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Antioxidant Enzyme Responses against Abiotic and Biotic Stresses in *Rehmannia glutinosa* L. and *Glycine max* L.

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ABSTRACT : *Rehmannia glutinosa* shows a high level of resistance to the non-selective herbicide paraquat. To characterize the antioxidant enzyme system of *R. glutinosa*, we comparatively examined the responses of antioxidant enzymes to UV, wounding and a general elicitor yeast extract in *R. glutinosa* and soybean. The levels of enzyme activities of the two plant species were drastically different between those per fresh weight (general activity) and per protein (specific activity) bases. The general activities of superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), and glutathione reductase (GR) were lower, but that of ascorbate peroxidase (APX) was higher in *R. glutinosa* than in soybean. The specific activities of the enzymes, however, were about two- to seven-fold higher in *R. glutinosa* than in soybean, except that of CAT, which was about 12-fold higher in soybean. The general and specific enzyme activities of *R. glutinosa* relative to those of soybean showed a consistent increase in responses to the stresses only in SOD. The specific activities of SOD and APX were higher in *R. glutinosa* in all stress treatments. The results might suggest a relatively higher contribution of SOD and APX to the stress tolerance.

Key words : *Rehmannia glutinosa*, abiotic stress, biotic stress, paraquat tolerance

INTRODUCTION

Rehmannia glutinosa is a perennial herb native to Korea, China, and Japan. The fresh or dried roots have been used for medicinal purposes to replenish vitality and for the treatment of ailments, including diabetes, constipation, and anemia (Duke, 2002). Despite its long history of ethnobotanical use, however, little information is available on the botanical and agronomic characteristics of the plant species.

Recently, a unique feature of the species, resistance to non-selective herbicide paraquat, has been reported. The resistance of the species to paraquat is about 10- to 100-fold higher than that of soybean (Chun *et al.*, 1997). Furthermore, no accessions of the species are susceptible to paraquat (Kim, 2003). As the herbicidal activity of paraqut is manifested by the burst of reactive oxygen species (ROS) in the photosynthetic organs (Preston, 1994), the resistance to paraquat has been related to the capacity to scavenge the ROS. A considerable amount of direct evidences supports the role of antioxidant enzymes in the resistance (Aono *et al.*, 1995; Allen *et al.*, 1997; Van Breusegen *et al.*, 1999; Ye & Gressel, 2000; Chun *et al.*, 2002). The higher antioxidant enzyme activity, at least in part, is also attributed to the paraquat tolerance in *R. glutinosa* (Chun *et al.*, 1997; Choi *et al.*, 2004). Relative to the significance of tolerance to paraquat,

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however, little is known about the antioxidant enzyme system of the plant species.

Antioxidant enzymes are also implicated in the resistance to various abiotic and biotic oxidative stresses, such as wounding and fungal infections. Different oxidative stresses often share only a part of the complicated response pathways in common (Mittler, 2002; Apel & Hirt, 2004). Therefore, the feature of the antioxidant enzyme systems can be evaluated by a comparative analysis of the responses to the various oxidative stresses.

To characterize the antioxidant enzyme system of R. glutinosa, we comparatively examined responses of the cellular antioxidant enzymes of the species to the abiotic and biotic stresses such as wounding, UV, and a general elicitor yeast extract.

MATERIALS AND METHODS

Chemicals and enzymes

Chemicals and enzymes were purchased from Sigma (USA) unless otherwise indicated.

Plant materials

R. glutinosa (cv. Namwon) and soybean (cv. Danyeopkong) plants were grown for 2 months under normal growing conditions in a glasshouse at Chonbuk National University, Korea. The plants of *R. glutinosa* were propagated from the tubers harvested in the previous growing season. Fresh leaves were collected from the healthy plants of the two species, and used for the stress treatments.

Wounding, elicitor, and UV treatments

The fully expanded leaves of *R. glutinosa* and soybean were collected and subjected to wounding and elicitor treatments. For the wounding treatment, the leaves were punched with fine pins and floated on the sterile water for 12 h in the dark at 25℃. For the elicitor treatment, the leaves were incubated in the sterile solution containing 5 ㎎/㎖ yeast extract for 12 h. For the UV treatment, plants were irradiated with UV light at 1.35 E/㎡/s for 2 h and returned to the dark at 25℃. The irradiated leaves were collected after 10 h in the dark (Chung *et al.*, 2003). All tissue samples were ground into fine powder and kept at −80℃.

Enzyme activity assays

Total cellular extracts were prepared at 4℃ for each antioxidant enzyme and used for the activity assay. The activities of superoxide dismutase (SOD; EC 1.15.1.1), ascorbate peroxidase (APX; EC 1.11.1.11), peroxidase (POX; EC1.11.1.7), catalase (CAT; EC 1.11.1.6), and glutathione reductase (GR; EC 1.6.4.2) were determined spectrophotometrically as described (Choi *et al.*, 2004). SOD activity was determined by calculating the nitroblue tetrazoliumreduction rate (Oberley & Spitz, 1984). APX activity was determined by following the oxidation of ascorbate to dehydroascorbate (Nakano & Asada, 1981). CAT activity was determined by calculating H_2O_2 decomposition rate (Beers & Sizer, 1952). GR activity was determined by the reduction rate of the oxidized glutathione (O'Kane *et al.*, 1996). Protein contents in the enzyme extracts were determined with bovine serum albumin as the standard (Bradford, 1979).

RESULTS

Protein content

Protein content in the control leaves was about 3 and 11 g/㎎ FW in *R. glutinosa* and soybean, respectively (Fig. 1). The leaf protein content of *R.*

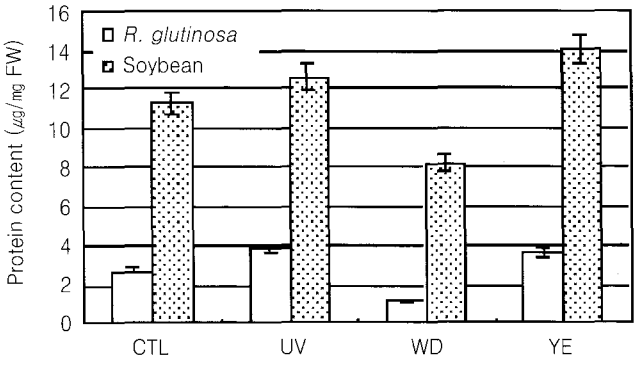

Fig. 1. Responses of protein content to UV, wounding (WD) and yeast extract (YE) in *R. glutinosa* and soybean. CTL, control. In the pair-wise comparisons between the species for each treatment, the protein contents of *R. glutinosa* and soybean were different significantly in all treatments at P = 0.05.

glutinosa was about one- forth of that of soybean in the control and all treatments. Protein content was decreased in the wounding treatment by about 60% and 30% in *R. glutinosa* and soybean, respectively. The decrease in the protein content might be caused by the secretion and dilution of cellular fluids during the incubation of the wounded leaves in water.

Superoxide dismutase activity

The specific SOD activity in the control was higher by about 50% in *R. glutinosa*. The activity was increased or remained unchanged in *R. glutinosa*, but was decreased in soybean in response to the stresses (Fig. 2). Thus, the specific activity of *R. glutinosa* relative to that of soybean was increased by about 33 to 66% by the UV, wounding and YE treatments.

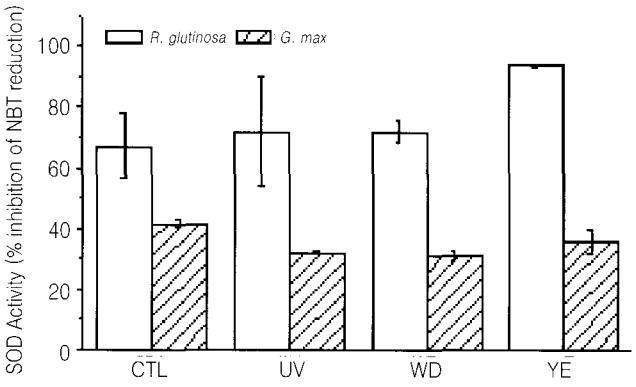

Fig. 2. Responses of SOD activities to UV, wounding (WD) and yeast extract (YE) in *R. glutinosa* and soybean. CTL, control. In the pair-wise comparisons between the species for each treatment, the activities of *R. glutinosa* and soybean were different significantly in all treatments at P = 0.05.

Ascorbate peroxidase activity

The specific and general APX activities of *R. glutinosa* were higher about by seven- and two-fold, respectively, than those of soybean inthe control treatment. The specific and general APX activities of *R. glutinosa* were remained higher under the stresses, except the general activity in wounding, where the activity was similar in the twospecies. The general and specific activities of *R. glutinosa* were increased in response to YE, but decreased in UV and wounding treatments. Those of soybean, however, were remained unchanged or decreased (Fig. 3). Therefore, the activities of *R. glutinos* relative to those of soybean were increased in YE but decreased in wounding relative to the control.

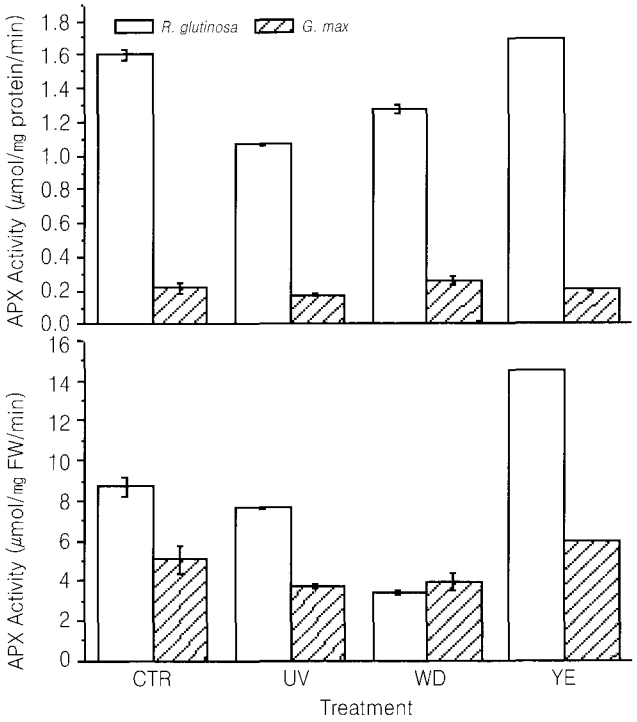

Fig. 3. Responses of specific and general APX activities to UV, wounding (WD) and yeast extract (YE) in *R. glutinosa* and soybean. CTL, control. In the pair-wise comparisons between the species for each treatment, the activities of *R. glutinosa* and soybean were different significantly in all treatments at P = 0.05.

Peroxidase activity

The specific POX activity was about three-fold higher in *R. glutinosa*, but the general activity was similar in the two species. In *R. glutinosa*, the specific activity was increased about three-fold by wounding, but the general activity by about two- to six-fold in all treatments. The specific activities were increased about two-fold in all treatments in soybean. However, the general activity was increased about two-fold in YE, but the activities were remained unchanged in UV and wounding (Fig. 4). The specific activity of *R. glutinosa* relative to soybean was increased two-fold in wounding, but decreased over two-fold in YE and UV treatments (Fig. 4).

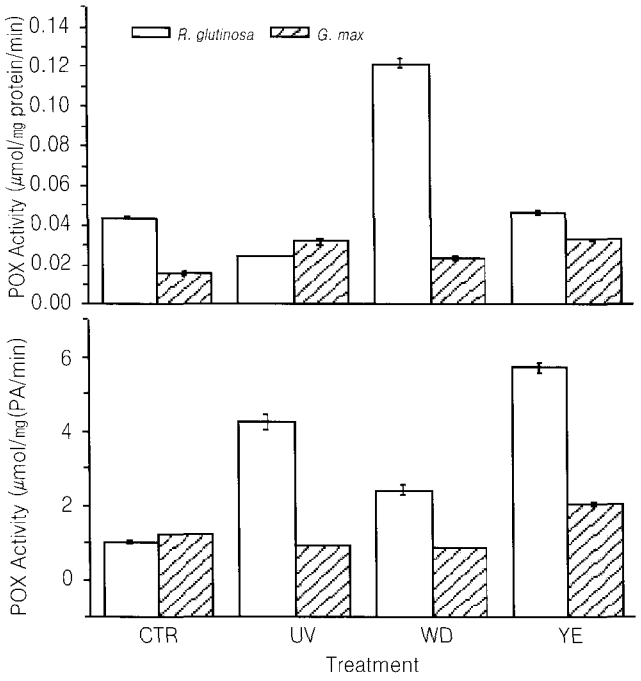

Fig. 4. Responses of specific and general POX activities to UV, wounding (WD) and yeast extract (YE) in *R. glutinosa* and soybean. CTL, control. In the pair-wise comparisons between the species for each treatment, the activities of *R. glutinosa* and soybean were different significantly in all treatments at P = 0.05.

Catalase activity

The specific and general CAT activities were about 10- or 25-fold lower in *R. glutinosa*. The specific activities were decreased in all treatments in the two species, except wounding, where it was remained unchanged. The general activity was increased by the treatments in soybean, but remained unchanged or decreased in *R. glutinosa* (Fig. 5).

Glutathione reductase activity

The specific GR activity was about five-fold higher in *R. glutinosa*, but the general activity was similar in the two species. Except wounding in *R. glutinosa*, the specific activities were decreased in all treatments in the two species. The general activities were also decreased in all treatments, except YE in soybean, where the activity was increased slightly (Fig. 6). As a result, the specific activity of *R. glutinosa* relative to soybean was increased about 40% in wounding, but decreased about 10-fold in UV and YE treatments.

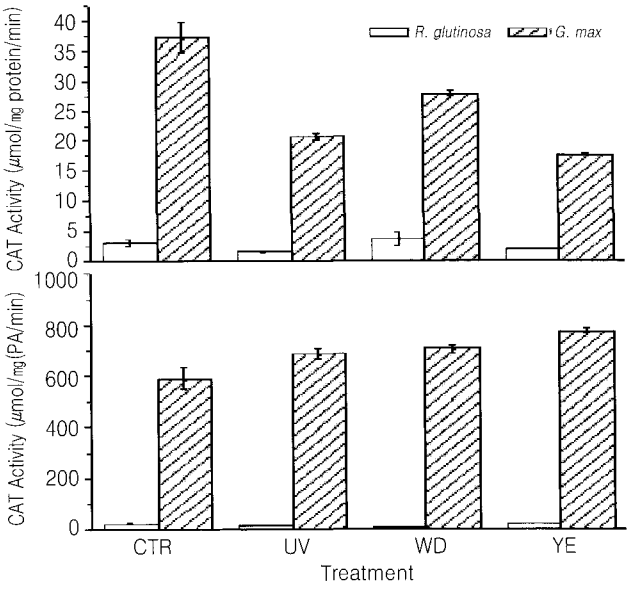

Fig. 5. Responses of specific and general CAT activities to UV, wounding (WD) and yeast extract (YE) in *R. glutinosa* and soybean. CTL, control. In the pair-wise comparisons between the species for each treatment, the activities of *R. glutinosa* and soybean were different significantly in all treatments at P = 0.05.

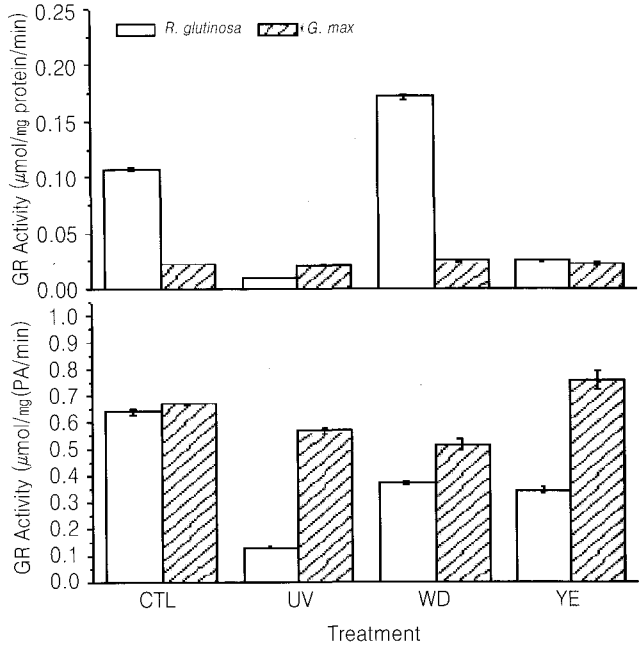

Fig. 6. Responses of specific and general GR activities to UV, wounding (WD) and yeast extract (YE) in *R. glutinosa* and soybean. CTL, control. In the pair-wise comparisons between the species for each treatment, the activities of *R. glutinosa* and soybean were different significantly in all treatments at P = 0.05.

DISCUSSION

The responses of antioxidant enzymes to abiotic and biotic stresses were drastically different between the plant species. Among the antioxidant enzymes examined, APX and CAT showed contrasting activity profiles between the species. The reduced responses of APX and CAT to abiotic and biotic stresses were similar in the two species, except APX to YE in *R. glutinos*, which was increased slightly. In the previous study, we observed qualitatively similar responses of APX and CAT to oxidative stresses and ethylene, but mixed responses of those to salicylicacid in the two species (Choi *et al.*, 2004). Thus, a little change in the APX and CAT activities of *R. glutinosa* relative to soybean in the stresses might indicate that the major H_2O_2 detoxification pathway is APX in *R. glutinosa* but CAT in soybean. The results also suggest that the role of the enzymes is mostly unchanged under the biotic and abiotic stresses.

An interesting result with SOD was that its specific activity of *R. glutinosa* relative to soybean is even increased under the stress conditions. The SOD activity responds similarly to paraqut and salicylic acid in the two species (Choi *et al.*, 2004). The observations might suggest that the capacity of SOD under stress conditions is maintained at higher levels in *R. glutinosa* than in soybean.

In general, the antioxidant enzymes activities are decreased under biotic stresses like pathogen infection, but they are increased under abiotic stresses like ozone exposure (Apel & Hirt, 2004). The responses of CAT and GR specific activities to YE in the two species are in agreement with the general observations. The responses of SOD and APX specific activities to YE are also consistent with the general responses, but only in soybean. The reduced responses of SOD, APX, and CAT specific activities to abiotic stresses were generally similar in the two species. However, the results were contrasting to the general observations of their increases in response to abiotic stresses.

The variable responses of antioxidant enzymes to stresses are frequently observed in different plant species, including those in *R. glutinosa* and soybean to the chemical oxidants and hormones (Choi *et al*, 2004). Fe-SOD expression in response to paraquat is contrasting in *N. tabacum* and *N. plumbaginifolia* (Kurepa *et al.*, 1997). Different responses of APX and CAT to ethylene are also observed in spinach, mung bean and carnations (Sylvestre *et al.*, 1989; Melhorn, 1990; Hodges & Forney, 2000).

Although there are quantitative and qualitative differences in the responses of the antioxidant enzymes to the different stresses, the results from this study confirm our previous observations on the antioxidant systems of *R. glutinosa* (Choi *et al.*, 2004). Most prominent features of the antioxidant system in *R. glutinosa* seem to be a very high level of specific APX activity and a capacity to maintain specific APX and SOD activities at higher levels under various stress conditions. It is noteworthy that the general activities of the enzymes of *R. glutinosa* were much lower than the specific activities under most stress conditions, showing moderate differences with those of soybean. This is most likely related to the low leaf protein content of *R. glutinosa*. Therefore, it is proposed that the localized antioxidant enzyme systems, possibly in the cellular organelles, are more responsible for the higherlevels of specific activities, thus contributing to the oxidative stress tolerances of the species.

ACKNOWLEDGMENTS

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