ALTERATION OF PHOTOSYNTHETIC AND OXIDATIVE METABOLISM UNDER LOW TEMPERATURE STRESS IN TWO *INDICA* RICE GENOTYPES CONTRASTING IN COLD TOLERANCE

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INTRODUCTION

About one million hectares of irrigated rice are cultivated annually in the State of Rio Grande do Sul, which corresponds to approximately 62% of the whole Brazilian production. The incidence of low temperatures during early stages of rice development is one of the major limiting factors to rice productivity in this State.

Numerous studies have shown the effects of low temperatures on metabolism and physiology of rice cultivars from ssp. *japonica* (Bonnecarrère et al., 2011), rarely cultivated in the State of Rio Grande do Sul, where cultivation of *Oryza sativa* ssp. *indica* prevails. However, *indica* rice is highly sensitive to cold.

One of the major effects of low temperature is the photosynthetic apparatus inhibition through reduced electron transport in the photosystem II (PSII) (Jeong et al., 2002). Due to reduced activity of electron transport proteins, the photosystem II (P680) is overloaded with energy excess and, damaging proteins. Moreover, as a consequence of excess excitation energy, triplet chlorophyll is often formed and reacts with molecular oxygen, generating free radicals (Foyer and Noctor, 2005). Free radicals toxicity can be attenuated by the activity of antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase and catalase.

The objectives of this study were to assess the oxidative stress responses and transient fluorescence of chlorophyll *a* in rice plants (*Oryza sativa* L.) of ssp. *indica* showing contrasting levels of cold tolerance.

MATERIAL AND METHODS

To identify low temperature susceptible and tolerant genotypes from *indica* subspecies, 100 genotypes were evaluated according to the percentage of three leaf-stage plant survival after ten days at 10°C and seven days of recovery under normal temperature (in greenhouse conditions).

To assess the oxidative stress and chlorophyll *a* fluorescence in seedlings of the genotypes characterized as susceptible and tolerant to low temperature stress, seeds were germinated and grown in a greenhouse under room temperature. After 20 days of growth, the seedlings were transferred to climatic chamber for chilling treatment for ten days.

The transient fluorescence of chlorophyll *a* was measured in the youngest fully expanded leaves of ten susceptible and tolerant plants, using a portable fluorometer (OS30p, Optisciences, UK). Catalase (CAT) activity was determined following the method of Aebi (1984). Superoxide dismutase (SOD) activity was assayed as described by Beyer and

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Fridovich (1987) and Ascorbate peroxidase (APX) activity was assayed as described by Zhu et al. (2004). Hydrogen peroxide concentration was determined following the method described by Loreto and Velikova (2001).

RESULTS AND DISCUSSION

After screening with 100 genotypes, two related genotypes, originated from the same cross, were characterized as tolerant (A) and susceptible (B) to low temperature stress. These genotypes were then subjected to low temperature.

After ten days of low temperature, most plants from the sensitive genotype showed leaf rolling and wilting. Fluorescence analysis of the susceptible genotype was performed before exposing the plants to low temperature (control), after 3 days and 8 days of low temperature exposure. Fluorescence was also evaluated in plants from the tolerant genotype after ten days of low temperature stress and after 72 hours and 7 days of recovery under room temperature in the greenhouse.

After eight days of cold stress, the energy flux absorption (ABS/RC) and energy dissipation per reaction center (DI_0/RC) increased compared to the control in both genotypes. The increase in ABS/RC could mean the inactivation of some RCs (Yusuf et al., 2010), being more pronounced in the susceptible genotype due to a reduction in electron transport per active RC (ET_0/RC) (Figure 1).

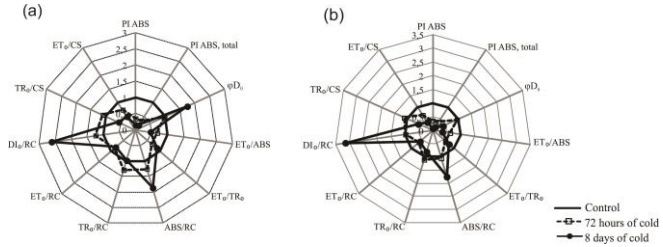

Figure 1. JIP-test parameters calculated from the chlorophyll *a* fluorescence transient in rice seedlings of *indica* genotypes. (A) Tolerant cultivar and (B) susceptible cultivar IRGA subjected to cold stress at 10^0C for different periods. For each parameter, values were normalized relative to values measured before the onset of the chilling treatment.

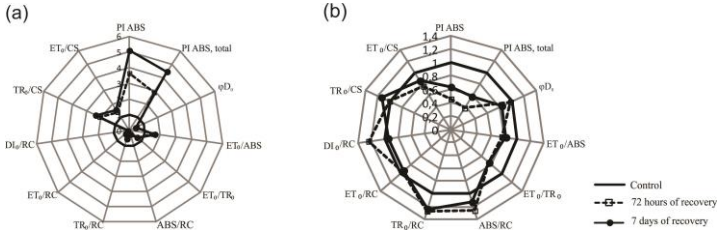

Figure 2. Tolerant cultivar subjected to cold stress for ten days followed by recovery at 28^0C for seven days. For each parameter, data were normalized using the values measured after ten days of cold stress (A) and using values measured before the onset of the chilling treatment (B).

The yield or flux ratio, $\varphi D_0 = F_0/F_M$ (quantum yield, at t = 0, of energy dissipation) increased in the tolerant genotype (Figure 1A) under cold treatment, whereas the value of this parameter in the susceptible genotype was smaller than in control plants after eight days

of cold (Figure 1B). This indicates that the excess energy was dissipated more efficiently in the tolerant genotype, acting as a possible protective mechanism, minimizing the over-excitation risks. At the recovery period, this parameter was smaller than under the control treatment in the tolerant genotype (Figure 2B).

In this study, the photosynthetic performance index in relation to absorption (PI_{ABS}) (Strasser et al. 2000) and total photosynthetic performance index ($PI_{ABS,total}$), that measures the electrons flux till the final electron acceptors of PSI (Tsimilli-Michael & Strasser, 2008), were extremely affected by low temperature in both genotypes (Figure 1). However, in the recovery period, PI_{ABS} and $PI_{ABS,total}$ in the tolerant genotype were, respectively, about 4-fold and 3.4-fold higher than in the 10°C treatment (Figure 2A).

Additionally, a rapid increase in SOD activity (53.10%) was detected in the tolerant genotype after 48h at 10°C, when compared to 24h (Figure 3A). After 72h of chilling exposure, there was a decrease in SOD activity, reaching values close to control. In the susceptible genotype, SOD activity was similar within the period evaluated. High SOD activity in plants has been associated with stress tolerance because it catalyzes the conversion of O^{2-} into H_2O_2 (Bowler et al, 1992). H_2O_2 levels were similar in both genotypes and the levels were maintained until 48h after the beginning of chilling treatment. However, after 72h at 10°C, the tolerant genotype accumulated higher levels of H_2O_2 (28.07%) (Figure 3D) probably due to SOD activity. High levels of H_2O_2 are toxic to the cell and must be detoxified. This process is carried out by catalase (CAT) and/or peroxidase (APX). CAT activity in the cold-tolerant genotype increased 46% after 72h at 10°C. No significant change in CAT activity was observed in the susceptible genotype during cold exposure (Figure 3C).

No differences were found in APX activity between the two *indica* genotypes in the evaluated periods of cold stress (Figure 3B).

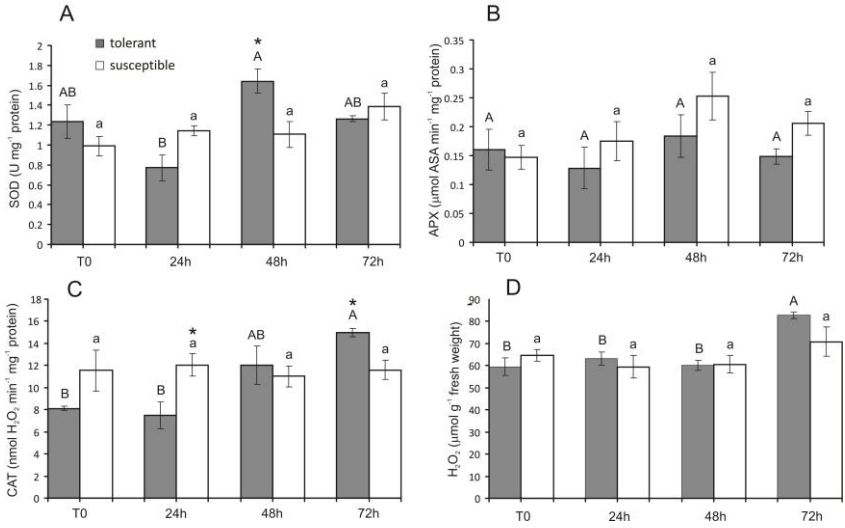

Figure 3. Enzyme activities (SOD, CAT and APX) and H_2O_2 concentration in rice seedlings of *indica* cultivars subjected to cold stress. Different letters indicate statistical difference among hours of treatment in the same genotype (uppercase letters indicate the tolerant genotype and lowercase letters indicate susceptible genotype). Asterisks indicate a statistical difference between genotypes at the same period. The results are expressed as means ± SE ($P \leq 0.05$, n=3).

CONCLUSION

Two rice genotypes of the subspecies *indica* were characterized as tolerant and susceptible to low temperature stress. The cold caused the inactivation of some RCs, loss of part of the excitation energy absorbed by PSII (DI_0/RC) and reduction in the photosynthetic performance index in both genotypes. However, enzyme activity (SOD and CAT) was higher in the tolerant genotype. During the recovery period, the values of PI_{ABS} and the $PI_{ABS,total}$ increased, demonstrating the ability of the tolerant genotype to recover its photosynthetic performance.

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REFERENCES

AEBI, H. Catalase in vitro. **Methods in Enzymology**, v. 105, p. 121-126, 1984.

BEYER, W.F., FRIDOVICH, I. Assaying of superoxide dismutase activity: some large consequences of minor changes in conditions. **Analytical Biochemistry**, 161: 559-566, 1987.

BONNECARRÈRE, V., BORSANI O., DÍAZ P., CAPDEVIELLE F., BLANCO P., MONZA J. Response to photoxidative stress induced by cold in *japonica* rice is genotype dependent. **Plant Science**, v. 180, p. 726–732, 2011.

BOWLER, C. Montagu MV, Inzé D. Superoxide dismutase and stress tolerance. **Annual Review in Plant Physiology and Plant Molecular Biology,** v. 43, p.83-116, 1992.

FOYER, C., NOCTOR, G. Redox homeostasis and antioxidant signaling: A metabolic interface between stress perception and physiology responses. **The Plant Cell**, v.17, p. 1866–1875, 2005.

JEONG, S. W., CHOI, S. M., LEE, D. S.,AHN, S. N., HUR, Y., CHOW, W. S., PARK, Yl. Differential Susceptibility of Photosynthesis to light-chilling stress in rice (*Oryza sativa* L.) depends on the capacity for photochemical dissipation of light. **Molecules and Cells**, v. 13, p. 419-428, 2002.

LORETO, F., VELIKOVA, V. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. **Plant Physiology**, v. 127, p. 1781–1787, 2001.

STRASSE, R.J., SRIVASTAVA, A., TSIMILLI-MICHAEL, M. The fluorescence transient as a tool to characterize and screen photosynthetic samples. *In*: Yunus, M, Pathre U, Mohanty P (Eds). **Probing photosynthesis: Mechanism, regulation and adaptation**, p.443-480. Taylor & Francis, London, 2000.

YUSUF, M. A., KUMAR, D., RAJWANSHI, R., STRASSER, R. J., TSIMILLI-MICHAEL, M., GOVINDJEE, SARIN, N. B. Overexpression of γ-tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: Physiological and chlorophyll a fluorescence measurements. **Biochimica et Biophysica Acta (BBA) Bioenergetics**, v. 1797, n. 8, p. 1428-1438, 2010.

TSIMILLI-MICHAEL, M.; STRASSER, R.J. In vivo assessment of plants vitality: applications in detecting and evaluating the impact of Mycorrhization on host plants. *In*: VARMA, A. (Ed.), **Mycorrhiza: State of the Art, Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics**, 3rd edition Springer, Dordrecht, p. 679-703, 2008.

ZHU, Z., WEI, G., LI, J., QIAN, Q., YU, J. Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). **Plant Science**, v. 167, p. 527-533, 2004.