



Physiological Mechanism of Salicylic Acid for Alleviation of Salt Stress in Rice

D. JINI¹, B. JOSEPH²

¹Department of Biotechnology, Manonmaniam Sundaranar University, Tirunelveli 627012, Tamil Nadu, India; ²Dean, Centre for Research and Consultancy, Hindustan University / Institute of Technology and Science, Padur, Kelambakkam, Chennai 603103, Tamil Nadu, India)

Abstract: Soil salinity is one of the most important problems of crop production in estuarine and coastal zones. Improvement in salt tolerance of major food crops is an important way for the economic utilization of coastal zones. This study proved that the application of salicylic acid (SA) improved the growth and yield under salt stress conditions and investigated its physiological mechanisms for salt tolerance. The investigation on the effect of SA for salt tolerance during germination showed that the decreased rates of germination and growth (in terms of shoot and root lengths) by the salt stress were significantly increased by the SA application (SA + NaCl). The treatment of SA to the high and low saline soils enhanced the growth, yield and nutrient values of rice. The effects of SA on Na⁺, K⁺ and Cl⁻ ionic accumulation were traced under salt stress condition by inductively coupled plasma optical emission spectrometry and ion chromatography. It was revealed that the increased accumulation of Na⁺ and Cl⁻ ions by the salt stress were reduced by SA application. An increased concentration of endogenous SA level was detected from the SA-treated rice varieties (ASD16 and BR26) by liquid chromatography electrospray ionization-tandem mass spectrometry. The activities of antioxidant enzymes such as superoxide dismutase, catalase and peroxidase were increased by salt stress whereas decreased by the SA application. The study proved that the application of SA could alleviate the adverse effects of salt stress by the regulation of physiological mechanism in rice plants. In spite of salt stress, it can be applied to the coastal and estuarine regions to increase the rice production.

Key words: salicylic acid; salt tolerance; *Oryza sativa*; NaCl; germination; physiology

Rice (*Oryza sativa* L.) is an important cereal which is consumed as a staple food by more than half of the world's population (Khush, 2005; Ma et al, 2007). The world population is increasing rapidly and may reach 6.0 to 9.3 billion by the year 2050 (<http://www.unfpa.org/swp/200/>), whereas the crop production is decreasing rapidly by the negative impact of various environmental stress, including biotic stress (such as insects, bacteria, fungi and viruses) and abiotic stress (such as drought, salinity, cold, heavy metals and pesticides). Therefore, it is now very important to develop stress-tolerant varieties

to cope with this upcoming problem of food security. To assure food security in the rice consuming countries of the world, rice production should be increased by 50% in these countries by 2025. This additional rice will have to be produced on less land with less usage of water, labour and chemicals.

Many estuarine and coastal regions in the world contain soils and water resources that are too saline for most of the crops, which affect plants' growth and productivity through osmotic effects, ion specific effects and oxidative stress (Lüttge and Läuchli, 2002; Munns, 2002). The injury to plants exposed to salt

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Corresponding author: D. JINI (jini@biotech.com)

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stress is connected with oxidative damage at the cellular and molecular levels (Foyer and Noctor, 2003). Salt stress induces cellular accumulation of reactive oxygen species (ROS), which can damage biomolecules of the cell (Hernandez et al, 2000; Mansour et al, 2005; Amor et al, 2007; Eyidogan et al, 2007). The salt stress increases the antioxidant enzyme activities and antioxidant content in salt-tolerant species whereas salt-sensitive species fail to do so (Shalata et al, 2001; Demiral and Turkan, 2005). Therefore, it is necessary to develop technologies to cultivate crop varieties against salt stress to meet the future food demand.

In recent years, there has been much interest in the development of salt-tolerant crop varieties. For this purpose, genetic improvement of salinity tolerance in current genotypes/cultivars has been proposed as the most effective strategy to overcome salinity problems (Marino et al, 2009; Sankar et al, 2011). Although a number of techniques have been developed (Ashraf, 2009), none of these approaches has been found to be fully effective under salt stress conditions. This is due to certain problems like the response of plants to salt stress are different at the cellular level, the tissue level or at the whole plant level, complex mechanism of salt tolerance, involvement of environmental factors in addition to salt stress, and lack of efficient selection criteria (Flowers, 2004; Ashraf et al, 2008; Munns and Tester, 2008). Therefore, it is necessary to develop methods and strategies to ameliorate deleterious effects of salt stress on plants.

Salicylic acid (SA) is an endogenous growth regulator (Sakhabutdinova et al, 2003) and belongs to a group of phenol compounds. It participates in the regulation of physiological processes (Hayat et al, 2010) and also provides protection against biotic and abiotic stress such as salinity (Kaya et al, 2009). It also has a role in germination under stressful conditions, although its definite role and the underlying physiological mechanisms have not been fully elucidated (Borsani et al, 2001; Rajjou et al, 2006; Alonso-Ramirez et al, 2009; Asadi et al, 2013). Even though the ameliorative effects of SA for salt tolerance have been studied in many crops such as bean (Azooz, 2009), tomato (Javaheri et al, 2012) and maize (Gunes et al, 2007; Bagheri, 2014), there is no study on the effect of SA from the germination to yield and its complete physiological mechanism in rice plants. This study was conducted with different assay conditions and rice genotypes to assess whether

the application of SA improved the growth and yield under salt stress conditions and to investigate the physiological mechanisms of SA for salt tolerance.

MATERIALS AND METHODS

Rice materials

Two indica rice varieties ASD16 (salt-sensitive variety) and BR26 (salt-tolerant variety) were used. Seeds of ASD16 were collected from Agricultural Block, Munchirai, India and BR26 seeds from Cox's Bazaar, Chittagong, Bangladesh. The collected dried seeds were subsequently stored at 2 °C and 40% relative humidity (RH) in the active collection storage vault of the gene bank until needed. Thereafter, seed samples were allowed to equilibrate at the room temperature (25 °C ± 1 °C and 68%–80% of RH) for 7 d before being subjected to germination test (Waheed et al, 2012).

Experimental soil and water

The physico-chemical parameters of water (Table 1) and soil (Table 2) samples were analyzed by using standard methods (van Reeuwijk, 2002).

Germination test

Seeds of ASD16 and BR26 were surface sterilized in 20% bleach for 10 min and washed repeatedly in distilled water. Seeds were laid out in 9 cm petri dishes (10 seeds per dish). The sodium chloride (NaCl) (0, 100, 200, 300 and 400 mmol/L), SA (1.0 mmol/L) and the combination of NaCl and SA were added as treatments (10 mL per petri dish). Treatments were replicated three times and placed in a growth chamber at 25 °C ± 2 °C for germination. The germination rate

Table 1. Physico-chemical parameters of water used in this study.

Property	Value
Dissolved oxygen (mg/L)	6.1 ± 0.2
pH	7.2 ± 0.0
Temperature (°C)	28 ± 2
Total hardness (mg/L)	346 ± 19
Free CO ₂ (mg/L)	2.2 ± 0.1
Ca (mg/L)	82 ± 22
Mg (mg/L)	34 ± 0
Na (mg/L)	20 ± 5
Salicylic acid	0
Sulphate (mg/L)	111 ± 13
Chloride (mg/L)	23 ± 2
Specific conductance	2 340 µS/cm at 2 °C

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