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COMPARATIVE EFFECTS OF NACL AND PEG STRESS ON LEAF AND ROOT GROWTH AND PROLINE CONTENT IN SEEDLINGS OF THREE RICE CULTIVARS.

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ABSTRACT

The effects of salinity and water stresses on the growth and proline content of seedlings of three rice cultivars were determined. Three cultivars of rice were used: Pokkali, a salt-tolerant cultivar; IR64, which was moderately salt-tolerant; and Khao Dawk Mali 105 (KDM105), which is salt-sensitive cultivar. Rice seedlings were grown in nutrient solution for 21 days, and subsequently in nutrient solution containing NaCl at the electrical conductivity (EC) of six desisiemens per meter (dS/m), 10%, and 20% of PEG for nine days. The relative water contents in leaves and roots were determined from their fresh and dried weights, and the proline content by a spectrophotometric assay. The dry weights of leaves and roots of rice seedlings decreased in response to stress induced by NaCl and PEG. Under most stress treatments, the relative water content of leaves and roots decreased until day 5, then slightly increased on day 9. However, the relative water content of leaves of IR64 grown in NaCl (12 dS/m) decreased more sharply than with other treatments until day 9. The relative water content of leaves of KDML105 grown on NaCl (12 dS/m) decreased continuously until day 9. The proline content of all three cultivars increased in response to both NaCl and PEG-induced stresses. Leaves and roots of KDML105 accumulated the highest amount of proline, followed by IR64 and Pokkali.

Keywords: Growth, relative water content, proline, stress, salt-tolerant, salt-sensitive cultivar.

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INTRODUCTION

Plants are exposed to various types of environmental stress, among which water and salt, in particular, are major factors causing stresses that dramatically limit plant growth and have negative impact on agricultural production throughout the world. This is probably resulted in subsistence or economic gain. In Northeastern Thailand, 35% of the land area faces salinity problems of varying degrees, especially for growing rice, which is considered to be moderately sensitive to salt (Munns and Tester, 2008). For most rice varieties, yields of grain are halved when the electrical conductivity of the irrigating water reaches 6 dS/m (Lu and Neumann, 1998; Yoshida, 1981). The expansion of agriculture in semi-arid and arid regions, using intensive irrigation and fertilization, tends to increase secondary salination through progressive salt accumulation in soil, because of dissolved salt in the applied water associated with high evapotranspiration rates (Chaves et al., 2008).

The response of rice plants to salt and water stresses consists of numerous processes that must be coordinated to alleviate both cellular hyper-osmolarity and ion disequilibrium. Several studies have shown that the Na-K selectivity of plant roots, which minimizes the entry of Na^+ into plants and maintains effective K^+ uptake, and also the low rate of salt transport to expanding shoots are very important mechanisms and are directly correlated with salt tolerance (Flowers et al., 1991; Munns, 2002). The effects of salt and water stresses on rice growth parameters vary, depending on the concentrations of NaCl (Miller and Gardiner, 1998) and polyethylene glycol 6000 (PEG) to which they are exposed, the duration of treatment, and the age of the leaves (Faustino et al., 1996). Comparative response studies with different cultivars could provide insights into the mechanisms of salt and water tolerance in rice. Proline accumulation in plant cells exposed to salt or water stress is a widespread phenomenon (Demir, 2000; Demiral and Turkan, 2004). Although there may be no causal relationship between stress conditions and the accumulation of proline and organic solutes, which may be simply a stress symptom without any adaptive implication, provoked by the interaction of some effectors concentrated in plant tissues under adverse conditions.

In this study, seedlings of three cultivars that differ in salt tolerance, Pokkali (salt-tolerant), KDML105 (salt-sensitive), and IR64 (moderately salt tolerant), were used to evaluate the comparative effects of salt and water stresses on several parameters, such as fresh weight, dry weight, relative water content, and on the proline content of leaves and roots.

MATERIALS AND METHODS

Plant materials

Seeds of rice, *Oryza sativa* L., cultivars Pokkali (salt-tolerant), KDML105 (salt-sensitive), and IR64 (moderately salt-tolerant), obtained from the Pathum Thani Rice Research Center (Rice Research Institute, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand), were used in this study. The methods of growing plants and salinization of the nutrient solution were modified from Gregorio et al. (1997). Rice seeds were surface-sterilized with 1.5% (w/v) calcium hypochlorite for 30 min, thoroughly washed and germinated in distilled water. The uniformly germinated seeds were transferred to a plastic grid placed over a 6-liter container filled with distilled water. The distilled water was replaced by Yoshida's nutrient solution after five days. The nutrient solution was adjusted to pH 5.0 with 1N KOH or 1N HCl and renewed every week. When the seedlings were 21 days old, they were subjected to stress treatments for nine days, during which nutritive solution was supplemented with 0, 60, and 120 mM NaCl (EC 0, 6, 12 dS/m) or PEG-6000 at 0, 10%, and 20% (w/v). The leaves and roots were harvested, frozen in liquid nitrogen and stored at -80°C for further use. Six replicate experiments were performed to obtain six independent samples of rice seedlings for the determination of the fresh weight, dry weight, relative water content, and proline content.

Measurement of fresh and dry weight of leaves and roots

Nine days after salinization, plants were randomly sampled and separated into leaves and roots. The fresh weight of each sample was taken; and the samples were subsequently oven-dried at 60°C for 72 h for the determination of dry weight.

Measurement of relative water content

The relative water contents (RWC) of the stressed leaves and roots were measured using samples of about 0.1 g which were cut into small pieces, and the fresh weights (FW) were recorded. The samples were subsequently floated in deionized water and left for four h in the refrigerator to allow the tissue to take up water, then re-weighted (turgid tissue weight, TW). Samples were then dried for 72 h at 60 °C after floating, and dry weights (DW) were measured. RWC was calculated using equation: RWC (%) = (FW-DW) / (TW-DW) x 100.

Measurement of Proline content

Proline content was analyzed according to the procedure of Bates et al. (1973). Approximately 0.1 g of fresh weight of leaves and roots were homogenized with 5 ml of 3% aqueous sulfo-salicylic acid, and the homogenate was filtered through Whatman No.2 filter paper. Two ml of filtrate was reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube, which was covered with a plastic cap and placed in a water bath maintained at 100°C for 1 h, then placed on ice. The reaction mixture was extracted with 5 ml of toluene, mixed vigorously with a vortex mixer for 15 sec. The toluene phase containing the chromophores was aspirated, warmed to room temperature, and the absorbance was measured at 520 nm, using toluene as a blank. Pure proline was employed to standardize the procedure for quantifying sample values. The proline content was determined from a standard curve and the concentration was expressed as μg/g FW.

Statistical analysis

Analysis of variance (ANOVA) was performed on all measurements. Significant differences between means were determined using the Duncan multiple range test (DMRT) at the $P \leq 0.05$ (n=6) level.

Results

Growth characteristics and free proline accumulation

The effects of salinity and water stress on leaf and root in seedlings of rice cultivars were compared. Fig. 1 shows the effect of NaCl on the water and proline content of rice seedlings, after nine days. Addition of NaCl to the growth medium significantly inhibited the growth of leaves and roots as judged by fresh weight and dry weight. Increasing concentrations of NaCl from 0 to 120 mM progressively decreased leaf growth, but only slightly decreased root growth. Similar findings were reported when seedling growth was measured in terms of root and leaf length, fresh weight and dry weight (Prajuabmon, 2009). These results are in general agreement with those reported by Demiral and Turkan (2006).

The effects of salt on proline levels in roots and leaves of rice seedlings were also investigated. Increasing concentrations of NaCl from 0 to 120 mM progressively increased proline levels in leaves. However, proline accumulation in roots was observed only when seedlings were treated with a high concentration (120 mM) of NaCl. Clearly, the reduction of leaf growth, but not of root growth, is associated with the accumulation of proline in leaves.

Inclusion of polyethylene glycol (PEG) in the growth medium inhibited leaf growth, but proline accumulation was observed only at a concentration iso-osmotic with 120 mM NaCl. Fig. 2 shows the effect of PEG on the growth and proline content after nine days. PEG treatment inhibited leaf growth but did not result in significantly increased accumulation of the proline. All these results suggest that proline accumulation in salt stress in roots and leaves is associated with the ionic strength rather than the osmotic component of salt stress.

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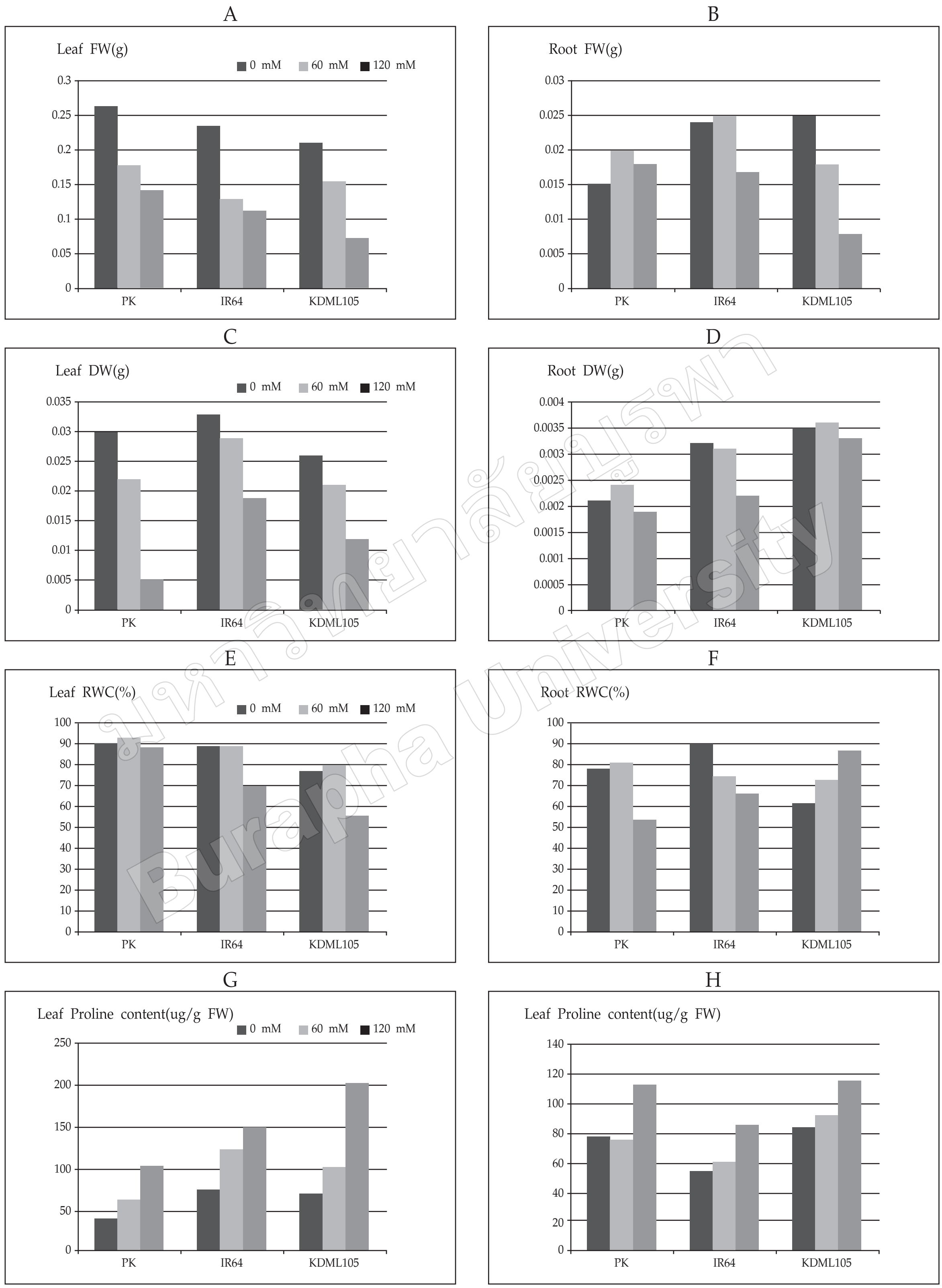

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Figure 1. The fresh weight (FW), dry weight (DW), relative water content (RWC), and proline content of leaves (A, C, E, G) and roots (B, D, F, H) of seedlings of three rice cultivars under NaCl stress conditions for nine days. Mean±SEM, n=6.

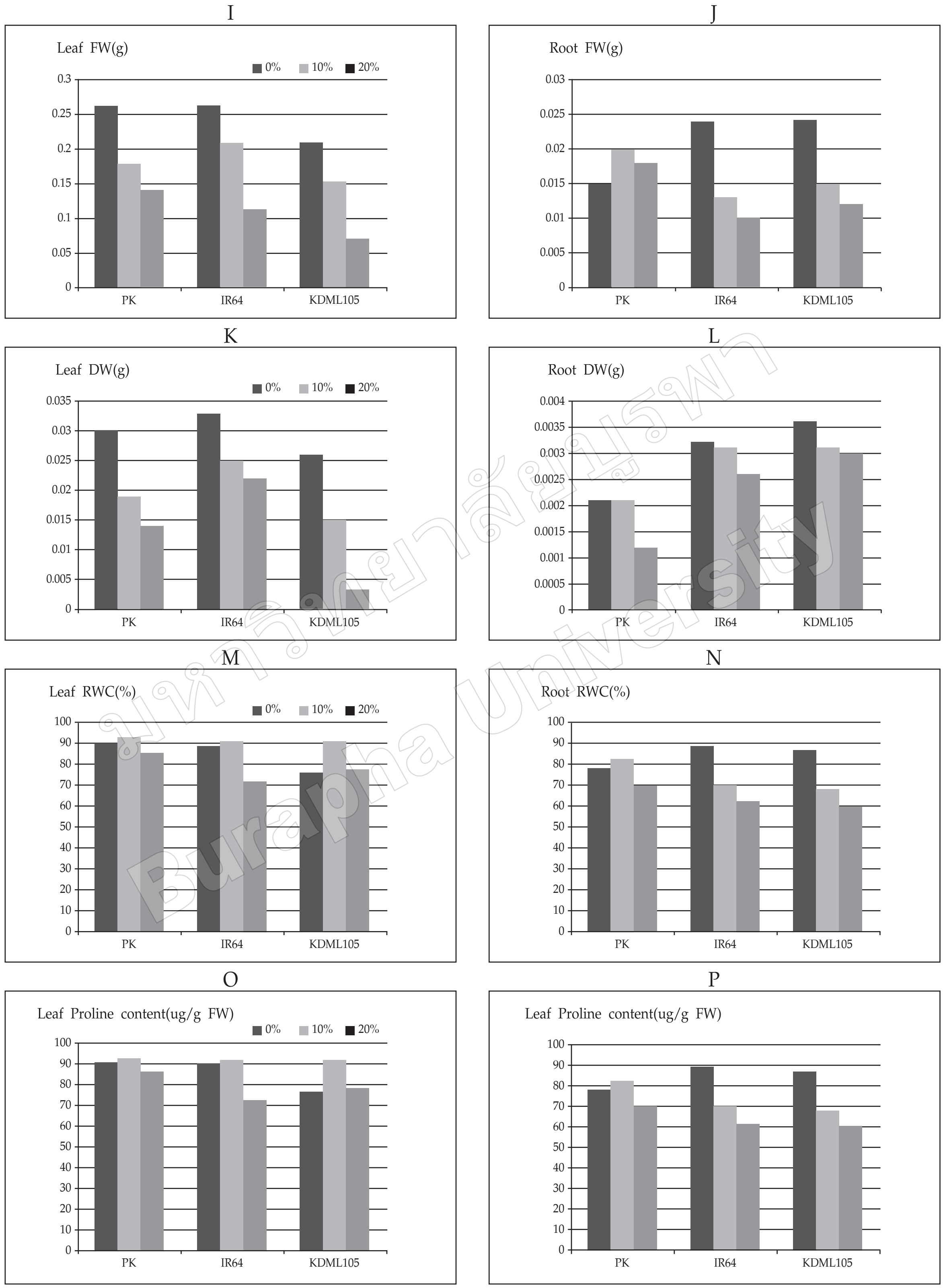

Figure 2. The content of fresh weight (FW), dry weight (DW), relative water content (RWC), and proline content of leaves (I, K, M, O) and roots (J, L, N, P) of seedlings of three rice cultivars, under the stress conditions with PEG-6000 for nine days. Mean+SEM, n=6.

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DISCUSSION

It appears that proline accumulation is important in regulating growth inhibition of leaves but not roots of rice seedlings under salt stress. In a previous report by Chuan and Ching (1996), proline accumulation was found to enhance NaCl-induced inhibition of root growth, but reversed the inhibitory effect of NaCl on leaf growth of rice seedlings. It seems that proline may play different roles in regulating leaf and root growth of rice seedlings under salt and water stresses.

An increasing in NaCl and PEG concentrations in the growth medium caused markedly decreased in the growth rates and the proline content of rice seedlings. It was shown that Pokkali cultivar was least affected than those of the IR64 and KDML105 cultivars at all NaCl and PEG concentrations, when compared with the control groups. Rice leaves accumulated larger amounts of proline with increasing NaCl or PEG concentration. Accumulation of proline was lowest with Pokkali and greatest with KDML105. This work suggests that the proline accumulation induced by osmotic stress treatment is related to the degree of salt- and water- tolerance shown in the differing responses of the three rice cultivars, and that measurement of its short-term accumulation may be a good indicator of rice osmotic stress tolerance (Hien et al., 2003). Salinity and water stress markedly increased the proline content of different salt-sensitive and salt-tolerant genotypes of maize (Mansour, 2005), *Arabidopsis thaliana* (Mohamed et al., 2008) and rice cultivars (Hsu et al, 2003), with more proline accumulation observed in salt-tolerant strains in contrast to the results reported here with rice. Our results imply that NaCl stress strongly increases proline accumulation in the leaves of the three rice cultivars, particularly in KDML 105, unlike the proline accumulation in the roots.

Both salt and water stresses affect plants through osmotic effects, ion specific effects and oxidative stress. Osmotic effects are due to salt-induced decrease in the soil water potential. Increased salinity results in a reduced K^+ and Ca^{2+} content and an increased level of Na^+ and Cl^-. Osmotic adjustment of rice is achieved through the accumulation of organic and inorganic solutes such as proline (Yeo, 1998). Therefore, a greater decrease potential in cell solute than in the external salt concentration may indicate an osmotic adjustment. Organic solutes are accumulated in the cytosol to balance the solute potential of vacuole, which is dominated by ions (Greenway and Munns, 1980). A large number of plant species accumulates proline in response to salinity stress, and this accumulation may play a role in combating salinity stress (Mansour, 2000; Ashraf and Harris, 2004). Although published data do not always indicate a positive correlation between the osmolyte accumulation and adaptation to stress, our results do suggest a correlation between the level of osmotic adjustment and the salt tolerance of rice cultivars.

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