Bangladesh J. Bot. **45**(2): 377-388, 2016 (June)

EFFECT OF DROUGHT STRESS ON ANTIOXIDANT MECHANISMS OF WEEDY RICE

GUOHUA DING[1], GUANG YANG[2], FENGMING ZHANG[1], LIANGMING BAI[1], SHICHEN SUN[1], SHUKUN JIANG[1], TONGTONG WANG[1], TIANSHU XIA[1], DIANRONG MA* AND WENFU CHEN*

Rice Research Institute, Shenyang Agricultural University, Shenyang 110866, China

*Key words***:** Weedy rice, Drought stress, Antioxidative enzyme, Physiological indicator

Abstract

The effect of varying levels of water stress simulated by polyethylene glycol (PEG) on oxidative parameters (malondialdehyde, MDA) and superoxide anion (O_2^-), the activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), as well as the content of non-enzymatic antioxidants ascorbate acid (ASA) and glutathione (GSH) were studied in the leaves and roots of weedy rice (*Oryza sativa* f. *spontanea*) heb07-2 and wr04-6, cultivated rice (*Oryza sativa* L.) variety yuefu and upland rice iapar9. Drought stress significantly increased MDA and O_2^- production. The MDA and O_2^- of heb07-2 increased significantly slower than other oxidants under drought indicating that heb07-2 accumulated lower ROS (reactive oxygen species) with intact cell membrane and lower level of lipid peroxidation. Under normal conditions, the activities of antioxidant enzymes in heb07-2, wr04-6, iapar9, and yuefu were similar. However, under stress, the activities of SOD, POD, CAT, APX, GR, and DHAR were significantly enhanced in heb07-2 than in iapar9 and wr04-6, whereas they were decreased in yuefu after 72 hrs of drought. The mechanism of drought tolerance in weedy rice involves enhanced antioxidant defense mechanisms.

Introduction

Increased levels of ROS such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen ($^1O_2$) are highly reactive and toxic, damaging proteins, lipids, carbohydrates and DNA, ultimately resulting in death of cell (Gill and Tuteja 2010). To counteract ROS accumulation induced by stress, plants have developed a series of defense systems including antioxidant enzymes (superoxide dismutase, SOD; peroxidase, POD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; and dehydroascorbate reductase, DHAR) and non-enzyme antioxidants (ascorbate, ASA and glutathione, GSH) that protect against oxidative stress damage (Gill and Tuteja 2010). When plants are under drought stress, the stress tolerance ability may be improved by the enhanced levels of antioxidant systems.

Weedy rice is thought as a weed which accompanies with rice and is widely distributed in every rice-growing areas worldwide (Zhang *et al*. 2012). Weedy rice is a good germplasm resource for improving rice varieties, as it contains several special genes for drought tolerance at seedling stage, germination characteristics, blast resistance, high salinity and cold tolerance (Hak-Soo 1993). Therefore, utilizing physiological traits for screening drought-tolerant weedy rice and further detecting and transferring unique genes for drought tolerance from weedy-rice to cultivated rice varieties may play an important role in enhancing tolerance to drought stress. However, the mechanism of tolerance in weedy rice to drought stress is poorly understood.

*Authors for correspondence: <ismadianrong@163.com>, <wfchen5512@126.com>, [1]Postdoctoral Scientific Research Station of Heilongjiang Academy of Agricultural Sciences /Northern Japonica Rice Molecular Breeding Joint Research Center/ Cultivation and Farming Research Institute, Heilongjiang Academy of Agricultural Sciences, HAAS, Harbin 150086, China. [2]Crop Institute of Liaoning Academy of Agricultural Science, Liaoyang 111000, Liaoning, China.

The purpose of the present study was to investigate the differences in response of antioxidant enzymes and non-enzyme antioxidants to drought stress using two weedy-rice and two cultivated rice varieties with different drought tolerance.

Materials and Methods

Two weedy rice varieties, namely heb07-2 and wr04-6; one cultivar, yuefu; and one upland rice, iapar9 (drought-resistance), were used. While heb07-2 and iapar9 are tolerant to drought stress, yuefu is sensitive, and wr04-6 is less tolerant than heb07-2.

Seeds of four varieties of rice were surface-sterilized with 10% (v/v) sodium hypochlorite solution for 10 min and washed with deionized water three times. Seeds imbibed in deionized water for 24 hrs at 25ºC and were germinated in Petri dishes with two layers of wet filter paper at 30ºC in dark. Uniformly germinated seeds were selected and cultivated in 96 PVP and placed in plastic box (24 cm × 24 cm × 10 cm), treated with distilled water for two weeks, half-strength Kimura solution for one week and Kimura nutrient solution for another week. The seedlings were grown at 29/24ºC (day/night) under a 12 hrs light/12 hrs dark cycle at 75% relative humidity and approximately 280 mol photons/m^2/s provided by fluorescent lamps in a plant growth chamber. When the third leaf expanded fully, the seedlings with uniform height and growth were selected for the following experiments.

On the day 28 of growth, rice seedlings were subjected to water stress by immersing roots in 20% PEG 6000 solution for 5, 24 and 72 hrs, respectively. Controls were held in distilled water. After the stress treatment, all leaves and roots were harvested and used either immediately or stored at –80ºC until used for various assays.

Fresh leaves and roots (0.25 g) were homogenized using 5 ml of 50 mM cold phosphate buffer (pH 7.8) containing 5 mM EDTA, 2 mM ASA and 2% (w/v) polyvinylpyrrolidone in a chilled mortar and pestle. The homogenate was centrifuged at 10000 g for 30 min at 4ºC, and the supernatant was subsequently used in assays for SOD, CAT, APX and POD activities.

Superoxide dismutase activity was assayed by measuring its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT) following Dhindsa *et al.* (1981) with modifications. The 3 ml reaction mixture contained 25 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 mM NBT, 0.1 mM EDTA, 4 µm riboflavin, 0.25 ml of distilled water and 0.05 ml of enzyme extract. This reaction was started by the addition of riboflavin, and the glass test tubes were shaken and placed under fluorescent lamps (60 µmol photons/m^2/s). The reaction proceeded for 20 min and then stopped by switching off the light. One unit of activity was defined as the amount of enzyme that inhibited 50% of NBT photoreduction at 560 nm.

CAT activity was assayed according to Li (Li *et al*. 2000). The CAT reaction solution (3 ml) containing 56 mm H_2O_2 and 0.2 ml of enzyme extract was catalyzed by the enzyme extract. Changes in absorbance of the reaction solution at 240 nm were read every 30 sec. One unit of activity was defined as µmol H_2O_2 reduced/min/gprotein.

APX was assayed using the method of Nakano and Asada (1981) with modifications. Three ml reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ASA, 0.1 mM EDTA, 0.1 mM H_2O_2 and 0.2 ml of enzyme extract. The reaction initiated by the addition of H_2O_2. Decrease in absorbance for a period of 30 sec was measured at 290 nm. One unit of activity was defined as µmol ascorbate oxidized/min/gprotein.

POD activity was assayed according to Li *et al.* (2000) with modifications. The POD reaction with a solution (3 ml) containing 0.1 ml enzyme extract and 2.6 ml 0.3% guaicol was initiated by adding 0.3 ml 0.6% H_2O_2. Changes in absorbance of the reaction solution at 470 nm were read

every 30 sec. One unit of activity was defined as the change in absorbance per minute and specific activity as enzyme units per g soluble protein.

GR was assayed using the method of Knörzer *et al.* (1996) with modifications. Fresh leaves and roots (0.3 g) were homogenized using 5 ml of 100 mM cold phosphate buffer (pH 7.5) containing 1 mM EDTA in a chilled mortar and pestle. The homogenate was centrifuged at 12000 g for 30 min at 4ºC, and the supernatant was subsequently used for assays of GR activity. The reaction mixture contained 3 ml of 100 mM cold phosphate buffer (pH 7.5), 0.1 ml of 5 mM oxidized glutathione (GSSG), 0.03 ml of 3 mM NADHP and 0.2 ml of enzyme extract. The reaction was started by the addition of NADPH. Changes in absorbance of the reaction solution at 340 nm were read every 30 sec. One unit of activity was defined as nmol NADPH oxidized/min/ gprotein.

DHAR was assayed using the method of (Doulis *et al*. (1997) with modifications. Fresh leaves and roots (0.2 g) were homogenized using 5 ml of cold phosphate buffer (pH 7.5) containing 1 mM EDTA, 0.1 mM Triton X-100, 2% PVP in a chilled mortar and pestle. The homogenate was centrifuged at 12000 g for 30 min at 4ºC, and the supernatant was subsequently used for assays of DHAR activity. The reaction mixture contained 0.2 ml enzyme extract, 2 ml potassium phosphate buffer (pH 7.0), 0.3 ml of 25 mmol/l GSH, 0.3 ml of 1 mmol/l EDTA. The reaction was started by the addition of 0.1 ml 2 mmol/l DHA. Changes in absorbance at 265 nm were read every 30 sec. One unit of activity was defined as mol dehydroascorbate reduced/min/g protein.

The ASA and GSH levels were measured according to the method of Li *et al*. (2003) with modifications. Fresh leaves and roots (0.25 g) were homogenized in 2.5 ml of 5% (w/v) ice-cold 5-sulfosalicylic acid with a chilled mortar and pestle and centrifuged at 20000 g for 20 min at 4ºC.

For total ascorbate measurement, 0.1 ml of supernatant was mixed with 24 µl, 1.84 mol/l triethanolamine, 250 µl of 50 mmol/l phosphate buffer (pH 7.5) containing 2.5 mmol/l EDTA, 50 µl 10 mmol/l DTT. The reaction mixtures were then incubated at 25ºC for 10 min, reducing DHA to AsA, and mixed with 50 µl of 0.5% N-ethyl maleimide (NEM) followed by addition of 0.2 ml of 10% TCA, 0.2 ml of 44% (v/v) H_3PO_4, 0.4 ml of 4% (w/v) bipyridyl in 70% (v/v) ethanol and 0.1 ml of 3% (w/v) $FeCl_3$, successively. The reaction mixtures were then incubated at 40ºC for 60 min in a water bath. The absorbance at 525 nm was read to determine ASA content according to the standard curve. For ASA determination, the same reaction was used but distilled water was used in place of DTT and NEM. The DHA level was estimated from the difference in total ascorbate and ASA.

For measurements of GSH, 0.05 ml of enzyme extract was added to 50 µl of 5% sulfosalicylic acid, 24 µl, 1.84 mol/l triethanolamine, 50 µl of 10% vinylpyridine in 70% (v/v) ethanol. After incubation for 60 min at 25 ºC, 706 µl 50 mmol/l phosphate buffer (pH 7.5) containing 2.5 mmol/l EDTA, 20 µl 10 mmol/l reduced nicotinamide adenine dinucleotide phosphate (NADP) and 80 µl 12.5 mmol/l dithiobis nitrobenzoic acid (DTNB) were added. Following incubation for 10 min at 25ºC, 20 µl 50 U/ml GR was added, and the absorbance was measured at 412 nm. For total glutathione determination, the same reaction was used but vinylpyridine was used in place of distilled water. The GSH was estimated from the difference in total glutathione and GSSG.

Superoxide radical was determined according to the method of Elstner and Heupel (1976) with modifications. Leaves and roots (0.5 g) were homogenized in 3 ml of 65 mmol/l phosphate buffer (pH 7.8) on an ice bath and were centrifuged at 4ºC and 5000 g for 10 min. The supernatant (0.75 ml) was mixed with 0.675 ml of 65 mmol/l phosphate buffer (pH 7.8) and 0.07 ml of 10 mmol/l hydroxylamine chlorhydrate and placed at 25ºC. After 20 min, 0.375 ml of 17 mmol/l sulphanilamide and 0.375 ml of 7 mmol/l α-naphthylamine were added, and the mixture was

placed at 25ºC for another 20 min before mixing with 2.25 trichloromethane. The absorbance was measured at 530 nm and the O_2^- concentration was calculated from a standard curve of $NaNO_2$.

Leaf and roots samples (0.5 g) were homogenized in 3 ml 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 15000 g for 20 min. The supernatant (1 ml) was mixed with 4 ml of 20% TCA containing 0.5% (w/v) TBA. The mixture was heated at 95ºC for 30 min and then cooled quickly on ice. After centrifugation at 10000 g for 10 min, the absorbance of the supernatant was recorded at 532 nm. The MDA content was calculated according to its coefficient of absorbance (0.155 μmol/ml). The values for non-specific absorbance at 600 nm were subtracted.

The experiment involved a randomized design with three replicates. Differences in treatments were analyzed by one-way ANOVA, taking $p < 0.05$ as significant according to Tukey's multiple range test.

Results and Discussion

To investigate the role of antioxidants in water stress tolerance in weedy rice, the MDA content was studied. The results indicated that drought stress induced by PEG increased MDA content in leaves and roots substantially. The MDA content of less drought-tolerant wr04-6 genotypes and drought-sensitive yuefu in leaves was increased more than that of the drought-tolerant genotypes of iapar9 and heb07-2. The wr04-06 and yuefu genotypes accumulated 1.73, 2.02 and 2.18-folds; and 2.13, 3.59, and 3.77-folds more MDA than their controls at 5, 24 and 72 hrs drought stress, respectively, compared with iapar9 and heb07-2, which accumulated 1.51, 1.76, and 1.95-folds; and 1.82, 1.64, and 1.84-folds, respectively (Fig. 1A).The MDA content increased in roots lower than in leaves under drought stress. Drought stress exhibited slightly but insignificant induction of MDA in heb07-2 at 5 hrs and 24 hrs, whereas MDA increased significantly at 72 hrs. The MDA content in wr04-6 significantly declined at 5 hrs, but increased slightly and significantly at 24 and 72 hrs compared with control, respectively. The MDA content in iapar9 increased first at 5 hrs and then decreased at 24 hrs, but increased significantly after 72 hrs. The MDA content was significantly increased in yuefu after drought stress at 5, 24 and 72 hrs, respectively (Fig. 1B).

The generation of superoxide anion in all genotypes increased significantly under drought compared with controls except wr04-6 leaves. The levels of superoxide anion in drought-sensitive genotypes of yuefu under stress were higher than in drought-tolerant genotype of heb07-2 and iapar9. Under drought conditions, the yuefu accumulated 2.10, 2.63 and 3.64-folds more superoxide anion free radical than its control at 5, 24 and 72 hrs, respectively, whereas heb07-2 accumulated the least superoxide anion free radical at 72 hrs (Fig. 1C). Under drought stress, a significant increase in superoxide anion production was observed in roots of all genotypes. In drought-sensitive genotypes of yuefu, the oxidative stress increased to a higher degree than in other genotypes, with only a small increase in heb07-2 seedlings compared with control (Fig. 1D).

The activity of antioxidant enzymes in leaves and roots was measured under drought stress. Fig. 2A, B shows that the SOD activities of leaves and roots were similar in heb07-2 and iapar9 under non-stress condition, which was markedly lower than in wr04-6 and yuefu after 24 and 72 hrs of drought stress, compared with other genotypes, the SOD activity of heb07-2 was significantly greater, whereas wr04-6 and yuefu were lower compared with control, respectively.

The leaf POD activity of heb07-2 increased significantly from 5 to 72 hrs of treatment. In contrast, drought stress caused a gradual but unremarkable increase in POD activities of roots in drought-tolerant genotypes of heb07-2, whereas it significantly increased in iapar9 and wr04-6. Further, the POD activities were significantly lower in drought-sensitive genotypes of yuefu at 24 and 72 hrs compared to control.

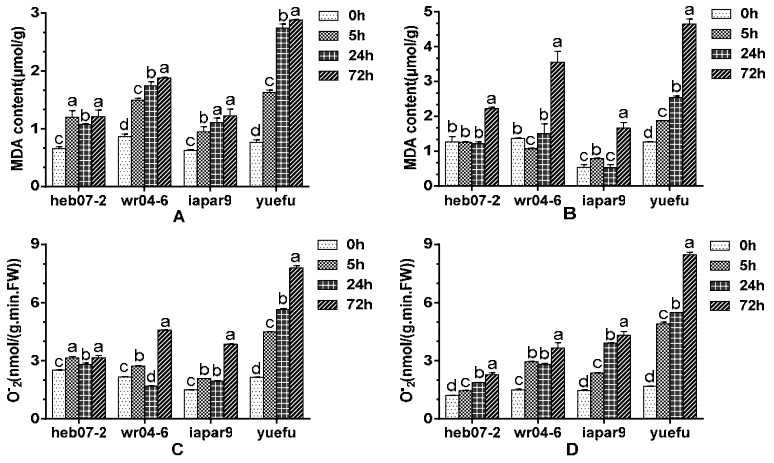

Fig. 1. MDA content in leaves (A) and roots (B), O_2^- generating rate in leaves (C) and roots (D). Values are mean ± Sd based on three independent determinations and bars indicate standard deviations. The different small letters indicate significant difference at p = 0.05% level.

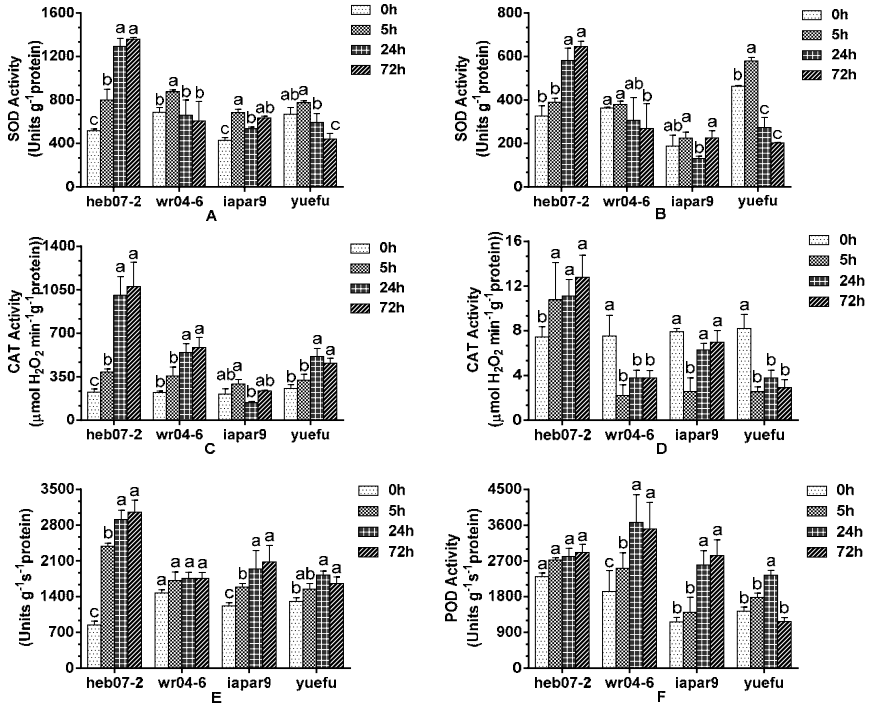

Fig. 2. SOD activity in leaves (A) and roots (B), CAT activity in leaves (C) and roots (D), POD activity in leaves (E) and roots (F) of heb07-2, wr04-6, iapar9, yuefu under drought condition for 0, 5, 24, 72 hrs. Values are mean ± Sd based on three independent determinations and bars indicate standard deviations. The different small letters indicate significant difference at p = 0.05% level.

No differences in CAT activity were observed among the genotypes in leaves and roots under unstressed conditions. Drought increased the leaf and root activities of CAT in heb07-2 at 5, 24, and 72 hrs compared with control, which was markedly higher than in wr04-6, iapar9 and yuefu.

Under drought stress, a significant increase in the activity of APX was observed in heb07-2 leaves, i.e., 1.47, 1.34 and 1.25-folds than its control at 5, 24, and 72 hrs. In contrast, drought stress decreased APX activity in wr04-6, yuefu and iapar9 (except at 5 hrs) leaves compared with their control at 5, 24 and 72 hrs, that is, 0.54, 0.67 and 0.51-folds; 0.80, 0.59 and 0.30-folds; and 1.24, 0.92 and 0.83-folds, respectively. A gradual increase in APX activity in heb07-2 root was observed, which was significantly higher than in other samples at 24, 72 hrs, while a gradual decrease in APX activity in wr04-6, yuefu, and iapar9 root was found at 5, 24 and 72 hrs compared with their control.

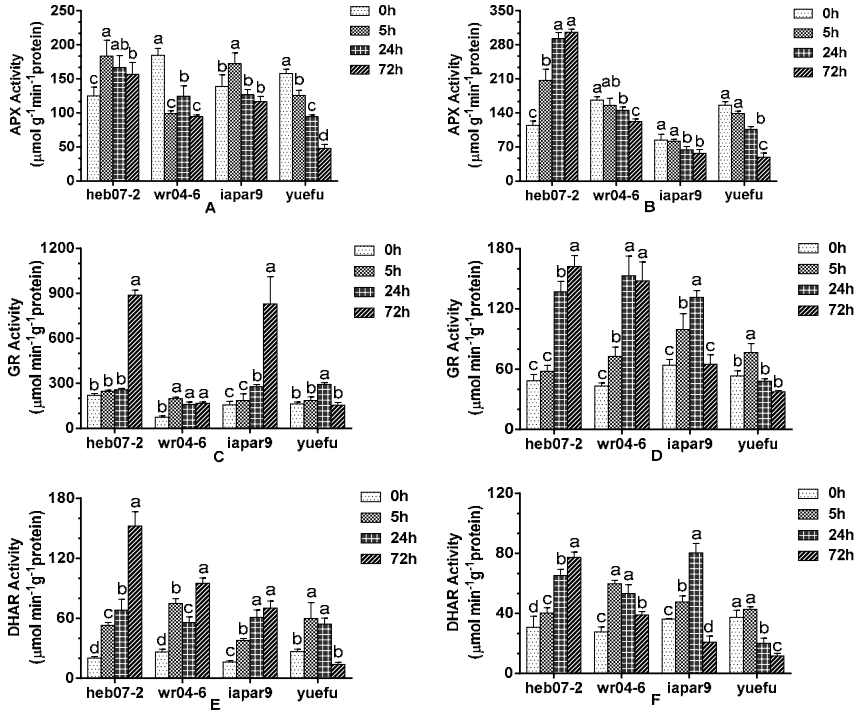

Fig. 3. APX activity in leaves (A) and roots (B), GR activity in leaves (C) and roots (D), DHAR activity in leaves (E) and roots (F) heb07-2, wr04-6, iapar9, yuefu under drought condition for 0, 5, 24, 72 hrs. Values are mean ± Sd based on three independent determinations and bars indicate standard deviations. The different small letters indicate significant difference at p = 0.05% level.

Effect of drought stress on GR activity in leaf and root was established in Fig. 3. The GR activity in heb07-2 and iapar9 leaf was significantly increased by 140.58 and 105.82% at 72 hrs drought stress, respectively but it was decreased in wr04-6 and yuefu leaf at 72 hrs of drought stress. The GR activity of heb07-2 in root increased during drought, by 182.78 and 234.77% compared to control at 24 and 72 hrs, respectively. Under drought conditions, the activity of GR in iapar9 and wr04-6 root significantly increased at 5 and 24 hrs and decreased at 72 hrs. The effect of drought stress on GR activity in yuefu root was different from other activities, and was significantly higher than control at 5 hrs, and decreased at 24 and 72 hrs compared with control.

Significant increases in DHAR activities up to 2.62, 3.36 and 7.51-folds; 2.33, 3.75, and 4.33-folds; and 2.84, 2.11 and 3.60-folds compared to control were found in heb07-2, iapar9 and wr04-6 leaves at 5, 24, and 72 hrs after drought stress, respectively. The leaf DHAR activity in yuefu significantly increased at 5 and 24 hrs, and decreased at 72 hrs. The change of DHAR activity in heb07-2 root was similar to change in leaf which accumulated 1.31, 2.12 and 2.52-folds at 5, 24, and 72 hrs after drought stress and wr04-6 increased up to 2.16, 1.92, 1.41-folds. The root DHAR activity was marginally increased at 5 hrs after drought stress, and significantly decreased at 24 and 72 hrs, respectively compared to control.

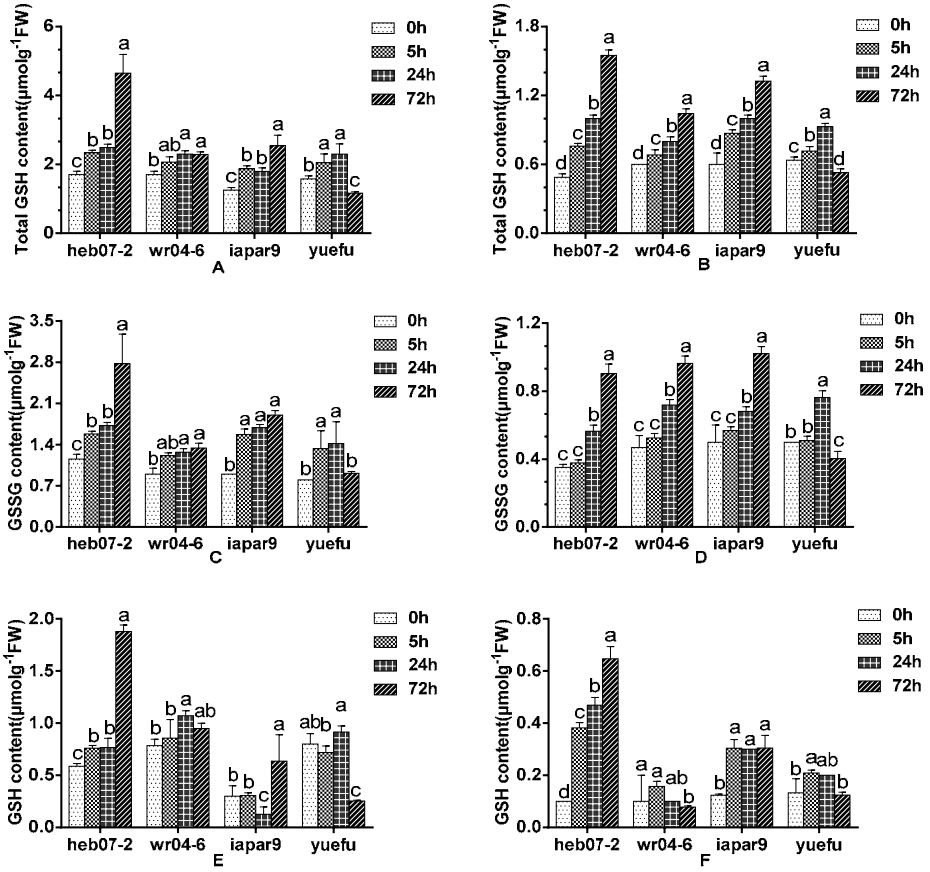

Fig. 4. Total GSH content in leaves (A) and roots (B), GSSG content in leaves (C) and roots (D), GSH content in leaves (E) and roots (F) heb07-2, wr04-6, iapar9, yuefu under drought condition for 0, 5, 24, 72 hrs. Values are mean ± Sd based on three independent determinations and bars indicate standard deviations. The different small letters indicate significant difference at p = 0.05 level.

The level of total glutathione (GSH + GSSG) pool increased consistently in the leaves of heb07-2 and iapar9 (except at 24 hrs) with an increase in during of water deficit, while in the leaves of wr04-6 and yuefu prolonged water deficit at 72 hrs caused a decline in total glutathione (GSH + GSSG) level. The water deficit in wr04-6 was also significantly higher than in control and in yuefu was markedly lower than in control. Water deficit for 5, 24 and 72 hrs led to 56.25,

112.51, 218.76, 10.95, 36.32, 69.41 and 34.46, 48.86, 104.60% enhancement in total glutathione (GSH+GSSG) level in heb07-2, wr04-6 and iapar9 roots, respectively whereas water deficit for 72 hrs led to 17.12% decline in total glutathione (GSH + GSSG) level in yuefu roots.

The altered level of GSSG in the leaves and roots of all genotypes was similar to total glutathione levels.

The GSH level increased coincidentally in the leaves of heb07-2 with an increase in during of water deficit. Water deficit for 5, 24 and 72 hrs led to 29.4, 30.6 and 220.1% enhancement in GSH level of heb07-2 leaves, respectively whereas GSH content of iapar9 declined at 5 and 24 hrs and significantly increased at 72 hrs. However, the GSH level changed in wr04-6 leaves in contrast to iapar9, and GSH content in yuefu was significantly lower compared to control at 72 hrs.

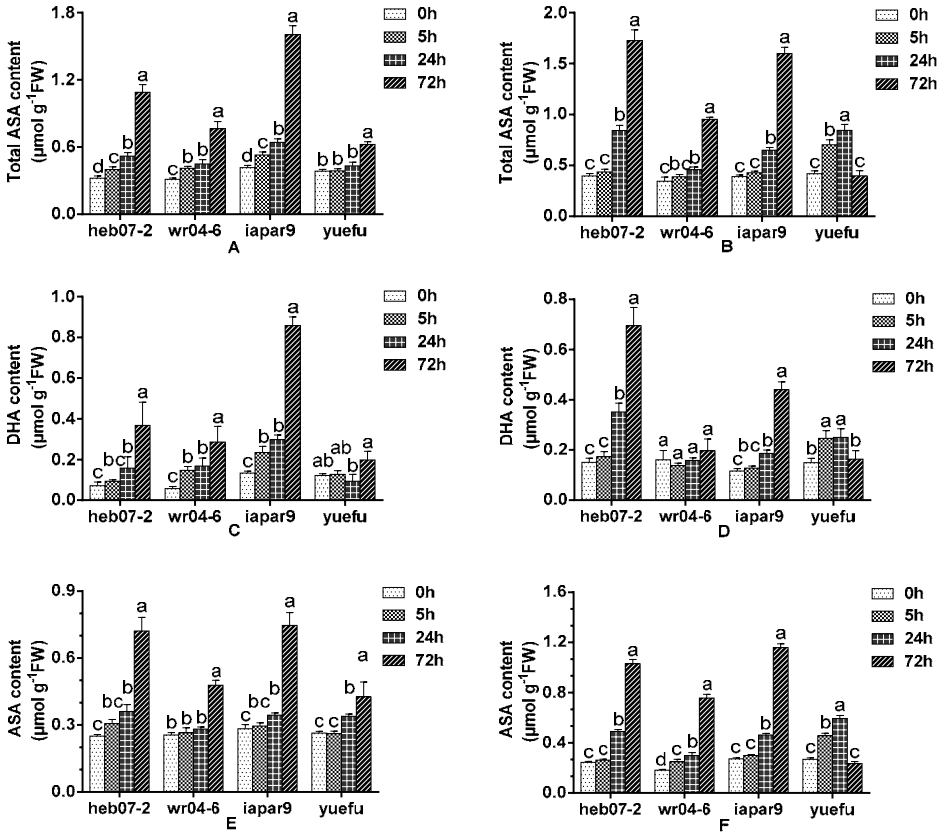

Fig. 5. Total ASA content in leaves (A) and roots (B), DHA content in leaves (C) and roots (D), ASA content in leaves (E) and roots (F) heb07-2, wr04-6, iapar9, yuefu under drought condition for 0, 5, 24, 72 hrs. Values are mean ± Sd based on three independent determinations and bars indicate standard deviations. The different small letters indicate significant difference at p = 0.05% level.

The total ascorbate content in drought-tolerant genotypes of heb07-2 and iapar9 in leaves increased by 24, 61, 238.1 and 27, 54, 285% than their controls at 5, 24, 72 hrs of drought stress, respectively whereas it was 31.37, 43.68, 144.25% for wr04-6, respectively and declined at 72 hrs for yuefu (Fig. 5A). The altered total ascorbate content in roots of heb07-2, iapar9, wr04-6 and yuefu was similar to that of leaves.

The DHA level significantly increased in the leaves of heb07-2, wr04-6 and iapar9 with an increase during water stress procession, while DHA content in yuefu had no significant effect on drought stress levels. DHA content in roots of heb07-2 increased by 132.98 and 361.34% after 24 and 72 hrs of water stress, and the DHA level in the roots of iapar9 was changed similar to heb07-2.

The ASA level increased consistently in the leaves and roots of all genotypes except in the roots of yuefu after 72 hrs of drought stress increased during water stress procession. In contrast, the drought-tolerant genotypes of heb07-2 and iapar9 showed higher ASA levels compared to drought-sensitive genotype of yuefu and less drought-tolerant genotype of wr04-6.

PEG is a long chain, inert substance, which is soluble in water, nonionic, and impermeable and therefore, used to simulated drought stress in soil (Couper and Eley 1948). PEG is commonly used to simulate drought stress in field conditions (Menconi *et al*. 1995).

Drought stress can lead to overproduction ROS in rice which causes protein, lipids and DNA damage (Gill and Tuteja 2010). The MDA level following membrane lipid peroxidation, is frequently used as an indicator of oxidative damage (Zhang *et al*. 2009). In our experiments, the levels of MDA, O_2^- increased in four genotypes after rice seedlings were subjected to drought, indicating the oxidative stress occurred. Moreover, drought-tolerant and drought-sensitive genotype, heb07-2 and yuefu accumulated least and most MDA and O_2^- under drought stress, respectively. Therefore, our results indicated that heb07-2 was more tolerant to drought stress than other genotypes, and yuefu was most sensitive to drought stress in four genotypes. It was similarly observed in previous report that drought-tolerant genotype showed higher tolerance to oxidative damage (Basu *et al*. 2010).

ROS can be scavenged efficiently by enzymes in plant, such as SOD, POD, CAT, and APX. SOD plays a very important role in scavenging superoxide anions to H_2O_2 and O_2^-, which contributed to be first line of defense against ROS. Higher SOD activity in tolerant genotypes indicated higher superoxide anion scavenging activity under stress. In this study, the SOD activity of heb07-2 increased with increasing drought stress. However, SOD activity of wr04-6, iapar9, and yuefu increased first and then decreased under severe drought. Increased SOD activity as observed in present study suggests that heb07-2 was more potent in its ability to scavenge ROS compared with wr04-6, iapar9 and yuefu. The findings indicated that the higher SOD activity in leaves and roots of heb07-2 scavenged ROS efficiently and mitigated oxidative damage compared with other materials, which accumulated higher ROS levels. Supratim Basu *et al*. (2010) also found that drought-tolerant genotypes of rice showed a higher SOD activity than drought-sensitive genotypes. Sharma and Dubey (2005) have also previously indicated that with increasing drought stress, SOD activity increased significantly in scavenging ROS.

The activity of CAT is closely related to stress resistance (Demiral and Türkan 2005, Egert and Tevini 2002, Wang and Huang 2004). Present results showed that CAT activity of heb07-2 in leaves and roots increased significantly with increasing drought stress compared to wr04-6, iapar9, and yuefu indicating that heb07-2 manifested higher CAT activity resulting in lower ROS production and lipid peroxidation. The activity of wr04-6, iapar9 and yuefu in leaves decreased with increasing drought stress possibly due to accumulated ROS triggering CAT inactivity, consistent with previous studies (Basu *et al*. 2010, Dwivedi *et al*. 1979, Yancey *et al*. 1982). In present study, the absolute value of CAT activity in roots was much smaller than that in leaves, which suggests, at least here, that CAT appears not to be an effective scavenger in roots in this case.

In tolerant plant species, a higher POD activity protected against oxidative stress, which was confirmed by a previous study (Hediye Sekmen *et al*. 2007). The content of GSSG, ASA and phenolic compounds increased under drought stress, which are POD substrates, are scavengers of

activated oxygen species (Elstner 1982, Shivrain *et al*. 2010). In our experiment, POD activities in heb07-2 leaves increased significantly with increasing drought stress compared with other materials indicating that heb07-2 manifested stronger ability to scavenge ROS, to maintain the metabolic and structural integrity of cells. However, enhanced POD activities in heb07-2 roots were lower than in leaves due to CAT and APX activities in heb07-2 roots were higher, which may partly replace POD physiological function.

APX is an enzyme of the ascorbate-glutathione pathway, which scavenges H_2O_2 efficiently (Foyer and Noctor 2005, RIAZI *et al*. 1985). Under drought or salt conditions, drought-tolerant or salt-tolerant potato, sugar beet and rice had higher (Demiral and Türkan 2005, Mallick and Mohn 2000, Shalata and Tal 1998). APX activity is resulting in lower lipid peroxidation. In our study, we found a significant increase in APX activity in heb07-2 leaves and roots compared to other antioxidants, which indicated that heb07-2 exhibited stronger free-radical scavenging capacity to mitigate oxidative damage.

ASA and GSH are widely used as potent non-enzyme antioxidants in the plant cell. ASA plays an important role in defense against $^1O_2$, O_2^-, HO^- and thiol radicals, which is a major primary antioxidant synthesized on the inner membrane of the mitochondria, and acts as the natural substrate for plant peroxidases (Foyer *et al*. 2001, Smirnoff 2005, Srivalli *et al*. 2003). GSH is another crucial low molecular weight nonprotein thiol which defense against oxidative damage by ROS-induced in intracellular. In our study, the content of ASA and GSH under drought stress increased significantly, especially in seedlings of drought-tolerant genotype heb07-2 because of the higher ROS level, which induced an antioxidant adaptive response. Results showed a reduction in GSH and ASA level in the sensitive genotype yuefu after 72 hrs of drought stress suggest a rapid and overwhelming increase in ROS levels (Noctor and Foyer 1998), which was consistent with another study (Sharma *et al*. 2012). The results indicated that heb07-2 scavenged ROS promptly and effectively compared with other materials because of a higher level of ASA and GSH content in the seedlings under drought stress. Similarly, other studies also reported a higher level of ASA and GSH in the drought-tolerant rice genotype under drought stress. In the present study, the ASA and GSH content in leaves was higher than in roots under normal and stress conditions, while their level in roots increased higher than in leaves under drought stress. The ascorbate-glutathione cycle occurs within cell including ASA, GSH, and specific enzymes MDHAR, DHAR and GR (Foyer and Halliwell 1976). DHA can be catalyzed to ASA by DHAR through oxidizing GSH. DHAR has been widely used as an indicator of oxidative stress in plant metabolism (Vadassery *et al*. 2009). GR is a flavor enzyme, in which oxidized cysteine residues exist in the active sites that catalyze GSSG to GSH. When plants are subjected to water stress, *de novo* synthesis of DHAR and GR is one of the primary responses to alleviate the oxidative stress through regeneration of potent antioxidants ASA and GSH. Results indicated that the GR activity of heb07-2 was enhanced significantly under drought stress, following the reduction of oxidized GSSG to GSH, increasing conversion efficiency. The GR activity of yuefu declined after 72 hrs drought stress, which prevented GSH generation, and decreased GSH conversion efficiency. The results showed that the weedy rice heb07-2 adapted to drought better by increasing the enzymes of ascorbate-glutathione cycle, which was consistent with the study of Pyngrope *et al*. (2013).

Taken together, drought-tolerant genotype of heb07-2 might survive better under drought stress in comparison to other genotypes of iapar9, wr04-6 and yuefu by retaining higher antioxidant enzymes activities and contents of antioxidants and alleviating oxidative damage. Weedy rice is available germplasm resource in rice breeding programme because hybrid sterility was not observed between weedy rice and cultivars due to their genetic similarity (Tang *et al*. 2011). Weedy rice heb07-2 was an excellent genetic resource for improving the drought tolerance of cultivated rice varieties.

Acknowledgements

This work was supported by the Rice Research Institute, Shenyang Agricultural University/Key Laboratory of Northeast Rice Biology and Breeding, Ministry of Agriculture/Key Laboratory of Northern Japonica Super Rice Breeding, Ministry of Education. The Postdoctoral Scientific Research Station of HAAS, Harbin, Heilongjiang, China also supported this work.

References

Basu S, Roychoudhury A, Saha PP and Sengupta DN 2010. Differential antioxidative responses of indica rice cultivars to drought stress. Plant Growth Regul. **60**: 51-59.

Couper A and Eley D 1948. Surface tension of polyoxyethylene glycol solutions. J. Polym. Sci. **3**: 345-349.

Demiral T and Türkan I 2005. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ. Exp. Bot. **53**: 247-257.

Dhindsa RS, Plumb-Dhindsa P and Thorpe TA 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot. **32**: 93-101.

Doulis AG, Debian N, Kingston-Smith AH and Foyer CH 1997. Differential localization of antioxidants in maize leaves. Plant Physiol. **114**: 1031-1037.

Dwivedi S, Kar M and Mishra D 1979. Biochemical changes in excised leaves of *Oryza sativa* subjected to water stress. Physiol Plantarum. **45**: 35-40.

Egert M and Tevini M 2002. Influence of drought on some physiological parameters symptomatic for oxidative stress in leaves of chives (*Allium schoenoprasum*). Environ. Expt. Bot. **48**: 43-49.

Elstner EF 1982. Oxygen activation and oxygen toxicity. Annual review of plant physiology **33**: 73-96.

Elstner EF and Heupel A 1976. Inhibition of nitrite formation from hydroxylammonium chloride: A simple assay for superoxide dismutase. Anal. Biochem. **70**: 616-620.

Foyer CH and Halliwell B 1976. The presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. Planta.+30

3++ **133**: 21-25.

Foyer CH and Noctor G 2005. Oxidant and antioxidant signalling in plants: a re evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ. **28**: 1056-1071.

Foyer CH, Theodoulou FL and Delrot S 2001. The functions of inter- and intracellular glutathione transport systems in plants. Trends Plant Sci. **6**: 486-492.

Gill SS and Tuteja N 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Bioch. **48**: 909-930.

Hak-Soo S 1993. Collection and evaluation of Korean red rices V. Germination characteristics on different water and soil depth. Korean J. Crop Sci. **38**: 128-133.

Hediye Sekmen A, Türkan İ and Takio S 2007. Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt tolerant *Plantago maritima* and salt sensitive *Plantago media*. Physiol. Plantarum. **131**: 399-411.

Knörzer OC, Burner J and Boger P 1996. Alterations in the antioxidative system of suspension cultured soybean cells (*Glycine max*) induced by oxidative stress. Physiol. Plantarum. **97**: 388-396.

Li H, Sun Q, Zhao S and Zhang W 2000. Principles and techniques of plant physiological biochemical experiment. Higher Education of Beijing press. p. 186.

Mallick N and Mohn FH 2000. Reactive oxygen species: response of algal cells. J. Plant Physiol. **157**: 183-193.

Menconi M, Sgherri C, Pinzino C and Navari-Lzzo F 1995. Activated oxygen production and detoxification in wheat plants subjected to a water deficit programme. J. Exp. Bot. **46**: 1123-1130.

Nakano Y and Asada K 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. **22**: 867-880.

Noctor G. and Foyer CH 1998. Ascorbate and glutathione: Keeping active oxygen under control. Ann. Rev. Plant Biol. **49**: 249-279.

Pyngrope S, Bhoomika K and Dubey R 2013. Reactive oxygen species, ascorbate-glutathione pool, and enzymes of their metabolism in drought-sensitive and tolerant indica rice (*Oryza sativa* L.) seedlings subjected to progressing levels of water deficit. Protoplasma **250**: 585-600.

Riazi A, Matsuda K. and Arslan A 1985. Water-stress induced changes in concentrations of proline and other solutes in growing regions of young barley leaves. J. Exp. Bot. **36**: 1716-1725.

Shalata A and Tal M 1998. The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt□tolerant relative Lycopersicon pennellii. Physiol. Plantarum. **104**: 169-174.

Sharma P and Dubey RS 2005. Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. Plant Growth Regul. **46**: 209-221.

Sharma P, Jha AB, Dubey RS and Pessarakli M 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J. Bot. **2012**: 1-26.

Shivrain VK, Burgos NR, Sales MA and Kuk YI 2010. Polymorphisms in the ALS gene of weedy rice (*Oryza sativa* L.) accessions with differential tolerance to imazethapyr. Crop Prot. **29**: 336-341.

Smirnoff N 2005. Ascorbate, tocopherol and carotenoids: Metabolism, pathway engineering and functions. Antioxidants and Reactive Oxygen Species in Plants **2005**: 53-86.

Srivalli B, Sharma G. and Khanna Chopra R 2003. Antioxidative defense system in an upland rice cultivar subjected to increasing intensity of water stress followed by recovery. Physiol. Plantarum. **119**: 503-512.

Tang L, Ma DR, Xu ZJ, Deng HF, Chen WF and Yuan LP 2011. Utilization of weedy rice for development of japonica hybrid rice (*Oryza sativa* L.). Plant Sci. **180**: 733-740.

Vadassery J, Tripathi S, Prasad R, Varma A and Oelmüller R 2009. Monodehydroascorbate reductase 2 and dehydroascorbate reductase 5 are crucial for a mutualistic interaction between *Piriformospora indica* and *Arabidopsis.* J. Plant Physiol. **166**: 1263-1274.

Wang Z and Huang B 2004. Physiological recovery of Kentucky bluegrass from simultaneous drought and heat stress. Crop Sci. **44**: 1729-1736.

Yancey PH, Clark ME, Hand SC, Bowlus RD and Somero GN 1982. Living with water stress: evolution of osmolyte systems. Science **217**: 1214-1222.

Zhang L, Dai W, Wu C, Song X and Qiang S 2012. Genetic diversity and origin of Japonica- and Indica-like rice biotypes of weedy rice in the Guangdong and Liaoning provinces of China. Genet. Resour. Crop Ev. **59**: 399-410.

Zhang W, Jiang B, Li W, Song H, Yu Y and Chen J 2009. Polyamines enhance chilling tolerance of cucumber (*Cucumis sativus* L.) through modulating antioxidative system. Sci. Hort. **122**: 200-208.

(Manuscript received on 14 March, 2016; revised on 21 April, 2016)