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Differential competence of redox-regulatory mechanism under extremes of temperature determines growth performances and cross tolerance in two indica rice cultivars

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ABSTRACT

The present study investigated the relationship between reactive oxygen species (ROS) accumulation (total and individual), antioxidant and radical scavenging capacity (total and individual), transcript abundance of some antioxidative genes and oxidative damages to membrane protein and lipid in germinating tissues of a salt resistant (SR26B) and salt sensitive (Ratna) rice cultivars under extremes of temperature to elucidate redox-regulatory mechanism governing differential oxidative stress tolerance associated with better growth and yield potential and identification of cross tolerance, if any. Imbibitional heat and chilling stress caused disruption of redox-homeostasis and oxidative damage to a newly assembled membrane system by increasing pro-oxidant/antioxidant ratio and by aggravating membrane lipid peroxidation and protein oxidation [measured in terms of accumulation of thiobarbituric acid reactive substances (TBARS), free carbonyl content (C=O groups), and membrane protein thiol level (MPTL)]. A concomitant increase in accumulation of individual ROS (superoxide and hydrogen peroxide) and significant reduction of radical scavenging activity (assessed in terms of ABTS, FRAP and DPPH methods), non-enzymatic and enzymatic anti-oxidative defense [assessed in terms of total thiol content and activities of superoxide dismutase (EC 1.15.1.1), catalase (EC 1.11.1.6), ascorbate peroxidase (EC 1.11.1.11), and glutathione reductase (EC 1.6.4.2)] are also noticed in both the salt sensitive (Ratna) and resistant (SR26B) germinating tissues of rice cultivars. When compared, salt resistant cultivar SR26B was found to suffer significantly less redox-imbalance and related oxidative damages to membrane protein and lipid as compared to salt sensitive cultivar Ratna. The salt tolerant cultivar SR26B resisted imbibitional chilling and heat stress due to its early preparedness to combat oxidative stress by up-regulation of gene expression of anti-oxidative enzymes and better capacity of redox-regulation and mitigation of oxidative damage to membrane protein and lipid as compared to salt sensitive cultivar Ratna, under the same magnitude of imbibitional heat and chilling stress. A model for redox-homeostasis in which the ROS-antioxidant interaction acts as a metabolic interface for up-regulation of gene expression necessary for cross tolerance is also proposed.

Introduction

Crop yield is severely affected by extremes of temperature as both heat and chilling cause loss of redox-homeostasis in tissues resulting in serious oxidative damages (Foyer and Noctor, 2013; Saidi et al., 2011; Long and Ort, 2010; Essemine et al., 2010; Boyer, 1972; Arora et al., 2002). To survive under these conditions plants have developed a complex signaling network involving different endogenous growth regulators that sense and protect them from environmental stresses. Antioxidants provide essential information on cellular redox-state, and influence gene expression associated with biotic and abiotic stress responses to maximize defense (Foyer and Noctor, 2013; Tokunaga et al., 2005).

Abbreviations: ABTS, 2,2′azinobis(3-ethylbenzthiazoline)-6-sulfonic acid; APOX, ascorbate peroxidase; CAT, catalase; DCFDA, 2′,7′-dichloroflorescin diacetate; DPPH, 2,2-diphenyl-1-pycryl hydrazyl; FRAP, ferric reducing ability of plasma; FCC, free carbonyl content; GR, glutathione reductase; HIS, imbibitional heat stress; ICS, imbibitional chilling stress; MPTL, membrane protein thiol level; MII, membrane injury index; OSI, oxidative stress index; RH, relative humidity; ROS, reactive oxygen species; SOD, superoxide dismutase; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances; TPTZ, 2,4,6 tripyridyl-s-trizine; TTC, total thiol content.

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Growing evidence suggests a model for redox-homeostasis in which the reactive oxygen species (ROS)-antioxidant interaction acts as a metabolic interface for signals derived from metabolism and from the environment. This interface modulates the appropriate induction of stress acclimation processes (Foyer and Noctor, 2005, 2013).

One of the common responses to different environmental stresses, both abiotic and biotic, is loss of redox homeostasis due to accelerated generation of ROS produced by many pathways with manifestation of oxidative injuries in plants (Bhattacharjee, 2005, 2010; Suzuki and Mittler, 2006; Foyer and Noctor, 2013; Jiang and Zhang, 2001). In fact, ROS play vital role in plant growth and development and also exhibit significant role in sensing environmental cues (Alvarej et al., 1998; Foyer and Noctor, 2013). ROS are constantly produced during the course of all basic life processes and their titer is tightly regulated by antioxidant system. Exposure of plants to unfavorable environmental cues cause activation of plasma membrane bound NADPH-dependent oxidases or respiratory burst oxidase homologue (rboh), producing superoxide and subsequently H_2O_2 by superoxide dismutase (Sagi and Fluhr, 2006). In most of the C_3 plants including rice, H_2O_2 may be generated by photosynthetic carbon oxidation cycle and cell wall associated peroxidases (Bhattacharjee, 2010). Another potential route of ROS and H_2O_2 generation in plant cell is via pseudocyclic electron flow of Z-Scheme of photosynthesis (Asada, 1994). The accelerated production of cytotoxic ROS disrupts redox-homeostasis and interrupts normal metabolisms during stress through oxidative damage of almost all important cellular macromolecules, ranging from membrane lipid to nucleic acid (Saidi et al., 2011; Long and Ort, 2010). So, redox regulation by up-regulating anti-oxidative defense and reducing ROS accumulation is required for restoring redox-homeostasis and normal metabolism under stress. A highly efficient antioxidant system comprising of antioxidant enzymes (SOD, CAT, APOX, and GR) and non-enzymatic constituents (thiol compounds, scavengers of oxy-free radicals and other ROS quenchers) is present in plant cells for restoring redox-homeostasis. In addition to its roles in removing ROS, antioxidant status appears to set the threshold for general plant defense responses, particularly those provoked by unfavorable environmental cues. Indeed, modulation of the ROS-antioxidant interaction plays a part in many stresses, as well as other responses to the environment, and in the regulation of plant development.

Germplasms of rice, in general, exhibit variation in their sensitivity toward abiotic stresses such as extremes of temperature, salinity, and drought (Roychoudhury et al., 2008; Turan and Tripathy, 2012). Indica rice cultivars, in particular, exhibit variations in temperature resistance or susceptibility during some vital phases of life cycle, including germination and flowering stages (Hurry and Hunar, 1992; Pareek et al., 1999; Roychoudhury et al., 2008; Turan and Tripathy, 2012). When sown under extremes of temperature that often prevails during sowing, rice seedlings exhibit symptoms of oxidative damage which is subsequently reflected in their low photosynthetic ability, altered physiology, and stunted growth (Hurry and Hunar, 1992). Although there are reports that indica rice varieties show differences in temperature sensitivity, especially during germination and flowering stages, there is very little research aimed at understanding the differences in physiology of these cultivars. The role of redox-regulatory mechanisms, particularly the ROS-antioxidant interaction, which acts at the metabolic interface for controlling defense reactions, needs to be explored in greater detail.

The indica rice variety SR26B is a recognized salt tolerant cultivar while Ratna is classified as salt sensitive (Bhattacharjee and Mukherjee, 1997, 2002). However, there are few reports regarding the sensitivity of these two rice cultivars under extreme temperatures. At present, our ability to improve temperature stress tolerance of indica rice cultivars is limited by our poor understanding of the extremely complex nature of its stress acclimation mechanism, particularly the ROS-antioxidant redox regulatory network. The objective of the present study was therefore to compare the redox-regulation properties, transcript abundance of important antioxidant enzymes along with the comparative oxidative damage of membrane components suffered by the seedlings of these two rice cultivars differing in salinity resistance under extremes of temperature. The impact of changes in pro-oxidant–antioxidant status, non-enzymatic radical scavenging properties, oxidation of membrane protein thiol, protein carbonylation, and membrane lipid peroxidation on early growth performances were studied.

Materials and methods

Plant growth and excess temperature treatment

Seeds of two cultivars of rice (*Oryza sativa* L., Cultivars Ratna and SR26B), selected as experimental material, have been collected from Central Rice research Institute, Cuttack, Orissa, India. Seeds of the two rice cultivars were washed with distilled water and were treated with 0.2% $HgCl_2$ for 5 min and then washed thrice with sterile distilled water. The surface sterilized seeds were imbibed in distilled water for 48 h in darkness at 25 ± 2 °C and thereafter, were sown on moist filter paper in Petri plates and were placed in two standardized conditions of thermostat-controlled seed germinator cum stability chamber (Remi 82 BL, India) maintained at 40 °C and 8 °C temperatures for duration of 16 h each to impose high and low temperature stress, respectively. For untreated control set, water imbibed seeds were sown directly in petri-plates and exposed at 25 ± 2 °C. Thereafter, all the seed lots were allowed to grow at 25 ± 2 °C with 12 h photo period (light intensity 270 μmol m^{-2} s^{-1}) and 78 ± 2% relative humidity. For all biochemical analysis 168 h old seedlings raised from aforesaid conditions were used (Fig. 1).

Determination of early growth performances

For studying early growth performances, relative growth index (RGI), biomass accumulation and vigor index (VI) were calculated according to Rubio-Casal et al. (2003) and Bhattacharjee (2008). RGI was calculated as: RGI = (average dry mass of ten treated seedlings/average dry mass of ten control seedlings) × 100. VI was measured as:

$$\mathrm{VI} = \frac{\text{Mean shoot length} + \text{mean root length}}{\text{Percentage of final germination}}.$$

RNA isolation and analysis of transcript profile by semi quantitative RT-PCR

RNA was extracted from an untreated control, imbibitional heat and chilling stress-raised seedlings of both the experimental cultivars of rice using guanidium isothiocyanate-phenol based reagent (RNA-XPress™ reagent, HiMedia) according to manufacturer's instructions. To ensure the comparability of the resulting band intensity, quantification of RNA was done using Nano drop Spectrophotometer (ND1000, Nanodrop technologies, USA) and confirmed by applying equal amounts of total RNA to an agarose gel using ethidium bromide staining.

Preparation of cDNA

First strand cDNA synthesis was done by using Revert Aid First Strand cDNA synthesis kit (Fermentas, Thermo Scientific) according to the manufacturer's protocol and quality of cDNA was checked by running 2 μL of total cDNA in 1.1% agarose gel using