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Physiology and proteome responses of two contrasting rice mutants and their wild type parent under salt stress conditions at the vegetative stage☆

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ABSTRACT

Salinity is one of the major environmental limiting factors that affects growth and productivity of rice (*Oryza sativa* L.) worldwide. Rice is among the most sensitive crops to salinity, especially at early vegetative stages. In order to get a better understanding of molecular pathways affected in rice mutants showing contrasting responses to salinity, we exploited the power of 2-DE based proteomics to explore the proteome changes associated with salt stress response. Our physiological observations showed that standard evaluation system (SES) scores, Na^+ and K^+ concentrations in shoots and Na^+/K^+ ratio were significantly different in contrasting mutants under salt stress condition. Proteomics analysis showed that, out of 854 protein spots which were reproducibly detected, 67 protein spots showed significant responses to salt stress. The tandem mass spectrometry analysis of these significantly differentially accumulated proteins resulted in identification of 34 unique proteins. These proteins are involved in various molecular processes including defense to oxidative stresses, metabolisms, photosynthesis, protein synthesis and processing, signal transduction. Several of the identified proteins were emerged as key participants in salt stress tolerance. The possible implication of salt responsive proteins in plant adaptation to salt stress is discussed.

Introduction

Salinity is one of the major environmental constraints for crop production worldwide (Munns, 2002). It is estimated that more than 800 million hectares of land throughout the world are affected by high salinity (Munns and Tester, 2008). High salinity causes water deficit, ion toxicity, and nutrient deficiency leading to molecular damage, growth arrest, and finally cell death. Salt stress accelerates production of reactive oxygen species (ROS), whose overproduction is detrimental for plant cells (Borsani et al., 2001; Gill and Tuteja, 2010). To cope with salt, plants have developed mechanisms to regulate its accumulation and partitioning to different organs. Plants can exclude Na^+ and Cl^- in their roots but the extent of this exclusion varies in halophytes and glycophytes (Munns and Tester, 2008). In addition, many plants have evolved strategies to accumulate compatible solutes (Zhu, 2002), which have a significant role in plant adaptation to osmotic stress. Accumulation of osmolytes in cytosol plays a crucial role in protecting the cytoplasm from detrimental effects of salt both in halophytes and glycophytes (Botella et al., 2007; Flowers et al., 1977).

Rice is generally categorized as salt sensitive crop but the extent of its sensitivity is varying during different growth phases. It is considered to be tolerant to salt stress during germination and active tillering, while it shows an enhanced sensitivity during early vegetative and reproductive stages (Heenan et al., 1988; Lutts et al., 1995; Zhu et al., 2001). Rice is recommended as a crop best suited for salt-affected soils because it can grow well under flooded conditions that can help in leaching harmful salts (Ismail et al., 2007).

Mutagenesis has been widely used to generate genetic diversity for plant breeding. Chemical mutagenesis has been known as the workhorse of traditional genetics (Greene et al., 2003). Induced mutations, with high efficiency in generating desirable variation, have recently been widely used in gene discovery and in the development of novel crop traits. Over the past 78 years, mutation breeding contributed to numerous valuable alterations in plant characteristics leading to increase in yield potential and

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improvement in qualitative traits of specific crops (Maluszynski, 2001). These efforts have resulted in over 434 mutant rice varieties developed by the end of the 20th century (Maluszynski, 2001). This clearly demonstrates the importance of this potent technique in plant breeding. Early maturity, shorter stature, tolerance to abiotic stresses, and resistance to pests and diseases are among the most important traits targeted through mutation breeding. Mutation breeding can significantly shorten the time for developing new varieties compared with conventional methods, in which certain genes have to be incorporated from other genetic backgrounds through a series of backcrossing (Zhu, 2000). Several single gene mutations conferring salt stress tolerance have been reported in soybean, barley, tobacco, fern, and Arabidopsis (Zhu, 2000). Apparently, considerable efforts have been made to increase salt tolerance in rice through conventional breeding but with slow progress. Combining mutation and conventional breeding methods might open new frontiers for developing salt-tolerant rice varieties. In recent years, some studies have successfully identified salt-tolerant mutants (Lee et al., 2003; Miah et al., 1996; Sathish et al., 1997; Zhang et al., 1995, 1999). A key challenge in a breeding program for salinity tolerance is to identify the genes that confer salinity tolerance and to use the identified genes efficiently to accelerate crop breeding (Bohnert and Cushman, 2000). Comparative proteomic analysis can provide a link to identify the genes that play a key role in salt stress tolerance.

As part of the rice functional genomics project, the International Rice Research Institute (IRRI) developed a large collection of mutants from the Indica rice variety, IR64, (Leung et al., 2001). This variety is widely grown in Asia because of its good agronomic traits and resistance to a wide range of pests and diseases. Some of these lines have been evaluated for various biotic and abiotic stresses to identify mutants with promising traits. For instance, few mutants with gain- and loss-of-resistance to blast, bacterial blight, and tungro virus were identified through forward genetics analyses (Zenna et al., 2008).

Two-dimensional gel electrophoresis-based proteomic approaches have been widely used to investigate salinity-induced responses in plants (Komatsu et al., 2003). In previous studies, proteomic approach was applied to study rice responses to salt stress at seedling stage (Kim et al., 2005; Moons et al., 1995; Salekdeh et al., 2002). In the current study, a comparative proteomic analysis was performed on shoots of IR64 as parent line and two IR64 derived mutant lines differing in susceptibility to salt stress to identify differentially expressed proteins during salt stress exposure. To the best of our knowledge, this is the first report that utilizes proteomics to discover the change in protein expression profile of a wild type parent and its mutant lines in response to salinity at the vegetative stage.

Materials and methods

Sample preparation

Two IR64 mutant lines, one (167-1-3) with better tolerance and the other (S-730-1) with higher sensitivity to salt stress along with their wild type parent (IR64), were selected and evaluated for salinity tolerance in a split plot experiment with three replications (Nakhoda et al., 2012). This experiment was conducted with plants grown in hydroponic condition at International Rice Research Institute (IRRI) during September and October 2006. During the experiment, the day/night temperature and relative humidity of culture room (glasshouse) were set at 29/21 °C and 70–75%.

Briefly, seeds were heat-treated at 50 °C for five days in an electrical oven to break seed dormancy and were then surface sterilized by immersing them in 50% hypochlorite solution (commercial bleach) and rinsed thoroughly with distilled water. Seeds were subsequently soaked in aerated distilled water at 25 °C for 24 h, then transferred to Petri dishes with two layers of Whatman filter paper number 5 at the bottom and incubated at 32 °C for 48 h in an incubator. Two pre-germinated seeds were sown per hole on a styrofoam seedling floats with the net at the bottom suspended on 12-liter plastic trays with distilled water for three days. Each entry was sown in two rows (20 holes) on seedling floats. Each replication consisted of 10 trays. On the fourth day, the distilled water was replaced with Yoshida nutrient solution and plants were grown on nutrient solution for 10 days. The solutions were renewed every 5–7 days and the pH maintained at 5.0 daily (by adding either 1 N NaOH or HCl). Weak or off-type seedlings were removed before imposing the stress to ensure uniformity at the start of the experiment. Salt stress was imposed 13 days after sowing by adding NaCl to the nutrient solution. Electrical conductivity (EC) of the nutrient solution was gradually increased in three steps (6, 8, and 12 dS m^{-1}) over a period of 5 days with two-day intervals until a final EC of 12 dS m^{-1} was reached which is equivalent to 120 mM. The EC of the nutrient solutions in stress treatment were monitored and maintained at 12 dS m^{-1} during the experiment using a handheld EC meter (WTW 340, Germany). The non-saline control treatment with Yoshida solution had an EC of 0.94–1.1 dS m^{-1}. All entries were monitored and scored based on the visual symptoms as described by (Gregorio, 1997) using modified Standard Evaluation System for rice (SES) at 10 and 16 d after salinization, as the initial and second scoring, respectively. Salt uptake of the mutant lines was examined to elucidate the physiological and biochemical bases of their tolerance/sensitivity at early vegetative stage.

Standard evaluation system (SES) and survival percentage

Modified SES for rice was used in rating the visual symptoms of salt injury as described by (Gregorio, 1997). Final scoring and sampling were done when the sensitive mutant scored 7. At final scoring, the number of surviving plants for each individual line was counted and the percentage of survival plants was calculated. For the rest of measurements five plants for each genotype were sampled at harvest time and divided into roots and shoots. Root and shoot samples were washed thoroughly with distilled water to remove salts from the surface. Samples were blotted dry and the following measurements were conducted for all of them. All the data are the average of five plants per replication.

Plant height, root length, and dry weight

The plant height (cm) was measured on the seedling samples from the base of the stem to the tip of the topmost or youngest fully expanded leaf of the plants using a meter stick. Root lengths were also measured for each plant and their means were computed. Root and shoot samples were oven dried at 75 °C for 72 h and their respective dry weights were determined.

Determination of sodium, potassium, and ion uptake in plant tissues

To determine the total uptake of sodium and potassium ions by roots and shoots, sodium and potassium concentrations and sodium to potassium ratio (Na^+/K^+) in roots and shoots were measured. For each genotype in each replication, five plants were uprooted and washed thoroughly as described before to remove salts from the surface of the tissues. Roots and shoots were separated and dried in an oven at 75 °C for 72 h. Root and shoot samples were ground to a fine powder and 10 mg of dried, ground materials was transferred into 15 ml Pyrex test tubes. Tissue extraction was done with adding 10 ml of 0.1 N acetic acid to the samples and boiling at 90 °C for at least 2 h in a water bath. The extracted tissues