Response of growth and antioxidant enzymes to osmotic stress in two different wheat (*Triticum aestivum* L.) cultivars seedlings

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ABSTRACT

To investigate the responses of growth and antioxidant enzymes to osmotic stress in two different wheat cultivars, one drought tolerant (Heshangtou, HST) and the other drought sensitive (Longchun 15, LC15), 15-day-old wheat seedlings were exposed to osmotic stress of –0.25, –0.50, and –0.75 MPa for 2 days. It is found that osmotic stress decreased shoot length in both wheat cultivars, whereas to a lesser degree in HST than in LC15. The contents of malondialdehyde (MDA) and the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) of shoot in both wheat cultivars were increased by osmotic stress. It is clear that MDA contents increased less in the more drought tolerant cultivar HST than in drought sensitive one LC15. On the contrary, POD and CAT activities increased more in HST than LC15 under osmotic stress. As the activity of SOD, however, no significant differences were found between HST and LC15. These results suggest that wheat cultivar HST has higher activities of antioxidant enzymes such as POD and CAT to cope with oxidative damage caused by osmotic stress compared to sensitive LC15.

Keywords: malondialdehyde; superoxide dismutase; peroxidase; catalase; drought tolerance

Drought is one of the major environmental factors that can limit the growth and physiological characteristics of plants and recent global climate change has made this situation more serious (Martínez et al. 2003, Ren et al. 2007, Tadina et al. 2007, Wu et al. 2009). It was shown that drought may increase the formation of free radicals of oxygen in plant cells. These reactive oxygen species (ROS) involved superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$) (Collakova and DellaPenna 2003, Wu et al. 2008), which mediate the degradation of membrane components, the oxidation of protein sulphydryl groups, the formation of gel phase domains, and the loss of membrane function (Blokhina et al. 2003, Jaleel et al. 2009).

To protect cellular membranes and organelles from the damaging effects of ROS, plants developed different non-enzymatic and enzymatic antioxidants (Ali et al. 2008). It was reported that the non-enzymatic antioxidants include lipid soluble membrane associated antioxidants (e.g., α-tocopherol and β-carotene), and water soluble reductants (e.g., glutathione, ascorbate and phenolics) (Jaleel et al. 2009). The antioxidant enzymes such as superoxide dismutase (SOD, EC 1.15.1.11), peroxidase (POD, EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6) were considered as a defensive team, whose combined purpose is to protect cells from oxidative damage (Mittler 2002). It was accepted that SODs are localized in chloroplasts, mitochondria, peroxisomes and the cytosol; POD activities are distributed in vacuoles, the cell walls and the cytosol, whereas CAT enzymes are presented only in peroxisomes (Vaseva et al. 2012). The drought-induced changes in activities of SOD, POD and CAT were detected in a large number of plant species, such as *Oryza sativa* (Srivalli et al. 2003), *Sesamum indicum* (Fazeli et al. 2007), *Carthamus tinctorius* (Hojati et al. 2011), *Mentha*

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pulegium (Hassanpour et al. 2012), and *Trifolium pratense* (Vaseva et al. 2012). These findings suggest that the induction of ROS-scavenging enzymes, such as SOD, POD and CAT, is the most common mechanism of drought tolerance for detoxifying ROS synthesized.

Wheat is one of the most important grain crops in the world and it is widely cultivated in the arid and semi-arid areas of Northern China. Drought seriously affected growth and development of wheat in many regions (Wu et al. 2009). Genotypic variation observed in wheat is a factor that contributes to their wide range of adaptation capacity to limited water regions. Therefore, it is imperative to compare the activities of ROS-scavenging enzymes among wheat genotypes with different drought tolerance, as it will tell us the intrinsic role of antioxidant enzymes against drought tolerance.

Sorbitol, a six carbon sugar alcohol, was used in osmotically induced water stress studies in plants (Hsu and Kao 2003, Kolarovič et al. 2009, Jain et al. 2010). The main objective of this study was to investigate the responses of growth and antioxidant enzymes to osmotic stress induced by sorbitol in two wheat cultivars with different tolerance to drought. The results suggest that the drought tolerant cultivar Heshangtou (HST) has higher activities of antioxidant enzymes such as POD and CAT to cope with oxidative damage induced by osmotic stress compared to the drought sensitive cultivar Longchun 15 (LC15).

MATERIAL AND METHODS

Plant materials, growth conditions and treatments. Seeds of two wheat (*Triticum aestivum* L.) cultivars, one drought tolerant (Heshangtou, HST) and the other drought sensitive (Longchun 15, LC15), were collected in the Yongdeng County, Gansu Province, China in August 2011. Seeds of both cultivars were germinated at 25°C on filter paper wetted with sterile water in Petri dishes (2 cm high × 15 cm diameter) – germination took 2–3 days. After emergence, seedlings were cultured in the same dishes but with the modified Hoagland's solution containing 2.5 mmol/L KNO_3, 0.5 mmol/L $NH_4H_2PO_4$, 0.25 mmol/L $MgSO_4 \cdot 7\,H_2O$, 2.5 mmol/L $Ca(NO_3)_2 \cdot 4\,H_2O$, 0.5 mmol/L Fe-citrate, 92 µmol/L H_3BO_3, 18 µmol/L $MnCl_2 \cdot 4\,H_2O$, 1.6 µmol/L $ZnSO_4 \cdot 7\,H_2O$, 0.6 µmol/L $CuSO_4 \cdot 5\,H_2O$, and 0.7 µmol/L $(NH_4)_6Mo_7O_{24} \cdot 4\,H_2O$. Once plants had two leaves, they were transferred to black-painted plastic containers with the same modified Hoagland's solution. All the seedlings were grown in the same chamber. The environmental conditions were as follows: temperature 28°C at day and 23°C at night, photon flux density 600 µmol/m^2/s, photoperiod 16/8 h for day/night cycle, and relative humidity 70%. 15-day-old wheat seedlings were used for following osmotic stress for 2 days. The Hoagland's solution was supplemented with 0, 80, 160, and 240 mmol/L sorbitol; its osmotic potential measured by a cryoscopic osmometer (Osmomat-030, Gonotec GmbH, Berlin, Germany), was 0, –0.25, –0.50, and –0.75 MPa, respectively. 40 seedlings were grown in each treatment. The treatment solution was changed everyday to maintain constant of osmotic stress.

Assay of growth parameters. At the end of treatments, 8 plants from each group were divided into separate shoot and root fractions. Fresh weights (FW) of shoot and root were weighed, and lengths of shoot and root were measured. The samples were then dried in oven at 80°C for 72 h and dry weights (DW) were determined.

Determination of shoot malondialdehyde (MDA) content. The MDA content in shoot of wheat seedlings was measured using the thiobarbituric acid (TBA) protocol as described by Peever and Higgins (1989) with slight modifications. The absorbance at 450, 532, and 600 nm (A_{450}, A_{532}, and A_{600}, respectively) was determined using an ultraviolet spectrophotometer (UV-2102C, Unico Instrument Co., Shanghai, China). The content of MDA in nmol/g FW was calculated according to the following equation as described by Bao et al. (2009):

$$\text{MDA content (nmol/g FW)} = C\ (\mu\text{mol/L}) \times V\ (\text{L})/\text{FW}\ (\text{g}) \times 1000$$

Where: $C = 6.45 \times (A_{532} - A_{600}) - 0.56\ A_{450}$, and V – volume of extracting solution.

Assay of SOD, POD, and CAT activities in shoot. SOD activity was determined according to the method as described by Beauchamp and Fridovich (1971). One unit of SOD was defined as the enzyme activity that inhibited the photoreduction of nitroblue tetrazolium (NBT) to blue formazan by 50%. POD activity was detected according to the method as described by Sakharov and Ardila (1999) with slight modifications. A unit of POD activity was expressed as the change in absorbance at 470 nm per min. CAT activity was measured following the change of absorbance at 240 nm for 1 min due to H_2O_2 (Aebi 1984). The activities of SOD, POD, and CAT were expressed as enzyme units per gram fresh weight (U/g FW).

Statistical analysis. Data were performed by one-way analysis of variance (ANOVA) using statistical software (SPSS 13.0, Chicago, USA). Duncan's multiple range test was used to detect asignificant difference between means at a significant level of $P < 0.05$.

RESULTS AND DISCUSSION

The results of present investigation demonstrate that with the increase of osmotic stress shoot length in both wheat cultivars showed decreasing trend, to a lesser degree in more tolerant cultivar HST than in more sensitive one LC15. For example, under osmotic stress of –0.75 MPa shoot length of HST was 89% of the control while that of LC15 was 79% (Figure 1a). It seems that osmotic stress has some slight effect on root length in both cultivars (Figure 1b). There was no significant effect on fresh weight of two cultivars seedlings exposed to osmotic stress of –0.25 MPa, whereas osmotic stress of –0.75 MPa significantly decreased fresh weight in both cultivars (Figure 1c). Dry weights of HST and LC15 showed similar trends under osmotic stress (Figure 1d). It was demonstrated that sorbitol-induced stress could reduce the total chlorophylls, chlorophyll *a* as well as chlorophyll *b* in maize (Jain et al. 2010). On the basis of these results, our findings suggested that osmotic stress can inhibit the normal growth and development of wheat cultivars.

It is observed that osmotic stress significantly increased MDA contents in both wheat cultivars (Figure 2a). As MDA is an end product of membrane lipid peroxidation (Peever and Higgins 1989, De Vos et al. 1991), the content of MDA represents the degree of cell membrane damage under osmotic stress and is a common physiological indicator in evaluation of drought tolerance (Luo et al. 2008). It is clear that when subjected to osmotic stress, MDA contents of shoot in drought tolerant cultivar HST were lower than those in drought sensitive one LC15 (Figure 2a). For example, under osmotic stress of –0.25 MPa, HST seedlings showed a smaller increase of MDA contents than LC15. The lower level of MDA in shoot of HST suggests that this cultivar is better protected against oxidative damage under osmotic stress than LC15. This result is in agreement with results observed by Sairam et al. (2005) on salt tolerant genotype of wheat and Fazeli et al. (2007) on drought tolerant cultivar of sesame (*Sesamum indicum* L.) under water stress.

SOD is one of several important antioxidant enzymes with the ability to repair oxidative dam-

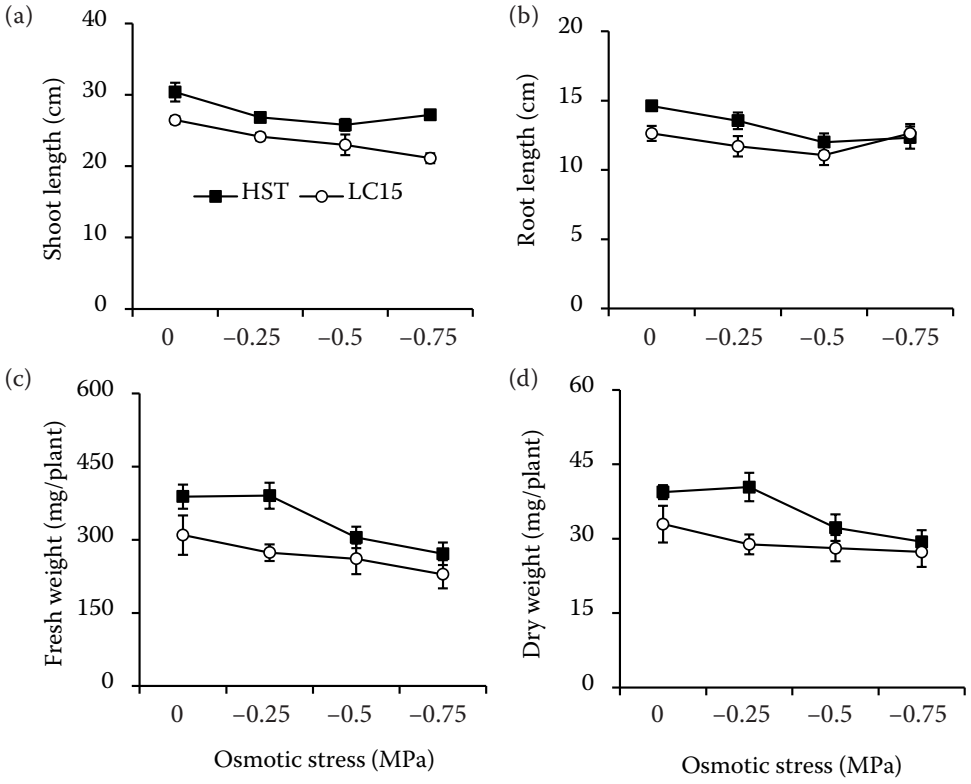

Figure 1. Effects of osmotic stress on shoot (a) and root (b) length, and fresh (c) and dry (d) weight in two wheat cultivars Heshangtou (HST) and Longchun 15 (LC15). 15-day-old wheat seedlings were exposed to osmotic stress of –0.25, –0.50, and –0.75 MPa for 2 days. Two wheat seedlings were pooled in each replicate ($n = 8$). Values are means ± SE and bars indicate SE

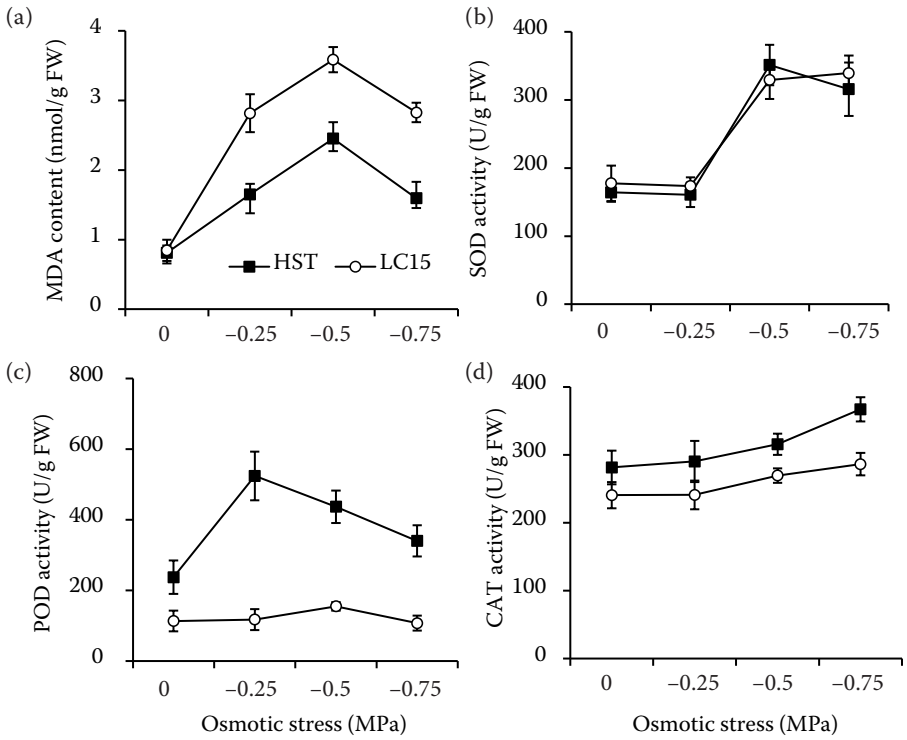

Figure 2. Effects of osmotic stress on (a) malondialdehyde (MDA) content; (b) superoxide dismutase (SOD) activity; (c) peroxidase (POD) activity and (d) catalase (CAT) activity of shoot in two wheat cultivars Heshangtou (HST) and Longchun 15 (LC15). 15-day-old wheat seedlings were exposed to osmotic stress of –0.25, –0.50, and –0.75 MPa for 2 days. Two wheat seedlings were pooled in each replicate (n = 8). Values are means ± SE and bars indicate SE; FW – fresh weight

age caused by ROS (Jaleel et al. 2009). Thus, SOD is considered as a key enzyme for maintaining normal physiological conditions and coping with oxidative stress in the regulation of intracellular levels of ROS (Mittler 2002). In this study, although osmotic stress of –0.25 MPa had no effects on SOD activity of shoot, osmotic stress of –0.50 MPa and –0.75 MPa significantly increased its activity in both wheat cultivars (Figure 2b). However, no significant differences in the SOD activity were found between HST and LC15 under either normal growth condition or osmotic stress (Figure 2b).

POD activity is considered a useful biomarker for environmental stress in examined plant species (Jaleel et al. 2009). It is shown that the POD activity of shoot in the tolerant cultivar HST exposed to osmotic stress of –0.25, –0.50, and –0.75 MPa was 2.2-, 1.8-, and 1.4-fold of that in corresponding control seedlings, respectively, whereas only osmotic stress of –0.75 MPa induced slightly its activity in LC15 (Figure 2c). Peroxidases are widely distributed in plant tissues where they are involved in growth, development, and senescence processes of plants (Mittler 2002). In drought-tolerant plant species, POD activity was found to be sufficiently high to enable the plants to protect themselves against oxidative stress (Fazeli et al. 2007, Vaseva et al. 2012). Activity of one or more antioxidant enzymes generally increases in plants exposed to drought conditions, and this elevated activity correlates with increased drought tolerance (Srivalli et al. 2003, Hojati et al. 2011, Hassanpour et al. 2012). Findings from the present study showed that POD activity in the tolerant cultivar HST was higher than those in the sensitive one LC15 (Figure 2c). A number of studies indicated that POD activity response to osmotic stress vary among plant species and among different cultivars (Hojati et al. 2011). It is concluded that the higher activity of POD in the tolerant cultivar HST might be better to protect proteins, chlorophyll and lipids of some parts of plants against ROS attack compared to that in the sensitive LC15.

The CAT activity of shoot in the tolerant cultivar HST was increased by 12.1% and 30.4% under osmotic stress of –0.50 MPa and –0.75 MPa compared to corresponding control, respectively (Figure 2d). Its activity in the sensitive cultivar LC15 was increased by 11.9% and 19.1% at osmotic stress of –0.50 MPa and –0.75 MPa, respectively (Figure 2d). However, osmotic stress of –0.25 MPa did not enhance remarkably the CAT activity in

both cultivars. CAT is one of the most effective antioxidant enzymes that can degrade H_2O_2 into water and molecular oxygen in the peroxysomes, where H_2O_2 is produced from β-oxidation of fatty acids and photorespiration (Fazeli et al. 2007). It was suggested that the higher activities of CAT reduced H_2O_2 level in the cell and enhanced the stability of membranes and CO_2 fixation because several enzymes of the Calvin cycle in chloroplasts are very sensitive to H_2O_2 (Bhutta 2011). A high level of H_2O_2 can directly inhibit CO_2 fixation (Yamazaki et al. 2003). It was shown that CO_2 laser pretreatment increased the activity of CAT in wheat seedlings under osmotic stress (Qiu et al. 2011). In this study, CAT activity in HST was significantly higher than that in LC15 under osmotic stress (Figure 2d). These results suggest that the drought tolerant cultivar HST has more catalases to protect against oxidative damage caused by osmotic stress.

In conclusion, osmotic stress inhibits the growth, increases the contents of MDA, and induces the activities of SOD, POD, and CAT in both wheat cultivars. It is clear that the degrees of growth inhibition and oxidative damage in the tolerant cultivar HST are less than those in the sensitive LC15 under osmotic stress, whereas activities of POD and CAT are contrary – they are higher in the former than in the latter. These results suggest that HST has higher capacity to cope with osmotic stress compared to LC15.

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