closely related with increase of $O_2^{\cdot-}$ generating rate and strengthening of membrane lipid over-oxidation induced by MDA content accumulation.

Influences of drought stress on protective enzyme system of pigweed leaf

SOD and POD are important enzymes of plants to defend damage from active oxygen. From Figure 6 we can see that, as the drought stress increased, the activity of SOD and POD assumes a first-up-then-down trend, indicating that drought stress of certain level may enable some adaption of SOD and POD protective enzymes of pigweed and that severe drought stress may suppress the activity of SOD and POD. ascorbic acid (ASA) is a kind of important antioxidant in the non-enzymatic protective system (Liu and Yang, 2000). Figure 7 indicated that under different drought stress, the ascorbic acid (ASA) content of pigweed leaves experienced a sharp decrease, which was consistent with the results of observation of 11 non-xerophytes by Price (Price and Hendry, 1991). There was a positive correlation between

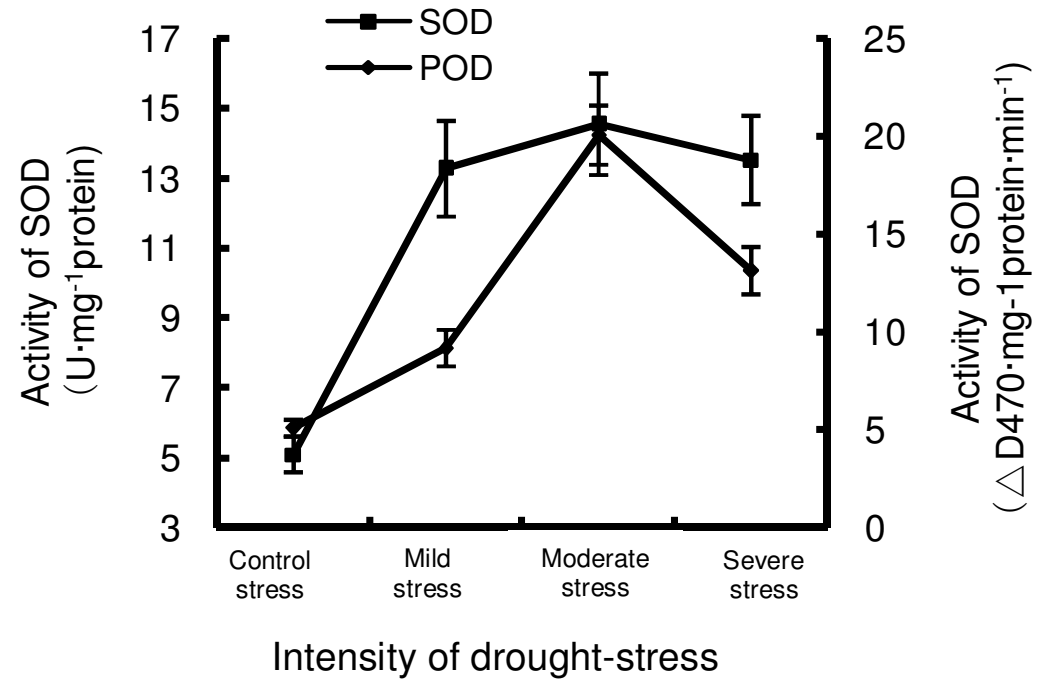

Figure 6. Effects of drought stress on activity of SOD and POD in leaves of pigweed.

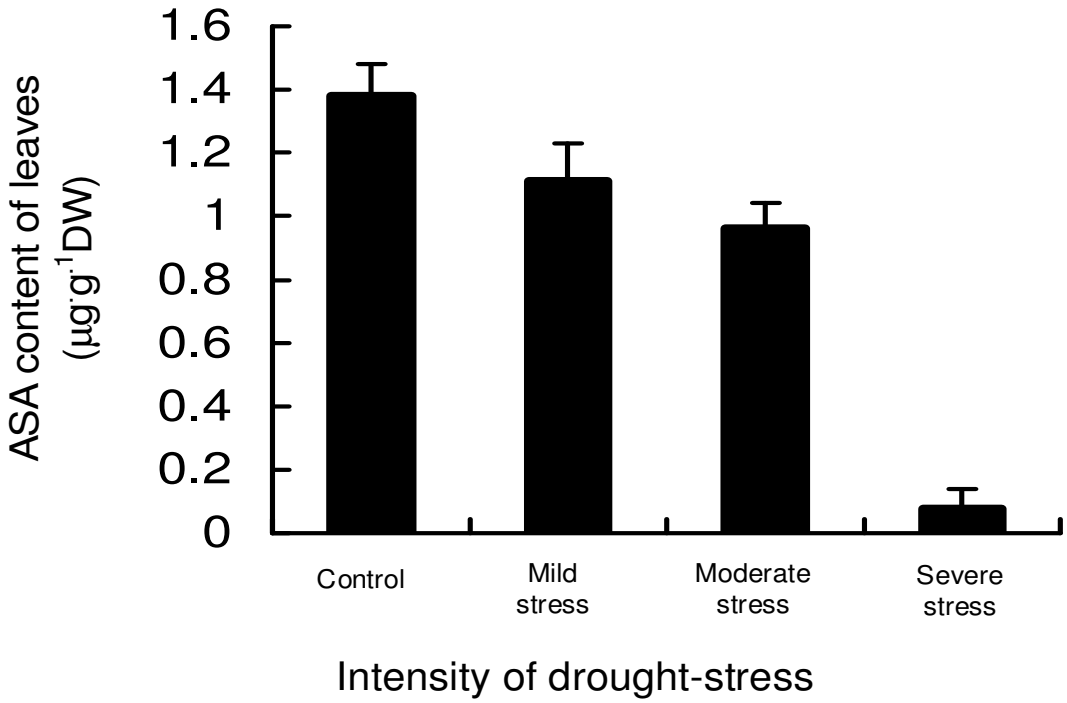

Figure 7. Effect of drought stress on the content of ASA in leaves of pigweed.

ASA content decreases with the intensity of drought stress, which indicates sensitivity of ASA in pigweed leaves to drought stress.

DISCUSSION

The intensity of metabolism is restricted by FWC, while

there is a close relationship between BWC and plant resistance (Pan et al., 2008). Under drought stress, the relative water content of pigweed leaves decreased, FWC decreased and BWC increased relatively. From the trend of water content changes of pigweed leaves, we can see that pigweed was adapted to drought stress by decreasing FWC and thus metabolism intensity, and increasing BWC to strengthen resistance.

Osmoregulation is an important physiological regulation system of plant to be adapted to drought stress. In dry conditions, plants can maintain the normal operation of physiological processes including cell growth, pore opening and photosynthesis by accumulating solutes, decreasing osmotic potential and maintaining certain turgor pressure (Sun et al., 2002). Those solutes involved in osmoregulation mainly include: organic osmotic solutes like soluble sugar, proline, betaine and abio-ions like K^+ and Ca^{2+} (Wang et al., 2001). The increase of soluble sugar, proline, K^+ and Ca^{2+} in pigweed indicated that it was capable of certain osmoregulation and some adaptation to drought. The level of relative plasma membrane permeability is an important indicator of membrane damage. As MDA is one of major products of membrane lipid peroxidation, the accumulation of MDA reflects the toxic action by active oxygen and MDA content is often used as one of major indicators to assess membrane lipid peroxidation (Zhang et al., 1996). Under the drought stress, the membrane permeability of pigweed leaves increased, and MDA content, together with free radical generating rate, experienced remarkable increases. Moreover, the increase of membrane permeability is significantly positively correlated with the increase of MDA content and $O_2^{\cdot-}$ generating rate, which indicated membrane damage of pigweed leaves caused by membrane lipid peroxidation due to drought stress. However, the increase of membrane permeability, MDA content and free radical generating rate did not assume a linear relationship with the intensity of stress. Under moderate stress, the membrane permeability, MDA content and free radical generating rate was lower than that under mild stress. However, the membrane permeability, MDA content and free radical generating rate was highest under severe stress, which may be explained by the adaptation of protective enzymes to the intensity of stress.

The elimination of active oxygen in plant cells is mainly realized by protective enzyme system and antioxidants. In the protective enzyme system, SOD enzyme can eliminate $O_2^{\cdot-}$ to form H_2O_2, while POD and CAT can turn H_2O_2 into H_2O. Their coordination will help to maintain active oxygen content to a relatively low level. This experiment indicated that under the drought stress, SOD enzyme activity of pigweed first increased with the intensity of drought stress, which began to decline after reaching the climax at moderate stress. This might be explained as that under moderate stress, some accumulation of $O_2^{\cdot-}$ would induce the SOD enzyme activity to increase, which resulted in the rapid dismutation of $O_2^{\cdot-}$ and reduced the damaging effects; therefore, there were relatively smaller increase of the membrane permeability and MDA content to be adapted to stress. However, excessive drought would suppress the activity of SOD and there would be a remarkable increase of membrane permeability, MDA content and generating rate. The POD activity of pigweed assumes the similar trend with that of SOD, which would be explained by the fact that SOD and POD were coordinate protective enzymes.

As one of the most important antioxidants in plants, ASA can deoxidize $O_2^{\cdot-}$, eliminate ·OH, quench $^1O_2$ and H_2O_2. V_E is the best $^1O_2$ quencher so far as we know and ASA can re-generate V_E. Under drought stress, the decrease of ASA in plants is a kind of common phenomenon (Jiang and Guo, 1996) and the ASA of pigweed leaf also decreased. The decrease of ASA may be taken as one indicator for the overall degeneration of antioxidation capability of plant (Wang et al., 2001). Under severe drought stress, the sharp decrease of ASA content in pigweed leaf would also represent the decreasing overall antioxidation capability; therefore, the membrane lipid peroxidation worsens and the membrane system was damaged.

SOD and POD was most active under the moderate stress; in contrast, the membrane permeability, MDA content and $O_2^{\cdot-}$ generating rate were relatively lower. Meanwhile, there was a rapid increase of osmoregulating materials, indicating that pigweed is somewhat adaptative to drought stress in terms of osmoregulation and membrane protection.

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