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ORIGINAL RESEARCH

Examination of Antioxidative System's Responses in the Different Phases of Drought Stress and During Recovery in Desert Plant *Reaumuria soongorica* (Pall.) Maxim

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Abstract The aim of this study was to test the protective roles of superoxide dismutases (SODs), guaiacol peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) against oxidative damage and their activities in different phases of the dry down process in *Reaumuria soongorica* (Pall.) Maxim. leaves. Drought stress was imposed during 100 consecutive days and rewatering after 16, 72, and 100 days. The concentration of hydrogen peroxide (H_2O_2), malondialdehyde, and SODs activities were elevated significantly with progressing drought stress. POD and CAT activities increased markedly in the early phase of drought and decreased significantly with further drought stress continuation, and POD activity was unable to recover after rewatering. Ascorbate, reduced glutathione, APX, and GR activities declined in the initial stages of drought process, elevated significantly with further increasing water deficit progression and recovered after rewatering. These results indicate that: (1) iron SODs-removing superoxide anion is very effective during the whole drought stress; (2) CAT scavenges H_2O_2 in the early phase of drought and enzymes of ascorbate–glutathione cycle scavenge H_2O_2 in further increasing drought stress; and (3) POD does not contribute to protect against oxidative damage caused by H_2O_2 under drought stress.

Keywords Antioxidant enzymes · Drought stress · Hydrogen peroxide · Reactive oxygen species · *Reaumuria soongorica*

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Plants growing in desert areas have to survive extremely high irradiance, high air temperature, and severe soil drought (Streb et al. 1997). Out of these, water availability is the most limiting factor influencing the growth of desert plants (Mittler et al. 2001). Drought-induced process of stomatal closure increases the oxidative load on the plant tissues causing imbalance in biochemical pathways and consequently formation of reactive oxygen species (ROS), such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen, and hydroxyl radicals (Foyer and Noctor 2005; Luna et al. 2005) and further give rise to oxidative damage to lipids, proteins, and nucleic acids. Thus, desert plants must acclimate to a variety of arid environments specifically, either by anatomical characteristics which enable them to lower the absorption of solar radiation or by physiological and biochemical means through which photo-oxidative damage of plant tissues is either avoided or rapidly repaired (Streb et al. 1997). These means include xanthophylls cycle, photorespiration, other changes in metabolic activity, and a number of enzymatic and non-enzymatic antioxidants (Munné-Bosch and Peñuelas 2004). Enzymatic antioxidants, such as superoxide dismutases (SODs, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), guaiacol-type peroxidase (POD, EC 1.11.1.7.), ascorbate peroxidase (APX, EC 1.11.1.11), and glutathione reduc-

tase (GR, EC 1.6.4.2), cooperate to minimize the concentrations of superoxide and hydrogen peroxide (Gajewska et al. 2006; Rajendran et al. 2007). Drought stress may increase the specific activity of these antioxidant enzymes and induce new isoenzymes to overcome the increased oxidative stress (Srivalli et al. 2003). Based on the metal cofactor used by SODs enzymes, they are classified into three groups: iron SODs (FeSODs), manganese SODs (MnSODs), and copper–zinc SODs (Cu/ZnSODs), and these SODs are located in different compartments of the cell. SODs are crucial for the removal of O_2^- (Alscher et al. 2002). The antioxidants such as ascorbate and glutathione are involved in scavenging ROS primarily via the ascorbate–glutathione (AsA–GSH) cycle, while GR is involved in the regeneration of ascorbate (Horemans et al. 2000; Khanna-Chopra and Selote 2007).

Although several studies have provided evidence for effective protective roles of some antioxidant enzymes against oxidative stress in diverse plant species, the responses of the antioxidant enzymes remain debatable (Cavalcanti et al. 2004). First, the data concerning SODs activity changes under drought stress are quite contradictory. It was reported that SODs activity increased in wheat and rice (Shao et al. 2007; Srivalli et al. 2003). However, a decrease in SODs activity has been reported in *Corchorus capsularis* L., *Corchorus olitorius* L., *Acacia holosericea*, *Bauhinia variegate*, and *Cassia siamea* (Roy Chowdhury and Choudhuri 1985; Sinhababu and Kumar Kar 2003). Second, it is questionable whether the increase of POD activity is related to elimination of H_2O_2. Some researchers support the theory that elevated POD activity scavenges H_2O_2 (Jung 2004), whereas other researchers suggest that it promotes a higher lignification process (Cavalcanti et al. 2004; Lin and Kao 2002). Third, the response of CAT activity to osmotic stress has frequently been contradictory. Some reports have shown enhanced CAT activity in rice (Vaidyanathan et al. 2003), whereas some works have displayed reduced CAT activity in *Crithmum maritimum* L. (Ben Hamed et al. 2007). Fourth, the effects of water stress reported in the literature on APX and GR activities were disputable and depended both on the degree of tolerance of the plant and on the way of water stress (Contour-Ansel et al. 2006). Another controversial point is the lack of studies on oxidative enzymes during recovery from drought stress (Jung 2004).

Reaumuria soongorica (Pall.) Maxim., an extreme xeric shrub of Tamaricaceae, is the dominant species of the desert in China. It exhibits strong sand fixation ability and survives extreme arid and high salinity conditions, which are important characteristics for maintaining the stability and continuity of the desert ecosystem (Ma et al. 2005). Previous workers have studied the genetic diversity (Xu et al. 2003) and the distribution characteristics of the stable carbon isotope (Ma et al. 2005) in *R. soongorica* population. In addition, the protective mechanism of desiccation tolerance mainly focuses on photosynthetic characteristics and sugar content in *R. soongorica* (Liu et al. 2007). Furthermore, photorespiration in *R. soongorica* consumed excess electrons and protected photosynthetic apparatus under moderate drought stress, whereas it accelerated H_2O_2 accumulation markedly and induced the leaf abscission under severe drought stress (Bai et al. 2008). However, we have not found studies on mechanisms of antioxidative defense under drought stress in *R. soongorica*. Stomatal closure induced by drought stress reduces the CO_2/O_2 ratio in leaves and inhibits CO_2 fixation, which increase the rate of ROS formation and further result in oxidative stress (Foyer and Noctor 2003). *R. soongorica* is characterized by very high water use efficiency and photosynthesis and maintains a high capacity of dissipation of the excitation energy by non-photochemical quenching under desiccation. Moreover, when *R. soongorica* is subjected to desiccation, leaves are wilted and die completely. But even then, they are still able to recover and develop new leaves upon rainfall (Liu et al. 2007). These observations suggest that *R. soongorica* is an excellent material for studying the mechanism of drought resistance in desert environment.

Many researchers have reported the effective protective roles of antioxidant enzymes against oxidative damage under drought stress in plants. However, to our knowledge, there are a few studies that have been carried out to elucidate the physiological protective roles of antioxidant enzymes in plants in the different phases of drought stress in detail. Consequently, we aimed to investigate the response and mechanisms of antioxidative protection in *R. soongorica*. The object was to test the different protective roles of SODs, POD, CAT, APX, and GR against oxidative damage in this shrub in different phases of drought and their recovery abilities after rewatering. The studies on mechanisms of antioxidative defense to drought stress will promote us to better understand drought-resistance mechanisms in desert plants and to exert all their capacities to maintain the stability and continuity of the desert ecosystem.

Materials and Methods

Plant Material, Growth Conditions, and Drought Stress

The research was conducted from 14 May to 8 September 2007 in the Botanical Garden at Lanzhou University. Environmental conditions in the experiment field are typically semiarid. The 3-year-old *R. soongorica* plants were transplanted from the field to individual 6-L plastic pots. The potted plants were transferred to a field tunnel

with the same depth as the height of pots. The bottoms of the plastic pots were mulched with plastic to avoid the roots spreading into ground and thereby absorb moisture from it. A rain shed was made over pots and covered with plastic to avoid precipitation. Our experimental design was similar to that of Kranner et al. (2002) and Munné-Bosch and Peñuelas (2004). To study the different effects of short- and long-term drought stress, 18 different plant individuals were subjected to drought stress for 16, 72, and 100 days and then rewatered, respectively. Leaves subjected to drought stress were collected at intervals of 0 (as "control"), 3, 9, 12, 16, 21, 26, 60, 66, 69, 72, 76, 86, and 91 days. Rewatering leaves previously subjected to drought stress for 16 days were collected at intervals of 0 (non-rewatered), 2, 5, 8, 12, 24, 48, and 96 h. Rewatering leaves subjected to drought stress for 72 days were collected at intervals of 0 (non-rewatered), 6, 12, 24, and 48 h. After 100 days, leaves died and abscised. New leaves developed after rewatering for 2 weeks. Leaf samples were frozen, stored in liquid nitrogen, and used for physiological and biochemical analysis.

Leaf Water Status

Leaf water status was expressed as the relative water content (RWC) of leaf samples collected according to the method of Munné-Bosch et al. (2001b). The relative leaf water content was determined as RWC=(FW − DW)/(TW − DW)×100, where FW is the fresh weight, TW is the turgid weight after rehydrating the samples for 24 h in darkness at 4°C, and DW is the dry weight after drying the samples to constant weight in an oven at 85°C.

Lipid Peroxidation and H_2O_2 Concentration Measurement

Lipid peroxidation was estimated as malondialdehyde (MDA) content (i.e., the concentration of thiobarbituric acid (TBA) reactive substances; Li 2000a). Leaf tissue (0.4 g) was homogenized in 8 ml of 5% trichloroacetic acid (TCA), and then the homogenate was centrifugated at 3,000×*g* for 10 min. The total reaction mixture of 4 ml contained 2 ml of extract and 2 ml of 0.67% (*w*/*v*). TBA was heated to 100°C for 30 min and then quickly cooled on ice. After centrifugation at 10,000×*g* for 10 min, the absorbance of the supernatant was measured at 532 nm. A correction for nonspecific turbidity was made by subtracting the absorbance value taken at 600 nm. The lipid peroxides were expressed as nanomole per gram drought weight using an extinction coefficient of 155 mmol L^{-1} cm^{-1}. The concentration of H_2O_2 in leaves was measured by monitoring the absorbance of the titanium–peroxide complex at 410 nm, using the method of Patterson et al. (1984). Leaf tissues of 0.15 g were homogenized in an ice bath with 5 ml 5% (*w*/*v*) TCA. The homogenate was centrifuged at 12,000×*g* for 15 min, and 1 ml of supernatant was added to 0.2 ml 20% (*v*/*v*) $TiCl_4$–HCl and 0.2 ml ammonia. The mixture was centrifuged at 18,000×*g* for 10 min at 4°C. The deposition was dissolved by 3 ml 1 M H_2SO_4. The absorbance of solution was read at 410 nm. Absorbance values were quantified using a standard curve generated from known concentrations of H_2O_2.

Ascorbate and Reduced Glutathione Contents Measurement

AsA and reduced GSH contents were estimated according to the method of Chen and Wang (2002). Leaf tissues of 0.15 g were homogenized with 5 ml 5% (*w*/*v*) TCA. The homogenate was centrifuged at 15,000×*g* for 10 min at 4°C. The supernatants were used for assays of AsA and GSH contents. The method of determining AsA content is following as: supernatant (0.2 ml) was combined with 0.2 ml of 150 mM NaH_2PO_4 (pH 7.7). To this mixture, 0.4 ml of 10% (*v*/*v*) TCA, 0.4 ml of 42% (*v*/*v*) H_3PO_4, 0.4 ml of 4% (*w*/*v*) bipyridyl (in 70% alcohol), and 0.2 mL of 3% $FeCl_3$ (*w*/*v*) was successively added. The mixture was incubated at 42°C for 40 min. Absorbance was determined at 525 nm. AsA concentration was calculated by comparison to a standard curve. The method of determining GSH content is following as: supernatant (0.25 ml) was added to 2.6 ml of 150 mM NaH_2PO_4 (pH 7.4). 0.18 ml of 5,5′-dithio-bis(2-nitrobenzoic) (DTNB; 75.3 mg of DTNB was dissolved in 30 mL of 100 mM phosphate buffer, pH 6.8) was then added. The mixture was incubated at 25°C for 10 min. Absorbance was determined at 412 nm, and the GSH concentration was calculated by comparison to a standard curve.

Assay of Antioxidant Enzyme Activities Measurement

For determination of antioxidant enzyme activities, 0.4 g of leaf tissue was homogenized in 3 ml of extraction buffer containing 50 mM sodium phosphate buffer (pH 7.8), 0.6 mM EDTA, 0.3% (*w*/*v*) Triton X-100, and 2% (*w*/*v*) soluble PVP-10 (Grace and Logan 1996). Protein concentration was estimated according to the method of Bradford (1976) using bovine serum albumin as standard.

CAT activity was assayed after Aebi (1983). CAT activity was expressed as enzyme units per mg of protein using a H_2O_2 extinction coefficient of 0.04 $mmol^{-1}$ cm^{-1}. Guaiacol-type peroxidase (POD) activity was measured according to Wang et al. (2002). One unit of POD activity was determined as the increase 0.01 in A_{470} each minute and specific activity as enzyme units per mg of protein. APX was activity was estimated according to Nakano and Asada (1981). The assay depends on the decrease of absorbance at 290 nm as ascorbate was oxidized. APX

activity was expressed as enzyme units per milligram of protein using an ascorbate extinction coefficient of 2.8 $mmol^{-1}$ cm^{-1}. GR activity was measured according to Grace and Logan (1996). The assay depends on the rate of decrease in the absorbance of oxidized glutathione at 340 nm. GR activity was expressed as enzyme units per milligram of protein using a NADPH extinction coefficient of 6.2 $mmol^{-1}$ cm^{-1}. Total SODs activity was assayed according to methods of Li (2000a) by monitoring the capacity of the enzyme extract to inhibit the photochemical reduction of NBT to blue formazan. One unit of SOD activity was expressed as enzyme units per milligram of protein.

Native Polyacrylamide Gel Electrophoresis and Isoenzymes Staining SODs Activities in Gels

SOD isoenzymes were separated by native-polyacrylamide gel electrophoresis (PAGE) in non-denaturing 17% separating and 5% stacking gels at 4°C and stained as described by Beauchamp and Fridovich (1971) as modified by us. After PAGE, gels were soaked in 50 mM sodium phosphate buffer (pH 7.8), containing 1 mM EDTA, 0.12 mM NBT, 26.6 μM riboflavin, and 0.42% (*v*/*v*) TEMED for 30 min in the dark followed by immersing in 50 mM sodium phosphate buffer (pH 7.8), containing 1 mM EDTA which were then exposed to a light source at room temperature. The different SOD isoenzymes were distinguished by their sensitivity to inhibition by 2 mM KCN or 5 mM H_2O_2 before staining (Cn/Zn SODs are inhibited by KCN and H_2O_2, Fe SODs are resistant to KCN but inactivated by H_2O_2, and Mn SODs are resistant to both inhibitors; Fridovich 1986). The gels were scanned and analyzed after staining.

Statistical Analysis

Statistical analysis was performed by one-way ANOVA using SPSS (version 13.0). Significant differences among values were determined at $P < 0.05$, according to least significant difference test. All figures were plotted using Origin (version 6.1).

Results

Effect of Drought Stress and Rewatering on Leaf Water Status

Plants were exposed to the progressive drought stress and were rewatered in different drought phases. After 16 days of drought stress, *R. soongorica* leaves showed a decrease in the RWC from 94% to 79.8% ($P < 0.05$), and 91 days of further drought stress declined in the RWC to 62% ($P < 0.001$) of the initial value (Fig. 1A). After 100 days of drought stress, *R. soongorica* leaves wilted and abscised. When plants that had been exposed to drought stress for 16 and 72 days, respectively, were rewatered, *R. soongorica* leaves RWC recovered to initial values within 24 h (Fig. 1B). However, after 100 days of drought stress, new leaves developed after rewatering for 2 weeks.

Effect of Drought Stress and Rewatering on Lipid Peroxidation and Generation of Hydrogen Peroxide

Lipid peroxidation (MDA content) increased markedly along with progressive drought stress (Fig. 2A). MDA contents were 2.4-, 6.4-, and 8.4-fold ($P<0.001$, $P<0.001$, $P<0.001$) of the initial value in plants subjected to drought stress for 16, 72, and 91 days, respectively. The concentration of hydrogen peroxide in *R. soongorica* leaves increased significantly with the increase of drought stress (Fig. 2C). The concentrations of H_2O_2 were 2.7-, 4.1-, and 11.8-fold ($P<0.001$, $P<0.001$, $P<0.001$) of the initial value in plants subjected to drought stress for 16, 72, and 91 days, respectively. When plants subjected to drought stress for 16 days were rewatered, MDA content recovered transitorily (Fig. 2B), as well as H_2O_2 concentration (Fig. 2D). When plants subjected to 72 days drought stress were rewatered, H_2O_2 concentration recovered transitorily (Fig. 2D), whereas MDA content did not recover (Fig. 2B).

Effect of Drought Stress and Rewatering on Antioxidant enzymes

SOD activity in *R. soongorica* elevated significantly with increasing drought stress. According to the statistics, SOD activity increased to 140%, 217%, and 248% ($P<0.05$, $P<0.05$, $P<0.01$) of the initial values in plants subjected to drought stress for 16, 72, and 91 days, respectively

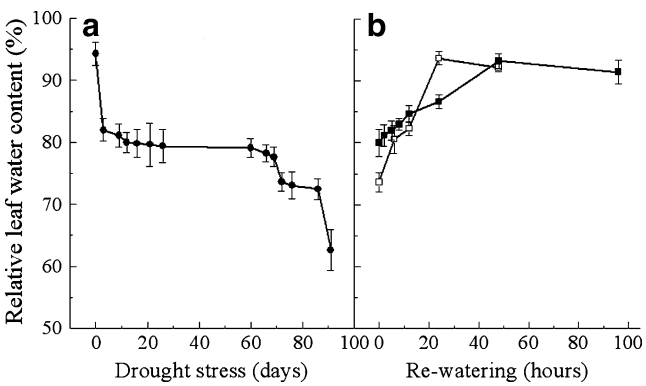

Fig. 1 Relative water content in *R. soongorica* leaves during drought stress (**A**) and rewatering (**B**) after 16 (*closed squares*) and 72 (*open squares*) days drought stress (mean ± SE; $n=6$)

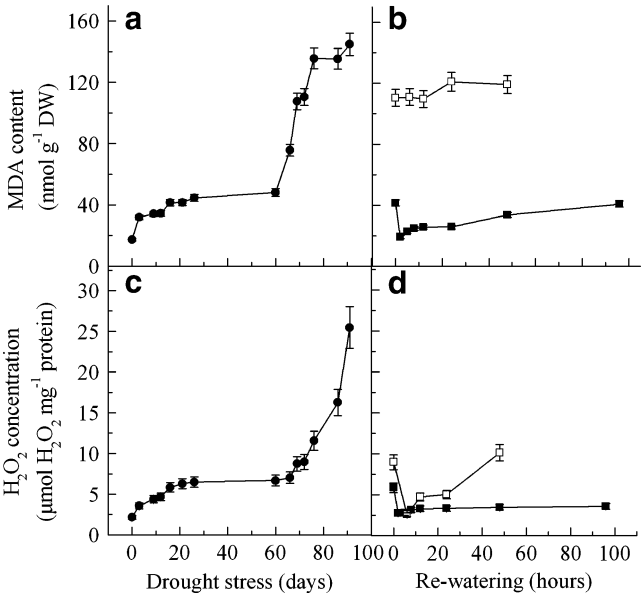

Fig. 2 MDA content and H_2O_2 concentration in *R. soongorica* leaves during drought stress (**A**, **C**) and rewatering (**B**, **D**) after 16 (*closed squares*) and 72 (*open squares*) days drought stress (mean ± SE; n=6). *DW* dry weight

(Fig. 3A). SOD isoenzymes only revealed FeSODs bands without MnSODs and Cu/ZnSODs bands by native-PAGE. FeSOD isoenzyme had four bands (FeSOD1, FeSOD4, FeSOD5, and FeSOD6) during the former phase of drought stress and six bands (FeSOD1, FeSOD2, FeSOD3, FeSOD4, FeSOD5, and FeSOD6) under extremely severe drought stress. Moreover, these bands were thicker along drought stress increasing (Fig. 4). CAT and POD activities increased markedly (P<0.01) compared with the initial value in plants subjected to drought stress for 26 and 60 days, respectively, and decreased with further increasing drought stress and even under the initial values (Fig. 3C, E). APX and GR activities declined during 16 days drought stress and elevated with further progressing drought stress (Fig. 3G, I). When plants subjected to drought stress for 16 days were rewatered, SOD, APX, and GR activities recovered rapidly, and even under the initial values (APX and GR; Fig. 3B, H, J), CAT activity recovered temporarily (Fig. 3D), and POD activity decreased yet did not recover to the initial level (Fig. 3F). However, when plants that had been desiccated for 72 days were rewatered, SOD, APX, and GR activities recovered for a while (Fig. 3B, H, J), but CAT and POD activity did not recover (Fig. 3D, F).

Effect of Drought Stress and Rewatering on Ascrobate and Reduced Glutathione

It is obvious from Fig. 5A, C that both ascrobate and reduced glutathione contents in *R. soongorica* leaves had a decrease under drought stress before 16 days and then an

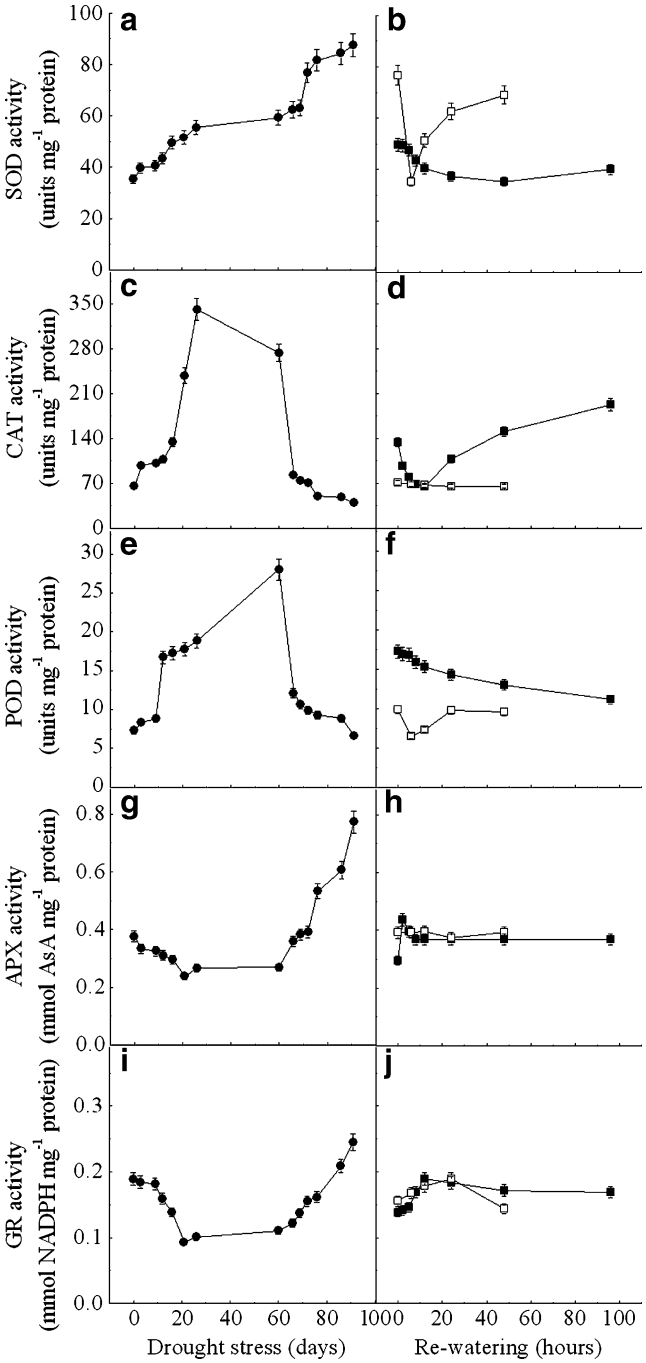

Fig. 3 Activities of SOD, CAT, POD, APX, and GR in *R. soongorica* leaves during drought stress (**A**, **C**, **E**, **G**, **I**) and rewatering (**B**, **D**, **F**, **H**, **J**) after 16 (*closed squares*) and 72 (*open squares*) days drought stress (mean ± SE; n=6)

increase. And Leaf ascrobate and reduced glutathione contents increased to 192% (P<0.01) and 162% (P<0.01) of their initial values in plants subjected to drought stress for 91 days, respectively. When plants that had been desiccated for 16 and 72 days were rehydrated, leaf ascrobate and reduced glutathione content recovered rapidly (Fig. 5B, D).

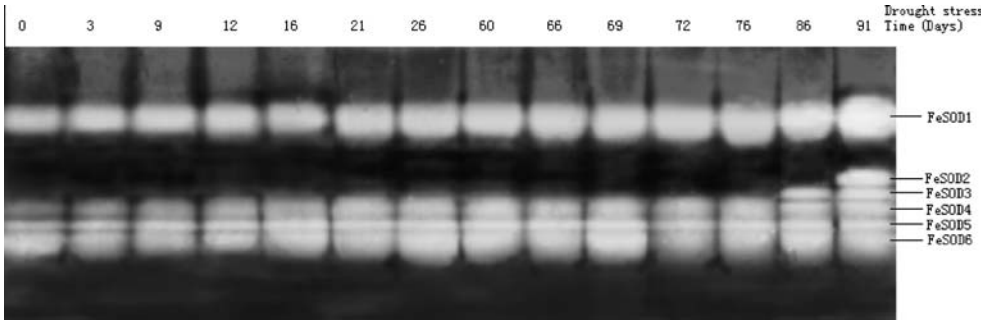

Fig. 4 SOD isoenzymes in *R. soongorica* leaves during drought stress. Nondenaturing activity gels were prepared and run as described in "Materials and Methods"

Discussion

R. soongorica, a perennial desiccation resistant shrub, is distributed widely in China (from the east to the west; Xu et al. 2003). The present study showed that in *R. soongorica*: (1) the concentration of H_2O_2, MDA, and FeSODs were elevated with increasing drought stress; (2) POD and CAT activities increased in the early phase of drought and decreased with further increase in magnitude of drought stress, and POD activity was unable to recover after rewatering; (3) AsA, GSH, APX, and GR activities declined in the early of drought and elevated significantly with further increasing water deficit and recovered after rewatering.

Lipid peroxidation is the systems most commonly ascertained to oxidative damage and, hence, is regarded as an indicator of oxidative stress (Sharma and Dubey 2005). Increased lipid peroxidation as a result of drought-induced oxidative stress has also been reported by various researchers (Sairam and Srivastava 2001; Selote and Khanna-Chopra 2006). In present work, increased MDA in *R. soongorica* indicated drought-induced oxidative stress. Hydrogen peroxide is a toxic compound produced because of the dismutation of the superoxide radical, which, in higher concentration, results in lipid peroxidation and cells' membranes injury. H_2O_2 has also been found to be involved in oxidative metabolism of mitochondria and peroxisomes of senescing leaves and to function in inter- and intracellular signaling (Munné-Bosch et al. 2001a). In present study, continual increase high levels of H_2O_2 was observed in *R. soongorica* leaves along with the progressive drought stress, and H_2O_2 level recovered after rewatering. These results suggested that H_2O_2 may be playing a secondary role in drought stress signaling network by inducing defense pathways in the early phase of drought. However, leaves wilted and abscised under extreme severe drought stress, which suggested that higher H_2O_2 levels might lead to toxicity and induced leaf senescing and abscising. In general, high H_2O_2 levels in *R. soongorica* might be playing two key roles in different phase of drought stress.

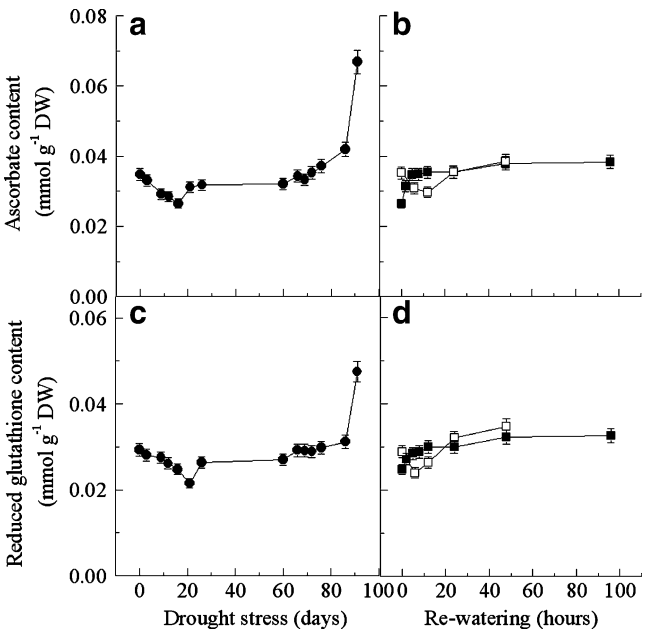

Fig. 5 Ascorbate content and reduced glutathione content in *R. soongorica* leaves during drought stress (**A**, **C**) and rewatering (**B**, **D**) after 16 (*closed squares*) and 72 (*open squares*) days drought stress (mean ± SE; n=6). *DW* dry weight

Plants subjected to drought stress, combined with high irradiance levels and high temperature, undergo an excess of reducing power responding to limitation of CO_2 assimilation, which in turn causes increased levels of ROS. During these stressful conditions, increased activities of ROS-scavenging enzymes might be expected (Sofo et al. 2005). Within a cell, the SODs constitute the first line of defense against ROS, which can react with superoxide radical and convert them to O_2 and H_2O_2. In fact, the activities of all the enzymes measured in *R. soongorica* leaves changed markedly under drought stress. In our work, the activity of SODs and the number of SODs isoenzyme bands in leaves showed an increase in parallel to the progressive drought stress. This result was in contrast to water-stressed plants (e.g., *C. capsularis* L., *C. olitorius* L., *A. holosericea*, *B. variegate*, and *C. siamea*) where SODs activity decreased (Roy Chowdhury and Choudhuri 1985; Sinhababu and Kumar Kar 2003), whereas our results are in agreement with other workers (Shao et al. 2007; Srivalli et

al. 2003). Most importantly, the results indicated that drought-related oxidative stress upregulated the activity of SODs and efficient superoxide anion removal by SODs in *R. soongorica* may be a key protective mechanism, specially, this is FeSODs. FeSODs is found both in prokaryotes and in eukaryotes; the group of FeSODs probably constitute the most ancient SOD group (Alscher et al. 2002). *R. soongarica* comes from tertiary and is one of the representative species in ancient Mediterranean region (Li 2000b). FeSODs are mostly located in the chloroplast. In addition, in some FeSOD-containing plant species such as tobacco, mustard, bean, and citrus, FeSODs is a major SOD isoenzyme in the leaf, indicating that FeSODs alone could remove superoxide from photosynthesis (Van Camp et al. 1997). FeSODs readily catalyses the dismutation of O_2^- to H_2O_2 and molecular oxygen because of the incidental shift between oxidative and reduced FeSODs. Accordingly, the increase and single expression of FeSODs isoenzyme suggested that drought acclimation in *R. soongorica* is probably being managed in chloroplast where FeSODs plays a pivotal role in removing superoxide anion.

In addition, CAT can scavenge H_2O_2 by converting it to H_2O and O_2, and PODs can oxidize and, thus, scavenge H_2O_2 using preferably some phenolic compounds (e.g., guaiacol) as primary reducing agents (Mehlhorn et al. 1996). In previous studies, CAT and POD activities increased in water-stressed plants (Ramachandra Reddy et al. 2004). However, in *R. soongorica* leaves, the activity of CAT and POD showed the trend that there was an increase and then a decrease along with the progressive drought stress. Furthermore, leaf POD activity did not recover after rewatering. Apart from toxic H_2O_2 scavenging via POD activity in plants, it is also involved in the biosynthesis of cell-wall components and lignification (Cavalcanti et al. 2004). Accordingly, the data presented suggested that POD activity in *R. soongorica* may be involved in the cell-wall biosynthesis rather than with protection of plant tissues against the oxidative damage caused by H_2O_2. In addition, the increase of CAT activity indicated that H_2O_2 diffused from the chloroplasts quickly. CAT in *R. soongorica* was very effective in scavenging H_2O_2 in the early phase of drought. However, CAT and POD did not confer protection against oxidative damage under severe drought stress. The decrease of CAT and POD activities might be the result of CAT and POD inactivation and degradation under severe drought stress (Feierabend et al. 1992).

APX, GR, AsA, and GSH in *R. soongorica* leaves played important roles in scavenging H_2O_2 under severe drought stress. APX and GR which act in AsA–GSH cycle and are responsible for H_2O_2 detoxification in leaves. APX is considered to be the primary H_2O_2 scavenging enzyme in plant cells and GR has a central role in maintaining the reduced glutathione pool during stress (Contour-Ansel et al. 2006). In present study, APX and GR increased under severe drought stress. Moreover, AsA and GSH are associated with hydrogen peroxide scavenging via AsA–GSH cycle. AsA is found to be one of the best characterized compounds required for many key metabolic functions in plant cells (Smirnoff and Wheeler 2000). In our study, AsA level decreased in the early phase of drought and increased markedly in further progressing water deficit, which is different from previous studies (Kranner et al. 2002; Munné-Bosch and Peñuelas 2004; Pinheiro et al. 2004; Ramachandra Reddy et al. 2004). The same change occurred in GSH level. On the contrary, GSH increased under mild drought stress and declined significantly during subsequent severe drought stress in *Myrothamnus flabellifolia* (Kranner et al. 2002). AsA and GSH increased during severe drought stress and recovered rapidly after rewatering, suggesting that AsA and GSH are very important in scavenging H_2O_2. Results presented here indicated that AsA–GSH cycle played an important role in scavenging H_2O_2 under severe drought stress although CAT and POD had been inactivated.

In conclusion, FeSODs-removing superoxide anion in *R. soongorica* leaves is very important under the progressive drought stress. In addition, overproduction of H_2O_2 was accompanied with superoxide anion removal. CAT plays an important role in scavenging H_2O_2 in the early phase of drought, whereas enzymes of AsA–GSH cycle are the efficient H_2O_2 removing mechanisms in further progressing in magnitude of drought stress (Fig. 6). Furthermore, the main role of POD is not the protection against oxidative damage caused by H_2O_2, but POD is perhaps more likely involved in the biosynthesis of cell-wall components and lignification. These results are essential for further studying the gene expression of the antioxidant enzymes and then providing evidence for the screening of drought-resistant genes and the selection of suitable genotypes for desert environment.

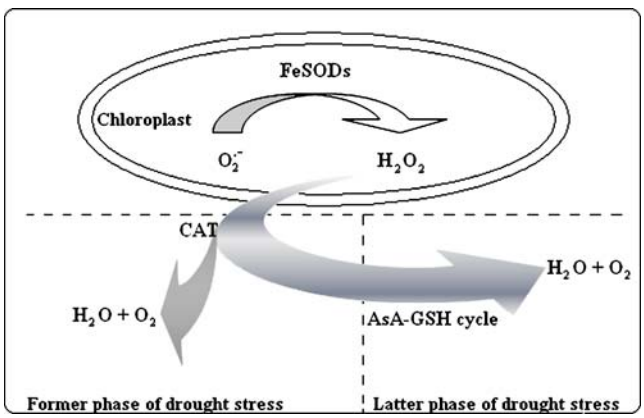

Fig. 6 Simplified schemes of antioxidative system's responses in the different phases of drought stress in desert plant *R. soongorica*

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