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Alteration of oxidative and carbohydrate metabolism under abiotic stress in two rice (*Oryza sativa* L.) genotypes contrasting in chilling tolerance

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Summary

Abiotic stress is a major limiting factor in crop production. Physiological comparisons between contrasting abiotic stress-tolerant genotypes will improve understanding of stress-tolerant mechanisms. Rice seedlings (S3 stage) of a chilling-tolerant (CT) genotype (CT6748-8-CA-17) and a chilling-sensitive (CS) genotype (INIAP12) were subjected to abiotic stresses including chilling (13/12 °C), salt (100 mM NaCl), and osmotic (200 mM mannitol). Measures of physiological response to the stresses included changes in stress-related sugars, oxidative products and protective enzymes, parameters that could be used as possible markers for selection of improved tolerant varieties. Metabolite analyses showed that the two genotypes responded differently to different stresses. Genotype survival under chilling-stress was as expected, however, CT was more sensitive to salt stress than the CS genotype. The CT genotype was able to maintain membrane integrity better than CS, perhaps by reduction of lipid peroxidation via increased levels of antioxidant enzymes during chilling stress. This genotype accumulated sugars in response to stress, but the accumulation was usually less than in the CS genotype. Chill-stressed CT accumulated galactose and raffinose whereas these saccharides declined in CS. On

Abbreviations: CAT, catalase; CS, chilling sensitive INIAP12 genotype; CT, chilling tolerant CT6748-8-CA-17 genotype; LOOH, lipid peroxides; MDA, malondialdehyde; POX, peroxidase; ROS, reactive oxygen species

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the other hand, the tolerance mechanism in the more salt- and water-deficit-tolerant CS may be associated with accumulation of osmoprotectants such as glucose, trehalose and mannitol.

Introduction

In nature, plants often experience harsh environmental conditions such as drought, salinity and chilling. These conditions can delay growth and development, reduce yield and, in extreme cases, can inflict lethal injuries to the plant. To ensure survival, plants have evolved a range of response strategies to the various abiotic stresses likely to be encountered. The responses to a specific stress may vary with the genotype; nevertheless, some general reactions occur in all genotypes. At the whole plant level, the effect of stress is usually perceived as a decrease in photosynthesis and growth associated with alteration in carbon and nitrogen metabolism (Cornic and Massacci, 1996; Mwanamwenge et al., 1999; Law and Crafts-Brandner, 2001), and reactive oxygen production (Turcsanyi et al., 2000; Schwanz and Polle, 2001). Oxidative stress products include reactive oxygen species (ROS) such as the superoxide radical ($^{\bullet}O_2-$), hydrogen peroxide, singlet oxygen and hydroxyl radicals ($^{\bullet}OH$) (Iturbe-Ormaetxe et al., 1998; Cho and Park, 2000). Moreover, damage to membrane integrity is a common effect of stress, especially in the case of low temperature (Morsy et al., 2005). ROS have a role in lipid peroxidation and membrane damage, and consequently in plant senescence (Fridovich, 1986; Thompson et al., 1987). Antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase and catalase (CAT) are involved in scavenging of ROS (Asada, 1992; Foyer, 1993). CAT, found predominantly in peroxisomes, converts H_2O_2 into H_2O and O_2, whereas peroxidase (POX) decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants (Sudhakar et al., 2001). Lipid peroxides (LOOH) and malondialdehyde (MDA) are the first products formed during radical induced damage and decomposition of polyunsaturated fatty acids in membranes. Thus, these compounds are commonly used to monitor human oxidative stress diseases or in food freshness control. Much less is known about the toxic effect of MDA in plants than in humans. Nevertheless, they have been found in numerous species, such as *Helianthus annuus* (Santos et al., 2001), *Arabidopsis thaliana* (Muckenschnabel et al., 2002) and *Populus tremula* (Jouve et al., 2004), and their presence is considered to be a good indication of the occurrence of oxidative stress.

An adaptive biochemical function of compatible solutes is the scavenging of ROS that are overproduced during hyperosmotic and ionic stresses. These compatible solutes, or osmoprotectants, can accumulate to high levels without disturbing intracellular biochemistry (Bohnert and Jensen, 1996). Additionally, they have the ability to maintain the activity of enzymes during stress. Carbohydrates are a major category of compatible solutes that include hexoses (mostly fructose and glucose), disaccharides (sucrose, trehalose), sugar alcohols (inositol, mannitol) and complex sugars (raffinose and stachyose), all of which accumulate during stress (Bohnert and Jensen, 1996; Jouve et al., 2004).

Unlike other cereal crops, such as wheat, barley and rye, rice (*Oryza sativa* L.) is not well adapted to cold weather and is damaged by temperatures below 15 °C (Howarth and Ougham, 1993). Moreover, soil and water salinity and a reduced supply of water are growing problems facing rice production (Flowers and Yeo, 1995; Munns, 2002). While the role of several osmoprotectants and ROS scavenging enzymes in abiotic stress tolerance has been well documented, data on genotypic differences are limited. In this study, we assessed stress-induced changes in ROS scavenging enzymes and osmoprotectants in two rice genotypes with contrasting levels of chilling tolerance. Changes associated with chilling, salt- and water-deficit stress were analyzed to obtain a better understanding of the general genotypic differences and to provide a basis for further analyses. Ultimately, physiological identification of stress-related responses of rice genotypes will provide markers for selection in breeding programs or genes for improvement through transgenic technology.

Materials and methods

Biological material and treatments

From among 20 genotypes of rice (*Oryza sativa* L.) cultivars CT6748-8-CA-17 and INIAP12 were previously identified as the most chilling-tolerant