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Effects of Sodium Fluoride on the Water Transport in Leaves of Barley and Rice under Salt Stress in the Light

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The kinetics of the loss of leaf fresh weight during incubation of barley and rice leaves in 9% or 15% NaCl solutions were biphasic, indicating the existence of a controlling mechanism for water transport. The first rapid phases reached their plateaus within 1 and 2 h in the case of rice and barley leaves, respectively. When barley leaves were fed with sodium fluoride, an inhibitor of phosphatase inhibitor, through their epicotyls for 3 h in darkness, prior to the treatment of NaCl, the biphasic pattern shown during NaCl treatment was disappeared resulting in linear decreases in the relative fresh weights. The results suggest that NaF accelerates salt-induced water efflux from plant cells, possibly by inhibiting the protection mechanism that may act in NaF-untreated leaves. The linear water loss can be explained in terms of phosphorylation of aquaporin by blocking its dephosphorylation in the presence of the phosphatase inhibitor to keep aquaporin in a phosphorylated form. However, the effect of NaF shown in barley leaves were not observed in rice. These results suggest that the regulation of water transport depends on plant species, and the mechanism for the controlling water transport in rice is different from that of barley.

key words: aquaporin, barley, NaF, phosphatase inhibitor, rice

INTRODUCTION

Water is the universal solvent and the most abundant molecule in all of the living tissues. Water is absorbed by roots and evaporates through stomatal pores in the leaves via transpiration stream. Water is indispensable for maintaining metabolism and structure of plants, and therefore plants require mechanisms for fine-tuning of the water balance. Radial water transport in roots is thought to occur along three parallel pathways, an apoplasmic, a symplasmic (via plasmodesmata), and a transcellular (vacuole to vacuole) path [1].

The water transport is supposed to be through a preferential route across cell membranes. Integral membrane proteins serving as specific water channels, referred to as aquaporins [2], have been reported to exist in plants at both plasma membrane [3] and tonoplast [4]. Aquaporins, which were first identified in animal cells [5], are water channels that facilitate passive movement of water across the membranes along the gradient in water potential [6, 7]. They are abundant in the vacuolar and plasma membranes in plant cells including mesophyll cells of the higher plants and the abundance of aquaporins change in response to the changes in the environmental conditions [7, 8].

Although high water permeability through membranes of elongating cells could be important for plant development, it carries the risk of excessive water loss on the other hand. Besides regulation at the transcriptional level, a possible mechanism for the control of aquaporin permeability might involve either blockage of aquaporins or the shift from an active state to an inactive one that is less-permeable. Such a post-translational molecular transition is considered to be mediated through its phosphorylation and dephosphorylation mechanisms, which is consistent with the existence of multiple and consensus phosphorylation sites in many aquaporins [9, 10]. The α-TIP from kidney bean and PM28 located in the plasma membrane of spinach are examples of aquaporins to be regulated by phosphorylation. They were responsible for the enhanced water permeability observed when expressed in oocytes in the presence of cAMP and forskolin, an activator of adenylate cyclase, in addition to a phosphatase inhibitor [11, 12].

In this study, we investigated the water transport kinetics of leaves under salt stress. To further examine the possible involvement of phosphorylation/dephosphorylation mechanisms in the process of water transport, we also investigated the effect of NaF, a phosphatase inhibitor. Interestingly, we found that the water transport possibly by aquaporin was influenced significantly by the treatment of NaF in the case of barley leaves, but not in the case of rice leaves.

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MATERIALS AND METHODS

Plant Material and growth conditions

Barley (*Hordeum vulagare* L. cv. Albori) and rice (*Oryza sativa* L. cv. Dongjin-byeo) seeds were sterilized in 1% sodium hypochloride solution for 30 min, and washed thoroughly in tap water for more than 10 times. To induce germination, the seeds were incubated in tap water at room temperature in the dark for 2 days. The germinated seedlings were transferred into pots containing fertilizer, vermiculite and peat moss with ratio of 1:1:0.5 and were grown in a growth chamber. The growth condition was kept at 14-h light period with photon flux density (PFD) of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at a day/night temperature regime of 28/23°C. For all experiments, expanded leaves of 2-week-old plants were used.

Treatment of chemicals and salt stress

Prior to the start of salt stress, the seedlings of 2-week-old plants were cut under water at the base of their epicotyls and then quickly immersed into either distilled water or a solution containing 25 mM NaF, as a phosphatase inhibitor. The leaves were then incubated at 25°C for 3 h in the dark. After the pretreatment step, leaves were subjected to salt stress by immersing them into 0%, 9% or 15% NaCl solutions, and further incubated at room temperature for 6 h with a PFD of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The changes in leaf fresh weights were measured after blotting water out from leaf surfaces.

RESULTS AND DISCUSSION

When barley leaves were exposed to salt stress by incubation of the leaves in NaCl solutions of either 9 or 15%, we could observe a time-dependent decrease in the leaf weights as shown in (Fig. 1A). The kinetics of the loss of leaf fresh weight seemed to be biphasic: a rapid decline in the leaf weight occurred within 2 h-incubation in NaCl solutions, which was followed by a slow phase during the incubation period for 6 h. The decline in the leaf weight during the first rapid phase was regarded as a result from water efflux from the leaf tissue. In other words, water within leaf cells was forced to move out to the apoplast for the osmotic adjustment of the leaf tissue against the external salt solution. After incubation for 2 h in the salt solutions, the decrease in the leaf weight was remarkably slowed down, indicating that the flow of water due to the osmotic pressure began to be counter-balanced with a certain protection mechanism against over dehydration that may lead to cell death.

In contrast, when the leaves pretreated with NaF were incubated in the NaCl solutions, we could not observe the biphasic decrease in the reduction of leaf weights. Instead, we found linear losses of leaf weights in the salt solutions (Fig. 1B). After incubation for 12 h in the salt solutions, the weights of the NaF-treated leaves reduced to 58% of their original levels, in contrast to the control leaves that showed

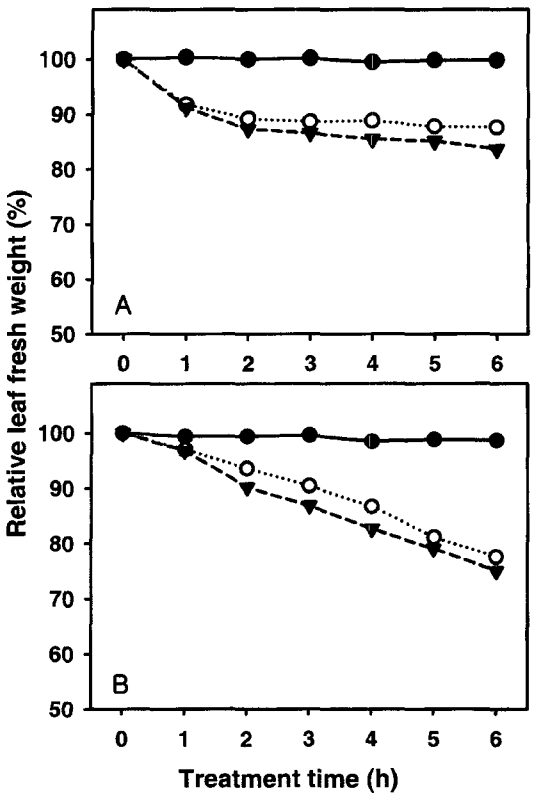

Figure 1. Time-dependent changes in the relative fresh weight of barley leaves during incubation in NaCl solutions and the effect of NaF. Leaves were incubated in 0% (●), 9% (○) or 15% (▼) NaCl solution after the pretreatment in a solution either without (A) or with 25 mM NaF (B) in the dark for 3h. The salt was treated in the light 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 25°C.

little changes in the leaf weight (data not shown). The results suggest that NaF, a phosphatase inhibitor, accelerates salt-induced water efflux from plant cells, possibly by inhibiting the protection mechanism that may act in NaF-untreated leaves.

A few aquaporins have been reported to be phosphorylated in vivo. Johansson *et al.* [12] suggested that water flow through the plasma membrane was regulated by phosphorylation of water channel PM28A. The model suggests that when the water potential in the apoplast is high, aquaporin is phosphorylated, leading to channel opening so that water flow across plasma membrane is essentially unrestricted. On the contrary, when leaves experience water deficiency, aquaporin is dephosphorylated, and water flow through the aquaporin becomes restricted. Moreover, water permeability increased in the presence of cAMP, the adenylate cyclase activator forskolin, and a phosphatase inhibitor [6, 12]. On the basis of these perspectives, the accelerated water transport by NaF we observed in salt-stressed barley leaves can be explained in terms of phos-phorylation of aquaporin by blocking its dephosphorylation in the presence of a phosphatase inhibitor, NaF, to keep aquaporin in a phosphorylated form. Therefore,

NaF-induced weight loss in salt-stressed barley leaves might be attributed to the enhanced water permeability of aquaporin in the presence of NaF.

When rice leaves were exposed to salt stress by incubation of the leaves in NaCl solutions of either 9 or 15%, we could observe a time-dependent decrease in the leaf weights as shown in (Fig. 2A).

When the control leaves of rice were incubated in NaCl solution of 9 and 15% NaCl, a biphasic change in the leaf weight could be also observed (Fig. 2A). The first rapid phase was rather fast, resulted in reaching its plateau after 1 h followed by a second slow phase for 6 h. However, the biphasic pattern was not disappeared in NaCl solutions in the rice leaves pre-treated with NaF (Fig. 2B). Instead, the first rapid phase became slower when compared with NaF-untreated leaves. The results suggest that water transport in rice leaves was rather insensitive to NaF treatment, and this observation was quite contrasting to the one we observed in barley leaves. Aquaporins are reported to be normally inactive or not expressed in rice plants that grow in the environment with a plentiful water supply [13]. In rice plants, we may speculate that aquaporins might be in dephosphorylated states or might even not exist. Therefore, these results suggest that the regulation of water transport depends on plant species, and the role and controlling mechanism of aquaporins or even the existence of aquaporins in rice need to be examined carefully.

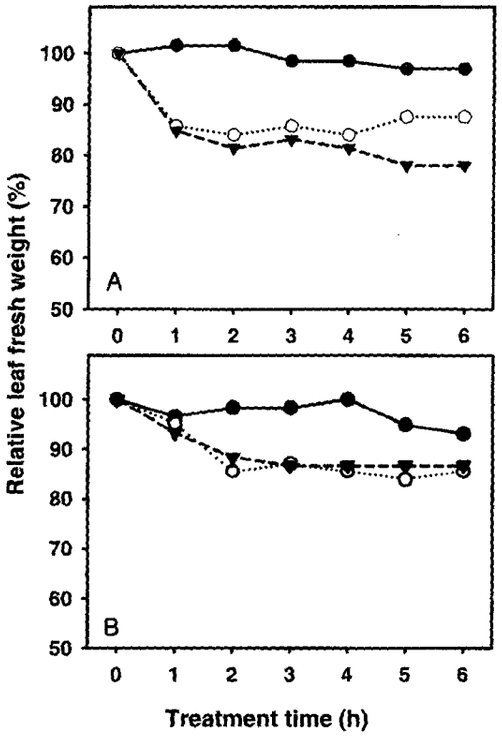

Figure 2. Time-dependent changes in the relative fresh weight of rice leaves during incubation in NaCl solutions and the effect of NaF. Leaves were incubated in solutions with varying concentrations of 0% (●), 9% (○) or 15% (▼) NaCl after the pretreatment in a solution either without (A) or with 25 mM NaF (B) in the dark for 3 h. The salt was treated in the light 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 25°C.

In summary, the kinetics of the loss of leaf fresh weight during incubation of barley and rice leaves in 9% or 15% NaCl solutions were biphasic with a rapid decline in the leaf weight saturating within 1 or 2 h, suggesting the existence of a controlling mechanism for water transport. The biphasic behavior in barley leaves was disappeared in the presence of NaF, and this result can be explained well by the involvement of aquaporin in the water transport we observed in leaves in high salt solution, and by the model of the phosphorylation of aquaporin to keep the enhanced water permeability of aquaporin in the presence of NaF. However, interestingly this effect of NaF was not observed in rice. These results suggest that the regulation of water transport depends on plant species, and the detailed mechanisms for the controlling water transport in rice require further investigation.

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