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Relative expression of genes related with cold tolerance in temperate rice at the seedling stage

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Low temperature is one of the main abiotic stresses affecting rice yield in Chile. Alterations in phenology and physiology of the crop are observed after a cold event. The objective of this work was to study the relative expression of genes related with cold stress in Chilean cultivars of rice. For this, we analyzed the expression of candidate genes using real-time polymerase chain reaction (rtPCR), after exposure to cold of the rice cultivars Diamante-INIA and Zafiro-INIA and one experimental line from INIA´s Rice Breeding Program in Chile, Quila 241701, with known high cold tolerance. For analysis, the Spanish cultivar, Susan, was used as check tolerance and Oryzica 1 as susceptible check. Oxidative stress was measured through lipid peroxidation. To find mechanisms of cold tolerance in Chilean cultivars, we determined the relative expression of genes related with oxidative stress, such as superoxide dismutase (SOD), glutathione reductase (GR), and catalase (CAT). Lipid peroxidation allowed the measurement of the physiological stress level of the genotypes under study. The results indicate that lipid peroxidation in Oryzica 1 was higher than in the other genotypes. No differences were observed in expression levels of gene-encoding SOD and GR between genotypes. Contrary to expected results, high level of the gene-encoding CAT enzyme in Oryzica 1 after cold stress was observed. Future experiment, related with enzymatic activity and non-enzymatic antioxidant mechanism, are necessary to elucidate the relationship between cold stress and expression levels of gene-encoding antioxidant enzyme in Chilean rice germplasm.

Key words: Cold stress, reactive oxygen species, antioxidant enzymes.

INTRODUCTION

Chile is one of the coldest regions where rice is cultivated in the Southern hemisphere and therefore, low temperature is the most important abiotic stress affecting rice production. Low temperatures are responsible for up to 40% yield loss in rice production in Chile (Alvarado and Hernaiz, 2007). Similar results are observed in temperate

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areas such as Australia and Japan (Jacobs and Pearson, 1994; McDonald, 1994). The rice area in Chile has a Mediterranean climate and it is located between the Maule (35° S lat) to Biobío region (36º S lat). Rice cultivation occurs from October to March mainly in a flooding regime by using pre-germinated seeds (Alvarado and Hernaiz, 2007).

Lately, the direct sowing started to be used by the farmers. Rice plants are frequently exposed to cold temperatures at germination, seedling and the reproductive stage. Commonly, the minimum temperature is below 12°C, below the optimum (20-30°C) required for rice cultivation (Yoshida et al., 1996). In all cases, the level of damage associated to cold stress will be dependent on the development stage and the intensity of the cold event (Jacobs and Pearson, 1994). For example, at the germination stage, chilling can cause slow growth and severely reduce seedling vigour (Ali et al., 2006); at the vegetative stage may cause, lower tillering (Shimono et al., 2002), high mortality (Mackill and Lei, 1997; Andaya and Mackill, 2003; Baruah et al., 2009), non-uniform maturity of the crop (Shimono et al., 2004), and yellowing of the plants (Shimono et al., 2002; Ali et al., 2006; Baruah et al., 2009). Night temperatures at germination and seedling stages are below 10°C (Alvarado and Hernaiz, 2007). In this condition, Chilean rice cultivars such as Zafiro-INIA and Diamante-INIA have shown high cold tolerance, especially at the seedling stage (Pulver, 2002).

At molecular levels, cold stress can cause an increase of reactive oxygen species (ROS) causing oxidative stress (O'Kane et al., 1996; Mittler, 2002; Apel and Hirt, 2004). The oxidative stress occurs due to an imbalance between oxidative species formation and antioxidant defences of the cell. In abiotic stress the principal causes of oxidative imbalance are the absorption of more photons than can be used by the photosynthetic apparatus (chloroplast) and the over-reduction of electron transport chain (mitochondria) (Møller et al., 2007). Accordingly, levels of ROS in the cell increase rapidly (Tsugane et al., 1999). ROS is produced in the electron transport chains of chloroplasts and mitochondria, some peroxidases and oxidases and excited chlorophyll molecules (Cheeseman, 2007). In the mitochondrial electron transport chain, NAD dehydrogenase and cytochrome reductase generate the superoxide anion, which is reduced to H_2O_2 by dismutation. H_2O_2 can react with reduced Fe^{2+} and Cu^{+} to produce hydroxyl radicals ($HO^{\bullet}$) (Sweetlove and Foyer, 2004). In chloroplasts, ROS is generated by univalent oxygen reduction in the Mehler reaction in the electron transport chain (Asada, 1996; Edreva, 2005). Generated $O_2^{\bullet-}$ is protonated to $HO_2^{\bullet}$ and these molecules can initiate lipid peroxidation. On the external stromal membrane surface $O_2^{\bullet-}$ may be dismutated to H_2O_2 and O_2 (spontaneously or by Superoxide dismutase) (Grant and Loake, 2000). Through the Fenton reaction, H_2O_2 is transformed to $HO^{\bullet}$, the most reactive species. To avoid production of $HO^{\bullet}$, H_2O_2 is scavenged by the antioxidant machinery (Noctor and Foyer, 1998; Dat et al., 2000).

Another source of ROS is excited chlorophyll, inducing the production of superoxide ($O_2^{\bullet-}$) and singlet oxygen ($^1O_2$) (Asada, 1996). ROS can cause damage to DNA, lipid membranes and proteins (Mittler, 2002; Apel and Hirt, 2004). Also, oxidative stress induces alterations in the redox status of proteins regulating protein functions and degradation (Møller et al., 2007).

Plants have developed numerous mechanisms to reduce the effects of the oxidative damage. Some of them are associated with enzymatic ROS scavenger systems, for example, superoxide dismutase (SOD), which catalyzes the conversion of superoxide to H_2O_2 and O_2, catalase (CAT), which convert H_2O_2 to water, glutathione reductase (GR) and the ascorbate peroxidase (APX), which eliminates the ascorbate H_2O_2 GSH cycle (Saruyama and Tanida, 1995; Hammond-Kosack and Jones, 1996; Huang and Guo, 2005; Ahmad et al, 2008).

Real time PCR is a powerful technique for evaluation gene expression based on the detection and quantification of the fluorescence emitted by PCR products accumulated through the amplification process (Higuchi et al., 1993). This technique can be used to quantify gene expression using two strategies: relative and absolute quantification. In relative quantification, gene expression of target gene is compared with one or more reference genes (Pfaffl, 2004). In the case of rice, the expression of the 18S rRNA gene is widely used as a reference gene (Kim et al., 2003; Welsch et al., 2008).

Tolerance to cold is a characteristic dependent on the genotype, which is controlled by more than one gene (Baruah et al., 2009). It has been demonstrated that cold tolerance is associated with capacity to remove or detoxify ROS (Malan et al., 1990). Therefore, the hypothesis of this research was that expression of genes associated with proteins that detoxify ROS (SOD, GR and CAT) is related to cold tolerant genotypes. The overall objective was to analyze gene transcription that encodes antioxidant enzymes in 3 cultivars and one cold tolerant experimental line, at the seedling stage.

MATERIALS AND METHODS

Plant and growth conditions

Seeds of rice genotypes Quila 241701 (Experimental line), Diamante-INIA, Zafiro-INIA, Susan (tolerant control) and Oryzica1 (susceptible control) were germinated on absorbent paper with a solution of the fungicide Benlate (2 ppm) at 28° C in the dark for 3 days. After, germination seedlings were transplanted into plastic pots of 500 mL with clay soil (Vertisol), fertilized with 100mg of Urea, 55 mg of potassium chloride and 45 mg of triple superphosphate (45% of P_2O_5).

Plants were grown in a greenhouse with a photoperiod of 14 h of light and 10 h of dark, at 28°C. The plants were illuminated with artificial light using metal halide lamps with a radiation of 300 µmol

Table 1. Primer sequences used for real-time PCR analysis.

Gen name	Primer sequence	Amplicon size (bp)
OsSODCu/Zn P93407	5' ACCATTGTTGATAAGCAGATTCC 3' 5' GTCGCCCACCAGCATTTC 3'	139
OsCAT Q0E4K1	5' AACTACCTCTCCCAGTGTGATGAG 3' 5' AGTTTCTTCACATGCTTGGCTTCA 3'	82
OsGR Q8S5T1	5' ACAGCAAACTTCAGGCCACTTAGG 3' 5' TGTCAGCCCAGCTTTAACAGCA 3'	171
18S rRNA*	5' ATGGTGGTGACGGGTGAC 3' 5' CAGACACTAAAGCGCCCGGTA 3'	159

* Primers developed by Todaka et al., 2012.

photons m^{-2} s^{-1}. Seedlings with 3 to 4 expanded leaves were treated at 5°C in a cold chamber in darkness (Baruah et al., 2009) for 72 h. Finally, three plants per pot were sampled and frozen in liquid nitrogen and stored at 80°C until analysis.

Lipid peroxidation

The protocol described by Dionisio-Sese and Tobita (1998), with modifications, was used to determine lipid peroxidation; the main purpose was to measure malondialdehyde (MDA) concentration. The leaves of the seedlings were ground into a fine powder with liquid nitrogen and weighed. A volume of 500 µL of 50 mM potassium phosphate buffer at pH 7 was added to the sample that was stirred in a Vortex and homogenized with a homogenizer (Heidolph, Diax 900). The extract was centrifuged at 16,000 *g* for 30 min at 4°C. This process resulted in a 300 µL aliquot of extract that was mixed with 300 µL 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA). Subsequently, the mixture was heated at 95°C for 30 min and reaction was stopped by placing tubes on ice. After, the samples were centrifuged at 10,000 *g* for 10 min. Absorbance of 532 and 600 nm was determined in the supernatant with spectrophotometer. The MDA concentration was determined by the extinction coefficient, which is equal to 155 $mM^{-1}cm^{-1}$ using the standard equation for weight in grams for each of the samples:

$MDA = [(A532 - A600)/ 155\ mM^{-1}cm^{-1}]$ [nmol g-1 FW]

FW = fresh weight tissue (g)

Analysis of genes by real- time PCR

Total RNA was extracted from 0.1 g of ground tissue using RNA-Solv Reagent (Omega Bio-tek). For removal of genomic DNA, DNase I (Applied Biosystems) treatment was applied and cDNA was retro-transcribed from 2 µg of RNA using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). Real time PCR was performed with a Stratagene Mx3000p system using GoTaq Green Master Mix (Promega). Expression was calculated using the formula $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001) with 18S rRNAas -the Housekeeping gene (Table 1). For amplification, GoTaq Mix from Promega was used according to manufacturer's instructions. For this, 3 ng of cDNA was used for amplification of 18S rRNA, 12.5 ng of cDNA for amplification of SOD, and GR, and 25 ng of cDNA for CAT amplification. Furthermore, dissociation curves were performed in each case to determine the absence of primer dimers and/or nonspecific products. All primers are described in Table 1.

Experimental design and statistical analysis

A completely random experimental design was used with three biological replicates. Also, for all Real time PCR analysis, three technical replicates were used. Two way ANOVA (treatment and genotype as factor of variations), was used for the statistical analysis of the MDA concentration. One way ANOVA was used for the analysis of the relative expression of each gene studied. All data were analyzed using Infostat® software (Di Rienzo et al., 2012).

RESULTS AND DISCUSSION

Lipid peroxidation showed values between 2.94 and 5.57 nmol g^{-1}PF in the analyzed genotypes (Figure 1). This result was similar to that found by Cabas (2012), in which lipid peroxidation of different rice genotypes showed variations between 1.39 to 5.56 nmol g^{-1}. Analysis of variance for MDA concentration showed that seedlings of Oryzica 1 were 1.86 times higher than Susan and 1.37 times higher than Diamante-INIA and 1.71 times higher than the control condition. Also, levels of MDA for Diamante-INIA exposed to cold was 1.28 times greater than the control plants and Quila 241701 exposed to cold.

No differences between Susan and Zafiro-INIA seedlings were found in control conditions and cold. Similar results were also observed by Kim and Tai (2011) and Wang et al. (2013), who found differences in MDA levels between tolerant and susceptible seedlings after cold treatment at 9°C for 14 days and at 5°C for three days, respectively. This suggests that cold treatment was enough to cause oxidative stress in the susceptible check, Oryzica 1 (Yun et al., 2010).

Expression levels of the gene-encoding the SOD enzyme in Susan (cold-tolerant genotype) was 2.7 times higher than Quila 241701, but no differences were

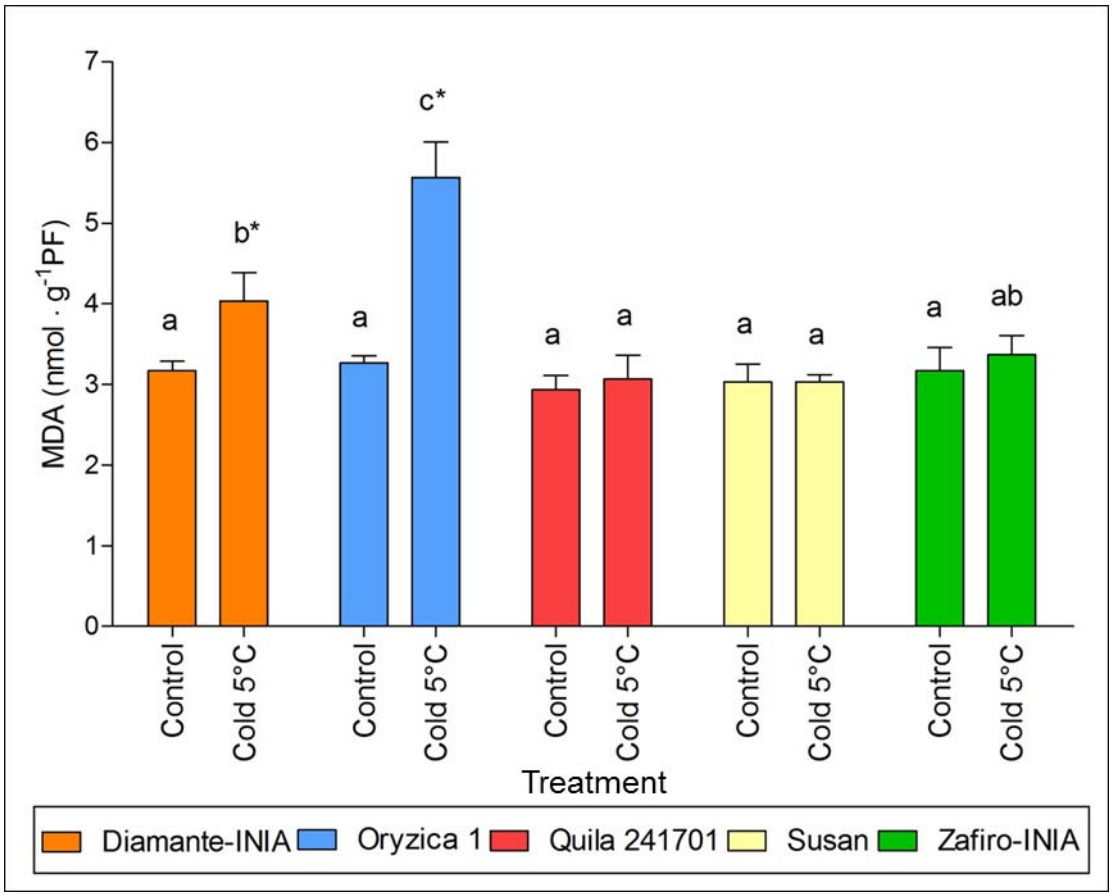

Figure 1. Lipid peroxidation after cold stress in rice seedlings. Letter and asterisk (*), represent differences between genotypes and treatment, respectively (Fisher LSD test, P≤0.05). Error bars represent the standard error of 3 biological replicates.

observed between other genotypes (Figure 2). The enzymatic activity of SOD showed that cold-tolerant genotypes increase enzyme activity with respect to a susceptible genotype (Wang et al., 2013). SOD is an intracellular enzyme that catalyzes the dismutation of O_2^- into H_2O_2 and O_2. This enzyme represents the first defense mechanism against ROS in comparison with GR and CAT that act in detoxifying H_2O_2. Some studies have revealed that tolerant genotypes express a higher level of antioxidant enzyme activity than sensitive genotypes (Guo et al., 2006).

No differences were observed between genotypes in the analysis of expression levels of the gene-encoding GR. This finding is not in agreement with other results that showed that the activity of the GR increases in the cold tolerant-genotypes (Wang et al., 2013).

The analysis of the level of the expression of the gene-encoding the CAT enzyme indicated that Oryzica 1 showed 4 to 10 times greater levels of expression than in the other genotypes (Figure 2). This high expression level of the gene-encoding CAT in Oryzica 1 is in agreement with other studies in rice (Wang et al., 2013), where a significant increase in expression levels of this enzyme was observed in a susceptible genotype.

Several mechanisms can contribute to the reduction or prevention of oxidative stress and its deleterious effects (Bonnecarrère et al., 2011). The first is related to prevention of ROS formation by the dissipation of excess energy via xanthophylls cycle involving synthesis of zeatin (Z) and antheraxanthin (A) from violaxanthin (V) (Demming-Adams and Adams, 1992).

A second mechanism is the detoxification of ROS by carotenoids or by the action of antioxidant enzymes (Saruyama and Tanida, 1995; Huang and Guo, 2005). For example, carotenoids such as lutein and neoxanthin have been associated with the process of elimination of ROS (Dall'Osto et al., 2007). Other studies have compared the contribution of enzymatic and non-enzymatic detoxification of ROS in cold susceptible genotypes (*Oryza sativa* L. spp. *indica*) and cold tolerant genotypes (*Oryza sativa* L. spp. *japonica*). However, the results have shown that there exist a complexity pattern, where enzymes increase or decrease their activity after cold stress (Bonecarreré et al., 2011).

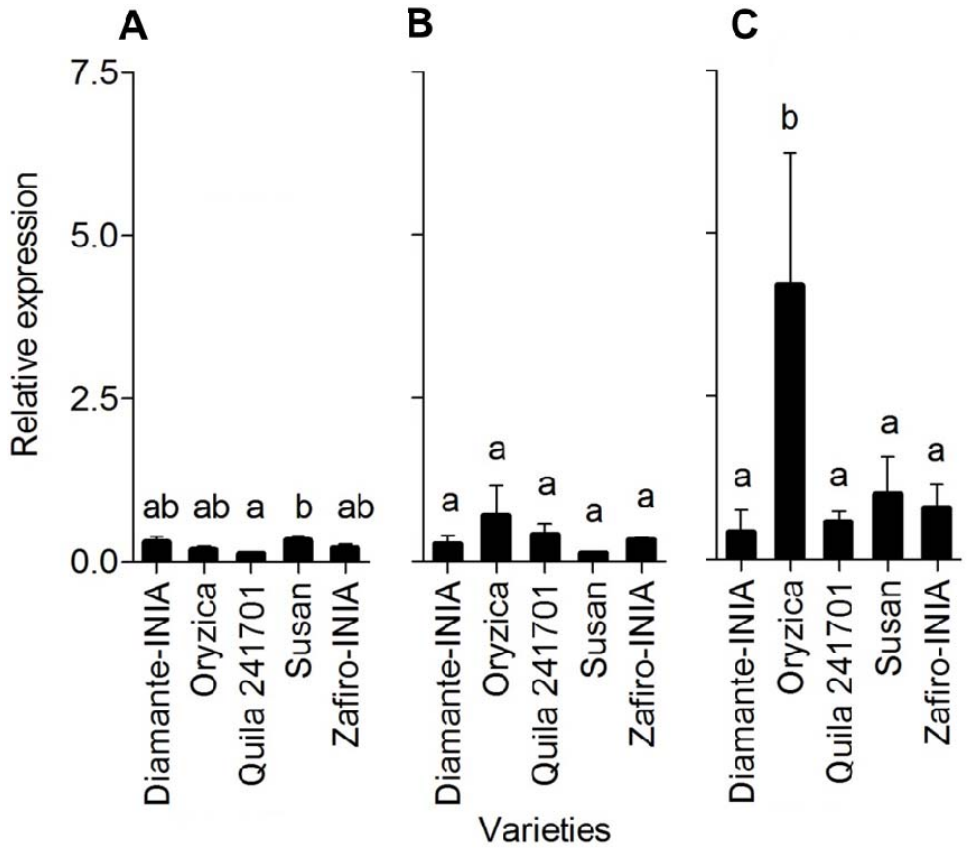

Figure 2. Relative expression levels of gene-encoding SOD (A), GR (B) y CAT (C). Different letters represent differences in expression levels between genotypes. Error bars represent the standard error of 3 biological replicates.

In general, the increase in expression levels of enzymes related with detoxification of ROS, can cause a decrease of lipid peroxidation. For example, the reduction of lipid peroxidation in rice plants after cold acclimation is related with the increase of antioxidant defense system as SOD, GR, and CAT (Kuk et al., 2003; Moradi and Ismail, 2007; Prasad, 1996). However, a high level of CAT transcript did not result in lower levels of lipid peroxidation in Oryzica 1.

The low correlation between mRNA, protein levels and enzyme activity could be explained mainly due to alternative splicing of mRNA precursors and post-translational modifications of proteins (Gygi et al., 1999; Washburn et al., 2003).The low increase in MDA concentration observed in Susan, Quila 241701 and Zafiro-INIA was not associated with the expression levels of genes-encoding antioxidant enzymes. Therefore, high levels of enzyme activities, non-enzymatic or other mechanisms could be related with cold tolerance of these genotypes. The high level of lipid peroxidation in the susceptible genotype Oryzica 1 could be the cause of the increase in the transcript levels of the gene that encodes the CAT enzyme. However, inefficient protein translation or low activity of CAT enzyme could be also explained by the low level of protection against ROS under cold stress in Oryzica 1.

Conclusions

The high level of lipid peroxidation observed in the genotype Oryzica 1 was associated with the susceptibility to cold stress. On the contrary, the low levels of MDA observed in Susan, Zafiro-INIA and Quila 241701 may indicate that these genotypes have a good mechanism to avoid lipid peroxidation after cold treatment. Unexpectedly, no relationship between lipid peroxidation and SOD and GR enzyme was observed. Also, contrary to expected results, high levels of the gene-encoding CAT enzyme in Oryzica 1 were not related to avoid lipid peroxidation. Future experiment related with enzymatic activity and analyses of non-enzymatic mechanism are necessary to elucidate the mechanism of protection against ROS in Chilean rice germplasm.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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REFERENCES

Ahmad P, Sarwat M, Sharma S (2008). Reactive oxygen species, antioxidants and signaling in plants. J. Plant Biol. 51:167-173.

Ali MG, Naylor REL, Matthews S (2006). Distinguishing the effects of genotype and seed physiological age on low temperature tolerance of rice (*Oryza sativa* L.). Exp. Agric. 42:337-349.

Alvarado JR, Hernaiz S (2007). Cultivars, sowing, certified seed, seed rate and sowing time. pp. 21-38. Bulletin INIA N° 162. INIA Quilamapu. Chillán, Chile.

Andaya VC, Mackill DJ (2003). Mapping of QTLs associated with cold tolerance during vegetative stage in rice. J. Exp. Bot. 54:2579-2585.

Apel K, Hirt H (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55:373-399.

Asada K (1996). Radical production and scavenging in the chloroplasts, Ed Kluwer Academic Publishers. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Baruah AR, Ishigo-Oka N, Adachi MY, Oguma Y, Tokizono Y, Onishi K, Sano Y (2009). Cold tolerance at the early growth stage in wild and cultivated rice. Euphytica. 165:459-470.

Bonnecarrère V, Borsani O, Díaz P, Capdevielle F, Blanco P, Monza J (2011). Response to photoxidative stress induced by cold in japonica rice is genotype dependent. Plant Sci. 180:726-732.

Cabas P (2012). Cold tolerance in rice genotypes (*Oryza sativa* L.) at seedling stage. Undergraduate thesis. University of Concepción. Chile.

Cheeseman J (2007). Hydrogen peroxide and plant stress: a challenging relationship. Plant Stress 1:4-15.

Dall'Osto L, Cazzaniga S, North H, Marion-Poll A, Bassi R (2007). The *Arabidopsis* aba4-1 mutant reveals a specific function for neoxanthin in protection against photooxidative stress. Plant Cell. 19:1048-1064.

Dat J, Vandenabeele S, Vranová E, Van Montagu M, Inzé D, Van Breusegem F (2000). Dual action of the active oxygen species during plant stress responses. Cell. Mol. Life Sci. 57:779-795.

Demmig-Adams B, Adams WW (1992). Photoprotection and other responses of plants to high light stress. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43:599-626.

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2012). InfoStat 2012. InfoStat Group, FCA, National University of Córdoba, Argentina. URL http://www.infostat.com.ar.

Dionisio-Sese ML, Tobita S (1998). Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135:1-9.

Edreva A (2005). Generation and scavenging of reactive oxygen species in chloroplasts:a submolecular approach. Agric. Ecosyst. Environ. 106:119-133.

Grant JJ, Loake GJ (2000). Role of Reactive Oxygen Intermediates and Cognate Redox Signaling in Disease Resistance. Plant Physiol. 124:21-30.

Guo Z, Ou W, Lu S, Zhong Q (2006). Differential responses of antioxidative system to chilling and drought in four rice cultivars differing in sensitivity. Plant Physiol. Biochem. 44:828-836.

Gygi S, Rochon Y, Franza R, Aebersold R (1999). Correlation between Protein and mRNA Abundance in Yeast. Mol. Cell. Biol. 19:1720-1730.

Hammond-Kosack KE, Jones JD (1996). Resistance gene-dependent plant defense responses. Plant Cell. 8:1773-1791.

Higuchi R, Fockler C, Dollinger G, Watson R (1993). Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. Biotechnology 11:1026-1030.

Huang M, Guo Z (2005). Responses of antioxidative system to chilling stress in two rice cultivars differing in sensitivity. Biol. Plant. 49:81-84.

Jacobs BC, Pearson CJ (1994). Cold damage and development of rice: a conceptual model. Aust. J. Exp. Agr. 34:917-919.

Kim BR, Nam HY, Kim SU, Kim SI, Chang YJ (2003). Normalization of reverse transcription quantitative-PCR with housekeeping genes in rice. Biotechnol. Lett. 25:1869-1872.

Kim SI, Thomas T (2011). Evaluation of seedling cold tolerance in rice cultivars: a comparison of visual ratings and quantitative indicators of physiological changes. Euphytica. 178:437-447.

Kuk I, Shin JS, Burgos NR, Hwang TE, Han O, Cho BH, Jung S, Guh JO (2003). Antioxidative enzymes offer protection from chilling damage in rice plants. Crop Sci. 43:2109-2117.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods. 25:402-408.

Mackill DJ, Lei X (1997). Genetic variation for traits related to temperate adaptation of rice cultivars. Crop Sci. 37:1340-1346.

Malan C, Greyling MM, Gressel J (1990). Correlation between Cu/Zn superoxide dismutase and glutathione reductase, and environmental and xenobiotic stress tolerance in maize inbreds. Plant Sci. 69:157-166.

McDonald DJ (1994). Temperate rice technology for the 21st century: an Australian example. Aust. J. Exp. Agric. 34:877-888.

Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7:405-410.

Møller IM, Jensen PE, Hansson A (2007). Oxidative modifications to cellular components in plants. Annu. Rev. Plant Biol. 58:459-481.

Moradi F, Ismail AM (2007). Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. Ann. Bot. 99:1161-1173.

Noctor G, Foyer CH (1998). Ascorbate and glutathione: Keeping Active Oxygen Under Control. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:249-279.

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

Pfaffl MW (2004). Quantification strategies in real-time PCR. pp:87-112. In: S.A. Bustin (Ed.). A-Z of quantitative PCR. International University Line. La Jolla, USA.

Prasad TK (1996). Mechanisms of chilling-induced oxidative stress injury and tolerance in developing maize seedlings:changes in antioxidant system, oxidation of proteins and lipids and protease activities. Plant J. 10:1017-1026.

Pulver EL (2002). Strategy for sustainable rice production in Latin America and the Caribbean. In: Proceedings of the 20th Session of the International Rice Commission, Bangkok, Thailand. Rome, Italy. FAO.

Saruyama H, Tanida M (1995). Effect of chilling on activated oxygen-scavenging enzymes in low temperature sensitive and -tolerant cultivars of rice (*Oryza sativa* L.). Plant Sci. 109:105-113.

Shimono H, Hasegawa T, Fujimura S, Iwama K (2004). Responses of leaf photosynthesis and plant water status in rice to low water temperature at different growth stages. Field Crops Res. 89:71-83.

Shimono H, Hasegawa T, Iwama K (2002). Response of growth and grain yield in paddy rice to cool water at different growth stages. Field Crops Res. 73:67-79.

Sweetlove LJ, Foyer CH (2004). Roles for reactive oxygen species and antioxidants in plant mitochondria. In Day DA, Millar AH, Whelan J (Eds.). Plant Mitochondria: From Genome to Function, Vol 1, Advances in Photosynthesis and Respiration. Kluwer Academic Press, Dordrecht, the Netherlands.

Todaka D, Nakashima K, Maruyama K, Kidokoro S, Osakabe Y, Ito Y, Matsukura S, Fujita Y, Yoshiwara K, Ohme-Takagi M, Kojima M, Sakakibara H, Shinozaki K, Yamaguchi-Shinozaki K (2012). Rice phytochrome-interacting factor-like protein OsPIL1 functions as a key regulator of internode elongation and induces a morphological response to drought stress. Proc Natl Acad Sci U S A. 2012 109:15947-15952.

Tsugane K, Kobayashi K, Niwa Y, Ohba Y, Wada K, Kobayashi H (1999). A Recessive *Arabidopsis* Mutant That Grows Photoautotrophically under Salt Stress Shows Enhanced Active Oxygen Detoxification. The Plant Cell 11:1195-1206.

Wang GJ, Miao W, Wang JY, Ma DR, Li JQ, Chen WF (2013). Effects of exogenous Abscisic acid on antioxidant system in weedy and cultivated rice with different chilling sensitivity under chilling stress. J.

Agron. Crop Sci. 199:200-208.
Washburn MP, Koller A, Oshiro G, Ulaszek RR, Plouffe D, Deciu C, Winzeler E, Yates JR (2003). Protein pathway and complex clustering of correlated mRNA and protein expression analyses in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U. S. A. 100:3107-3112.
Welsch R, Wüst F, Bär C, Al-Babili S, Beyer P (2008). A third phytoene synthase is devoted to abiotic stress-induced abscisic acid formation in rice and defines functional diversification of phytoene synthase genes. Plant Physiol. 147:367-380.
Yoshida R, Kanno A, Sato T, Kameya T (1996). Cool-temperature-induced chlorosis in rice plants (I. Relationship between the induction and a disturbance of etioplast development). Plant Physiol. 110:997-1005.
Yun K-Y, Park MR, Mohanty B, Herath V, Xu F, Mauleon R, Wijaya E, Bajic VB, Bruskiewich R, de los Reyes BG (2010). Transcriptional regulatory network triggered by oxidative signals configures the early response mechanisms of japonica rice to chilling stress. BMC Plant Biol. 10:16.