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Responses of antioxidative system to chilling stress in two rice cultivars differing in sensitivity

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Abstract

The responses of antioxidative system of rice to chilling were investigated in a tolerant cultivar, Xiangnuo-1, and a susceptible cultivar, IR-50. The electrolyte leakage and malondialdehyde content of Xiangnuo-1 were little affected by chilling treatment but those of IR-50 increased. Activities of suoperoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase, and ascorbic acid content of Xiangnuo-1 were remained high, while those of IR-50 decreased under chilling. The results indicated that higher activities of defense enzymes and higher content of antioxidant under stress were associated with tolerance to chilling.

Additional key words: ascorbate peroxidase, ascorbic acid, catalase, glutathione reductase, superoxide dismutase.

Introduction

Abiotic stresses are limiting in crops production. Much of the injury to plants caused by stress is associated with oxidative damage at cellular level (Bowler *et al*. 1992). It was suggested that oxidative stress may be a significant factor in relation to chilling injury in *Arabidopsis thaliana* and rice (Fadzillah *et al*. 1996, O'Kane *et al*. 1996). Chilling induced oxidative stress has also been observed in maize and coffee (Prasad *et al*. 1994, Queiroz *et al*. 1998). Higher plants have defense system to scavenge reactive oxygen species (ROS), consisting of several defense enzymes, such as superoxide dismutase (SOD), ascorbate peroxide (APX), glutathione reductase (GR) and catalase (CAT), and antioxidants, such as ascorbic acid (AsA) and reduced glutathione (GSH) (Bowler *et al*. 1992). Activities of oxygen-scavenging enzymes under chilling stress have been correlated with tolerance to the stress. Higher contents of defense enzymes were correlated with higher chilling tolerance of cucumber radicles (Kang and Saltveit 2002). The chilling-tolerant maize cultivar had higher activities of defense enzymes and contents of antioxidants than the susceptible cultivar (Hodges *et al*. 1997a,b). In our preliminary experiment quite different tolerance to chilling among rice cultivars was observed (Liu *et al*. 2003). In order to explore the mechanism of rice in chilling tolerance or susceptibility, the relationship between antioxidative system and chilling injury were investigated in this paper.

Materials and methods

Plants and treatments: The seedlings of two rice (*Oryza sativa* L.) cultivars, Xiangnuo-1 (chilling tolerant) and IR-50 (chilling susceptible), were cultured in Kimura B nutrient solution in a glasshouse under natural light conditions and temperature of 28 °C for 12 d. The seedlings were then grown for 5 d in the growth chamber at 70 % humidity, and 12-h photoperiod and photosynthetic photon flux density of 165 μmol m^{-2} s^{-1}. The growth chamber temperature was 8 °C for the chilling treatment and 28 °C for the control.

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Abbreviations: ROS - reactive oxygen species; AsA - ascorbic acid; APX - ascorbate peroxidase; CAT - catalase; GR - glutathione reductase; GSH - glutathione, reduced form; MDA - malondialdehyde; SOD - superoxidase.

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Determination of electrolyte leakage: Shoots of four seedlings were immersed in 15 cm^3 of distilled water in a test tube overnight at room temperature. The electrolyte leakage was determined as described by Lu *et al*. (2003).

Determinations of MDA content and enzyme activities: Rice shoots (1 g) were ground in a mortar and pestle in 5 cm^3 of 50 mM phosphate buffer (pH 7.8) at 4 °C. The homogenate was centrifuged at 13 000 *g* for 15 min. The supernatant was recovered for determinations of MDA content, SOD and CAT activity. MDA content was determined by spectrophotometer (*Model U-1100*, Hitachi, Tokyo, Japan) as described by Dhindsa *et al*. (1981). The concentration of MDA was calculated using coefficient of absorbance of 155×10^6 cm^2 mol^{-1}. SOD and CAT activity were assayed as described by Lu *et al*. (2003).

The extraction and assay of APX activity were performed as described by Nakano and Asada (1981). Rice shoots (0.5 g) were extracted in 3 cm^3 extraction solution (50 mM pH 7.0 phosphate buffer, 2 mM AsA and 5 mM EDTA) using a mortar and pestle at 4 °C. The reaction was initiated by addition of H_2O_2. GR activity was measured as described by Gamble and Burke (1984). Shoots (0.3 g) were extracted in 3 cm^3 of 0.1 *M* Tricine-NaOH (pH 7.8). The oxidation of NADPH was monitored at 340 nm during 2 min.

Determination of AsA content: Rice shoots (0.5 g) were ground in a mortar and pestle in 5 cm^3 of trichloroacetic acid at 4 °C. The homogenate was centrifuged at 13 000 *g* for 15 min. The supernatant was used for AsA determination as described by Law *et al*. (1983). AsA concentration was calculated from standard curve prepared from known concentrations of AsA.

Experimental design and statistical analysis: All assays were based on at least two readings from three independent replicates. Each sample was taken from four to five shoots. All data were subjected to analysis of means and standard errors (n = 3), using *Microsoft Excel* program.

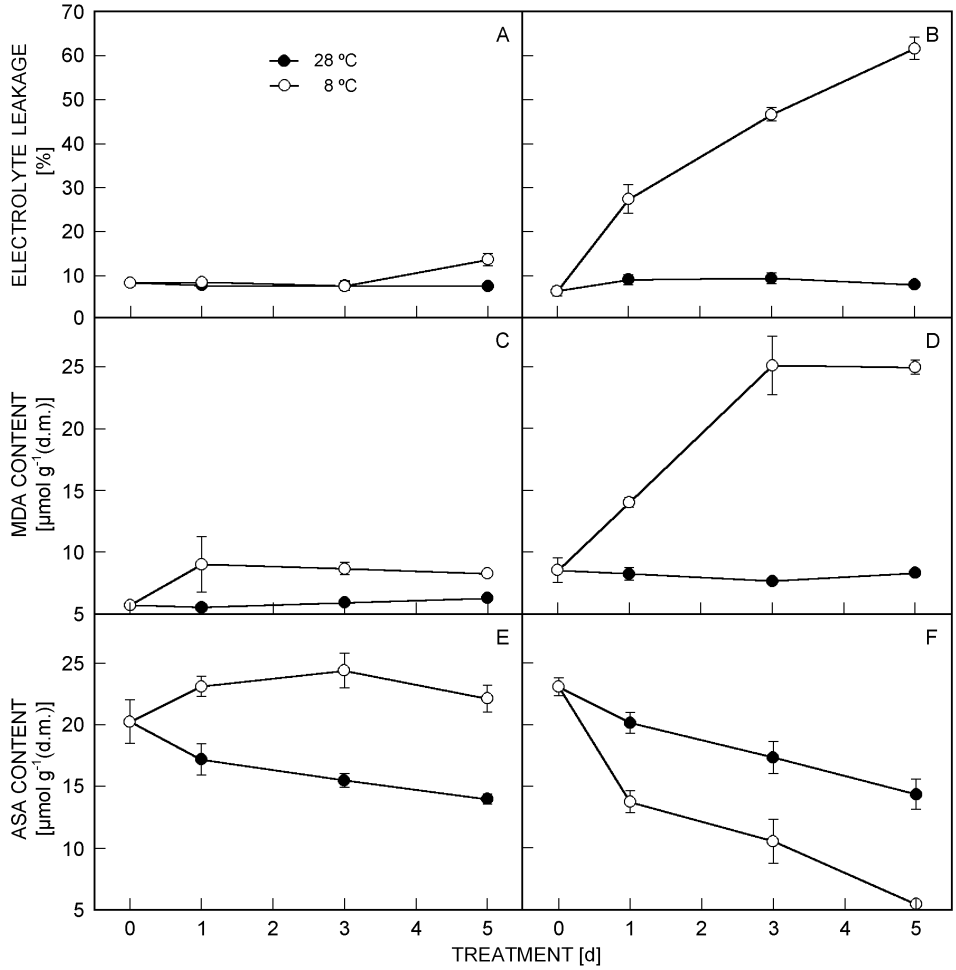

Fig. 1. Effects of chilling on electrolyte leakage and MDA and AsA content of rice seedlings of Xiangnuo-1 (*A*, *C*, *E*) and IR-50 (*B*, *D*, *F*). The treatment as described in Materials and methods. Means ± SE of three separate measurements.

Results and discussion

Under chilling conditions, tolerant cultivar Xiangnuo-1 showed little change of electrolyte leakage compared with its control plants, while the electrolyte leakage of IR-50 increased significantly (Fig. 1). Electrolyte leakage reflected the damage of stresses to the plasmalemma. MDA is one of the products of plant lipid peroxidation, and its content reflects the level of lipid peroxidation resulting from oxidative stress (Dhindsa *et al*. 1981). The MDA content in Xiangnuo-1 increased slightly but remained at low level after treatment by chilling, while that of IR-50 increased rapidly with treatment (Fig. 1). These results indicated that the membrane integrity in tolerant cultivar Xiangnuo-1 is better protected against injury of chilling damage than in susceptible cultivar IR-50.

SOD catalyzes the conversion of $O_2^{\cdot-}$ into H_2O_2. Under chilling conditions, SOD activity of the tolerant Xiangnuo-1 remained similar to that of control plants. On the contrary, SOD activity of IR-50 decreased after chilling and remained low throughout the chilling period (Fig. 2). H_2O_2 is still toxic to plants. It is scavenged by APX in the chloroplast and cytosol and by CAT in

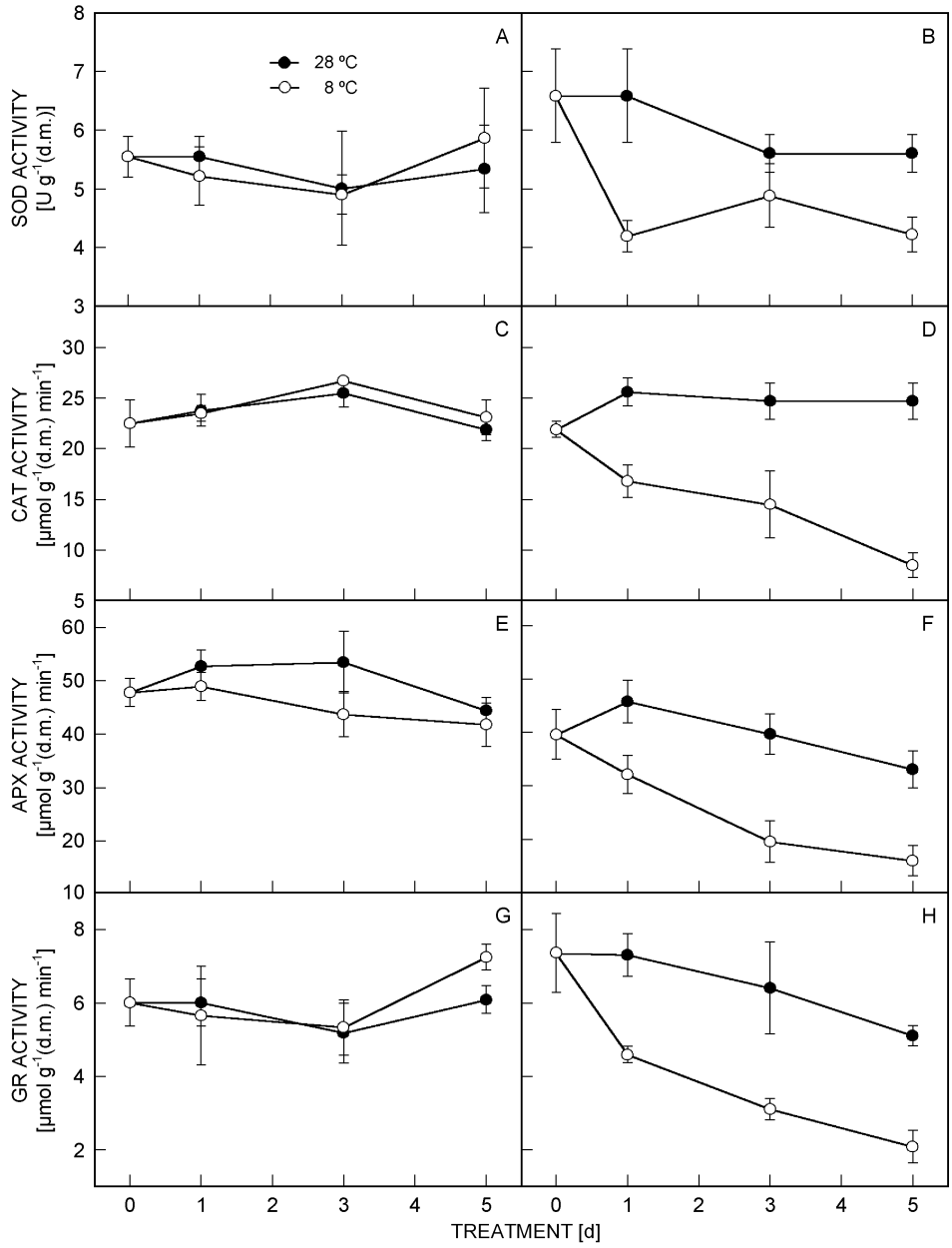

Fig. 2. SOD, CAT, APX and GR activities of Xiangnuo-1 (*A*, *C*, *D*, *G*) and IR-50 (*B*, *D*, *F*, *H*) as affected by chilling. The treatment as described in Materials and methods. Means ± SE of three separate measurements.

peroxisomes. GR is involved in the regeneration of ascorbic acid, the substrate of APX.

They have important roles in protection of plants from oxidative damage. Transgenic plants elevating APX or GR improved the recovery of cotton after chilling treatment (Payton *et al*. 2001). CAT activity decreased significantly in rice during chilling (Fadzillah *et al*. 1996), but remained similar to unchilled tissue in *A. thaliana* that was considered cold-hardened (O'Kane *et al*. 1996). Heat shock enhanced chilling resistance in rice was attributed to the induction of APX gene expression (Sato *et al*. 2001). The activities of CAT, APX and GR in Xiangnuo-1 remained similar to those in the controls, whereas those in IR-50 declined (Fig. 2). The results indicated that higher H_2O_2 scavenging enzyme activities in tolerant cultivar. The decline of activities of CAT, APX, and GR in IR-50 were linearly correlated with electrolyte leakage, indicating anti-oxidative enzyme activity is correlated with chilling injury in IR-50.

AsA is an important antioxidant in plants. Application of exogenous AsA improved the tolerance of *Cassia angustifolia* to water stress (Singh *et al*. 2001). AsA contents showed different responses between the two cultivars with respect to chilling. Xiangnuo-1 had high content of AsA under chilling, while IR-50 had decreased AsA content (Fig. 1).

In summary, higher activities of SOD, CAT, APX and GR and higher content of AsA under chilling is associated with tolerance to chilling in Xiangnuo-1. Decreased activities of defense enzymes and AsA content under chilling are correlated with susceptibility to chilling in IR-50.

References

Bowler, C., Van Montagu, M., Inze, D.: Superoxide dismutase and stress tolerance. - Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 83-116, 1992.

Dhindsa, R.S., Plumb-Dhindsa, P., Thorpe, T.A.: 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, 1981.

Fadzillah, N.M., Gill, V., Finch, B.P., Burdon, R.H.: Chilling, oxidative stress and antioxidant responses in shoot cultures of rice. - Planta **199**: 552-556, 1996.

Gamble, P.E., Burke, J.J.: Effect of water stress on the chloroplastic antioxidant system. I. Alterations in glutathione reductase activity. - Plant Physiol. **76**: 615-621, 1984.

Hodges, D.M., Andrews, C.J., Johnson, D.A., Hamilton, R.I.: Antioxidant enzyme responses to chilling stress in differentially sensitive inbred maize lines. - J. exp. Bot. **48**: 1105-1113, 1997a.

Hodges, D.M., Andrews, C.J., Johnson, D.A., Hamilton, R.I.: Antioxidant enzyme and compound responses to chilling stress and their combining abilities in differentially sensitive maize hybrids. - Crop Sci. **37**: 857-863, 1997b.

Kang, H.-M., Saltveit, M.E.: Reduced chilling tolerance in elongating cucumber seedling radicles is related to their reduced antioxidant enzyme and DPPH-radical scavenging activity. - Physiol. Plant. **115**: 244-250, 2002.

Law, M.Y., Charles, S.A., Halliwell, B.: Glutathione and ascorbic acid in spinach (*Spinacia oleracea*) chloroplasts. - Biochem. J. **210**: 899-903, 1983.

Liu, C.-L., Chen, H.-P., Liu, E.-E., Peng, X.-X., Lu, S.-Y., Guo, Z.-F.: Multiple tolerance of rice to abiotic stresses and its relationship with ABA accumulation. - Acta agron. sin. **29**: 725-729, 2003.

Lu, S., Guo, Z., Peng, X.: Effects of ABA and S-3307 on drought resistance and antioxidative enzyme activity of turfgrass. - J. Hort. Sci. Biotech. **78**: 663-666, 2003.

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

O'Kane D., Gill, V., Boyd, P.L, Burdon, R.: Chilling, oxidative stress and antioxidant reponses in *Arabidopsis thaliana* callus. - Planta **198**: 371-377, 1996.

Payton, P., Webb, R., Kornyeyev, D., Allen, R., Holadys, A.S.: Protecting cotton photosynthesis during moderate chilling at high light intensity by increasing chloroplastic antioxidant enzyme activity. - J. exp. Bot. **52**: 2345-2354, 2001.

Prasad, T.K., Anderson, M.D., Martin, B.A., Stewart, C.R.: Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. - Plant Cell **6**: 65-74, 1994.

Queiroz, C.G.S., Alonso, A., Mares-Guia, M., Magalhaes, A.C.: Chilling-induced changes in membrane fluidity and antioxidant enzyme activities in *Coffea arabica* L. roots. - Biol. Plant. **41**: 403-413, 1998.

Sato, Y., Murakami, T., Funatsuki, H., Matsuba, S., Saruyama, H., Tanida, M.: Heat shock-mediated APX gene expression and protection against chilling injury in rice seedlings. - J. exp. Bot. **52**: 145-151, 2001.

Singh, D.V., Srivastava, G.C., Abdin, M.Z.: Amelioration of negative effect of water stress in *Cassia angustifolia* by benzyladenine and/or ascorbic acid. - Biol. Plant. **41**: 141-143, 2001.