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Research article

# Divergences in morphological changes and antioxidant responses in salt-tolerant and salt-sensitive rice seedlings after salt stress

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ABSTRACT

Salinization plays a primary role in soil degradation and reduced agricultural productivity. We observed that salt stress reversed photosynthesis and reactive oxygen scavenging responses in leaves or roots of two rice cultivars, a salt-tolerant cultivar Pokkali and a salt-sensitive cultivar IR-29. Salt treatment (100 mM NaCl) on IR-29 decreased the maximum photochemical efficiency (Fv/Fm) and the photochemical quenching coefficient (qP), thereby inhibiting photosynthetic activity. By contrast, the salt treatment on Pokkali had the converse effect on Fv/Fm and qP, while increasing the nonphotochemical quenching coefficient (NPQ), thereby favoring photosynthetic activity. Notably, chloroplast or root cells in Pokkali maintained their ultrastructures largely intact under the salt stress, but, IR-29 showed severe disintegration of existing grana stacks, increase of plastoglobuli, and swelling of thylakoidal membranes in addition to collapsed vascular region in adventitious roots. Pokkali is known to have higher hydrogen peroxide ($H_2O_2$)-scavenging enzyme activities in non-treated seedlings, including ascorbate peroxidase, catalase, and peroxidase activities. However, these enzymatic activities were induced to a greater extent in IR-29 by the salt stress. While the level of endogenous $H_2O_2$ was lower in Pokkali than in IR-29, it was reversed upon the salt treatment. Nevertheless, the decreased amount of $H_2O_2$ in IR-29 upon the salt stress didn't result in a high scavenging activity of total cell extracts for $H_2O_2$, as well as $O_2^{\bullet -}$ and $^{\bullet}OH$ species. The present study suggests that the tolerance to the moderate salinity in Pokkali derives largely from the constitutively maintained antioxidant enzymatic activities as well as the induced antioxidant enzyme system.



## 1. Introduction

Salinity of soil or irrigation water is one of the major abiotic stresses that severely affects crop production worldwide [1]. Salinization plays a primary role in soil degradation, affecting up to 20% of irrigated land and 2.1% of dryland agriculture globally [2]. Salinity effects are more apparent in arid and semi-arid regions, where limited rainfall, high evapotranspiration, and high temperature (associated with poor water and soil management) contribute to the salinization of soil and greatly affect agricultural production [3]. High salt concentrations in soil cause various cooperative events that negatively impact agricultural production, such as delays in plant growth and development [4], inhibition of enzymatic activities, and reductions in photosynthetic rates [5]. Therefore, investigators are aiming to understand the mechanisms by which plants respond and adapt to such stresses. Plant responses to salt stress have generally been conducted using anatomical, ecological, physiological, and molecular approaches [6–8] in relation to regulatory mechanisms of ionic and osmotic homeostasis. Although several prior reports of morphological and biochemical responses of plants have displayed different salt sensitivities, few anatomical or ultrastructural studies in salt-tolerant and salt-sensitive rice cultivars under conditions of salt stress have been reported.

*Abbreviations:* AA, ascorbic acid; APX, ascorbate peroxidase; CAT, catalase; $CO_2$, carbon dioxide; Chl, *a*chlorophyll *a*; Chl, *b*chlorophyll *b*; $H_2O_2$, hydrogen peroxide; $^{\bullet}OH$, hydroxyl radical; MS, Murashige and Skoog; NPQ, nonphotochemical quenching; $HO_2^{\bullet}$, perhydroxyl radical; POD, peroxidase; PS II, photosystem II; ROS, reactive oxygen species; $O_2^{\bullet -}$, superoxide anion; $^1O_2$, singlet oxygen; qP, photochemical quenching coefficient; SOD, superoxide dismutase; TEM, transmission electron microscopy; tChl, total chlorophylls; tCar, total carotenoids.

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It has been reported that the ionic and osmotic effects of salinity cause growth impairment in plants [9,10]. Moreover, salt stress, like other abiotic stresses, results in oxidative stress through an increase in reactive oxygen species (ROS), such as the superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical (•OH). These ROS are highly reactive, altering normal cellular metabolism through oxidative damage to lipids, proteins, and nucleic acids [11]. To mitigate the ROS-mediated oxidative damage, plants have developed a complex antioxidative defense system, including low-molecular mass antioxidants – such as ascorbate, reduced glutathione, tocopherol, carotenoids, and flavonoids – as well as antioxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT) [12,13]. Therefore, the complex antioxidative defense system develops with a concomitant increase in ROS. In addition, salt adversely affects the metabolism of plants, resulting in substantial modifications in plant gene expression. These modifications may lead to the accumulation or depletion of certain metabolites, resulting in an imbalance in the levels of cellular proteins, which may increase, decrease, appear, or disappear after salt treatment [14]. A previous study showed that antioxidant responses under conditions of salt stress in salt-tolerant Pokkali rice plants included slight increases in SOD activity and decreases in POD activity, with virtually unchanged lipid peroxidation, electrolyte leakage, and $Na^+$ accumulation against salt-sensitive rice varieties Hitomebore and IR-28 [15].

Comparative investigations of the leaves and roots of the salt-sensitive IR-29 cultivar and the salt-tolerant Pokkali cultivar could result in comprehensive morphological, biochemical, and photochemical insights into response mechanisms induced by salt stress in rice plants. The results of this particular study may provide valuable information regarding how rice plants are damaged by salt stress and how they cope with the stress. Therefore, we investigated the effects of varying salt stress on the activities of various antioxidant (iso)enzymes analysis, antioxidants, scavenging capacity of ROS, and the morphological changes in leaves and roots of two rice cultivars, IR-29 and Pokkali.

## 2. Results

### 2.1. The effects of salt stress on seedling growth and photosynthesis of rice cultivars IR-29 and Pokkali

Salt stress is known to cause severe inhibition on plant growth and development [16]. A high concentration of salt in soil may induce three additional major types of stress: ionic, osmotic, and oxidative stress [17]. Salt stress also results in a considerable reduction in the fresh and dry weights of leaves, stems, and roots [18–20]. During 7 days of salt treatment (100 mM NaCl), salt-sensitive IR-29 showed early symptoms of wilting within 48 h after the treatment and the severity of the symptoms was directly related to the consecutive exposure to salinity, eventually leading to death. However, the newly developed leaves of the plant remained pale green until the end of the treatment. In contrast, salt-tolerant Pokkali did not develop wilting or any visible symptoms in response to the salt treatment up to 1 week after the treatment.

The effects of salt stress on photosynthetic pigment contents and photosynthetic activities in the two cultivars were measured at 7 days after the salt treatment. IR-29 showed that decreased chlorophyll content upon the salt stress, but increased in carotenoid content. In contrast, total chlorophyll and total carotenoid content in Pokkali remained unchanged by the salt stress (Table 1). The maximum photochemical efficiency (variable fluorescence/maximal fluorescence, Fv/Fm) of photosystem II (PS II) has been demonstrated as a reliable chlorophyll fluorescence parameter by measuring photosynthetic rates under stress conditions [21]. When the two rice cultivars were exposed to salt stress conditions for 7 days, the Fv/Fm of treated IR-29 was slightly lower than that of the control (Table 1). By contrast, Pokkali demonstrated an increase in Fv/Fm under conditions of salt stress. In addition, other parameters, including the photochemical quenching coefficient (qP) and non-photochemical quenching coefficient (NPQ), were also reduced in IR-29 but increased in Pokkali by the salt stress (Table 1). For protein contents under the salt stress condition, IR-29 showed a significant decrease in protein contents both leaves and roots, while Pokkali showed a slight decrease in leaves but negligible change in roots. Our findings revealed that the salt stress inhibited photosynthetic capacities or growth of IR-29, but, elevated photosynthetic activities in Pokkali, as described in the earlier study [15], suggesting that Pokkali was more resistant to the salt stress than IR-29.

**Table 1**
The effects of salt stress on the content of pigments and chlorophyll-fluorescence parameters in two rice cultivars. Rice seedlings were cultivated in half MS liquid medium for 3 weeks and were exposed salt stress by adding 100 mM NaCl to the hydroponic solution for 7 days. Fv/Fm, the maximum photochemical efficiency of PS II; qP, photochemical quenching coefficient; NPQ, nonphotochemical quenching coefficient. Each value represents the mean of three replications ± S.E. $n = 9$.

| Cultivars | IR-29 | | Pokkali | |
|---|---|---|---|---|
| NaCl (mM) | 0 | 100 | 0 | 100 |
| Total chlorophyll | 3.11 ± 0.03 | 2.46 ± 0.19 | 4.19 ± 0.05 | 4.29 ± 0.10 |
| Total carotenoids | 0.87 ± 0.08 | 1.19 ± 0.06 | 1.37 ± 0.04 | 1.29 ± 0.04 |
| Chlorophyll *a*/*b* | 3.19 ± 0.11 | 3.34 ± 0.12 | 3.13 ± 0.08 | 3.39 ± 0.05 |
| Fv/Fm | 0.794 ± 0.004 | 0.736 ± 0.029 | 0.740 ± 0.018 | 0.837 ± 0.004 |
| qP | 0.598 ± 0.019 | 0.428 ± 0.032 | 0.378 ± 0.004 | 0.465 ± 0.019 |
| NPQ | 0.609 ± 0.097 | 0.539 ± 0.045 | 0.379 ± 0.067 | 1.020 ± 0.066 |
| Protein (leaves) | 7.930 ± 0.423 | 2.808 ± 0.085 | 5.272 ± 0.386 | 4.957 ± 0.383 |
| Protein (roots) | 0.439 ± 0.027 | 0.190 ± 0.014 | 0.580 ± 0.022 | 0.561 ± 0.032 |

### 2.2. Effects of salt stress on cellular structures of IR-29 and Pokkali

While physiological effects of salt stress on the two rice cultivars have been reported [15], the changes in cellular ultrastructures under the stress have not yet been examined. This study examined the ultrastructural changes in IR-29 and Pokkali in response to salt stress by light microscopy and transmission electron microscopy (TEM) (Figs. 2 and 3). The ultrastructural alteration of chloroplasts in Pokkali was not conspicuous (Fig. 2C and D), whereas the thylakoidal membranes in IR-29 were severely disorganized by swelling and curling (Fig. 2A and B). The chloroplasts in the control IR-29 were located at mesophyll and parenchyma cells, containing large starch grains (Fig. 2A), while those in the salt-treated IR-29 showed a noticeable increase of plastoglobuli (shown by red arrows in Fig. 2B). When cross-sections of the segment at 10 mm from the tips of adventitious roots were imaged by bright-field microscopy, it was observed that the vascular cylinder region of the salt-treated IR-29 had entirely collapsed (Fig. 3F), which included the central metaxylem vessel, compared with that of the control plant (Fig. 3E). Consistent with this finding, disintegrated root cell structures were only observed in salt-treated IR-29 via TEM analysis (shown by red arrows in Fig. 2F). After the salt treatment, cortex cellular membranes were disintegrated, resulting in the release of cell organelles. The ultrastructural components of root cells were nearly the same between the control (Fig. 2G) and salt-treated Pokkali (Fig. 2H). These results indicated that leaf and root cells of IR-29 were more sensitive to the salt stress than those of Pokkali.