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NaCl stress in rice seedlings: starch mobilization and the influence of gibberellic acid on seedling growth

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Abstract. The growth of shoots and roots of rice (*Oryza sativa* L., cv. Taichung Native 1) seedlings was significantly inhibited when the seeds were subjected to NaCl stress. NaCl markedly decreased the mobilization of starch in endosperm. Results also showed that α-amylase activities in endosperm were reduced when rice seeds were germinated in the presence of NaCl. NaCl-inhibition of α-amylase activities was counteracted by gibberellic acid (GA_3). GA_3 reduced NaCl-inhibition of shoot growth, but not of root growth. Sugars (sucrose, fructose, and glucose) were able to reduce NaCl-induced growth inhibition of shoots and roots. The possible mechanism by which shoot growth and root growth in NaCl media respond differently to GA_3 is discussed.

Keywords: α-Amylase; Gibberellic acid; NaCl; *Oryza sativa* L.; Starch mobilization.

Abbreviation: GA_3, gibberellic acid.

Introduction

Mobilization of seed reserves, which occurs during early seed germination, is crucial because it supplies substrates for the proper functioning of different metabolic processes that are essential to growth of the embryonic axis (Mayer and Poljakoff-Mayber, 1975). Rice is a salt-sensitive crop species (Flowers and Yeo, 1981). The mechanism of NaCl inhibition of rice-seedling growth is unclear, but NaCl may inhibit mobilization of seed reserves (Prakash and Prathapasenan, 1988).

Gibberellic acid (GA_3) is known to induce the synthesis of α-amylase in embryo-less rice seeds (Palmiano and Juliano, 1972). It is not known whether GA_3 can reduce NaCl inhibition of rice-seedling growth, but GA_3 has been reported to promote the growth of cotton and some halophytes in saline condition (Agakishiev, 1964; Boucaud and Ungar, 1976a; 1976b; Zhao et al., 1986). Huber et al. (1974) also reported that GA_3 counteracted the influence of NaCl on the carbohydrate metabolism in leaves of *Pennisetum typhoides*. The present investigation was conducted to examine whether NaCl-inhibited growth of rice seedlings is mediated through diminishing mobilization of starch in endosperm, and to determine the influence of GA_3 on NaCl-inhibited rice-seedling growth.

Materials and Methods

Rice (*Oryza sativa* L. cv. Taichung Native 1) seeds were sterilized with 2.5% sodium hypochlorite for 15 min and washed thoroughly with distilled water. These seeds were then germinated for 1 day in petri dishes (20 cm) containing distilled water at 37°C in the dark. Uniformly germinated seeds were selected and transferred to petri dishes (9 cm) containing two sheets of Whatman No. 1 filter paper moistened with 10 ml of distilled water or test solution. Each petri dish contained 20 germinated seeds. Each treatment was performed 4 times. The germinated seeds were allowed to grow at 27°C in darkness, and 3 ml of distilled water or test solution was added to each petri dish on day 3.

Sugars and starch were extracted from the endosperm twice with hot ethanol (80%). The extract was evaporated to dryness and the residue was dissolved in 2 ml of distilled water. A portion of this extract was used for the estimation of total soluble sugars and reducing sugars using the methods of Yoshida et al. (1972) and Lindsay (1973), respectively. Total soluble sugars and reducing sugars are expressed as μg glucose equivalents per endosperm. The tissue residues were suspended in 2 ml of 20 mM sodium phosphate (pH 6.9) and 6 mM NaCl, and boiled for 15 min to gelatinize the starch. Crude boiled homogenates were then used to determine starch according to the method described previously (Hurng and Kao, 1993). Starch level is expressed as mg maltose equivalents per endosperm.

To extract α-amylase, endosperm was homogenized in a chilled (4°C) mortar and pestle with 0.2 M sodium acetate (pH 5.4) containing 3 mM $CaCl_2$. Crude extract was used to determine α-amylase activities by the method developed by Rinderknecht et al. (1967), which uses starch azure as substrate. The change in A_{595} was used to calculate the α-amylase activity. One unit of enzyme activity is defined as an increase of 1 A_{595} min^{-1}.

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For all measurements, each treatment was performed four times, and all experiments described here were performed three times. Similar results and identical trends were obtained each time. The data reported here are from a single experiment.

Results

The influence of NaCl concentrations on the levels of starch, total soluble sugars, and reducing sugars in endosperm of germinating seeds is shown in Figure 1. Endosperm of germinating rice seeds treated with NaCl contained higher levels of starch than that treated with distilled water—the higher the NaCl concentration, the higher the level of starch in endosperm. Figure 1 also shows that the levels of total soluble and reducing sugars were lower in endosperm of seeds treated with NaCl than in that of the non-stressed control. The starch level in endosperm decreased and the levels of total soluble and reducing sugars increased as germination progressed. This process was greatly inhibited by NaCl (Figure 2). In the endosperm of both non-stressed and NaCl-stressed seeds, starch level decreased with a parallel increase in the levels of total soluble and reducing sugars (Figure 2). The fresh weight

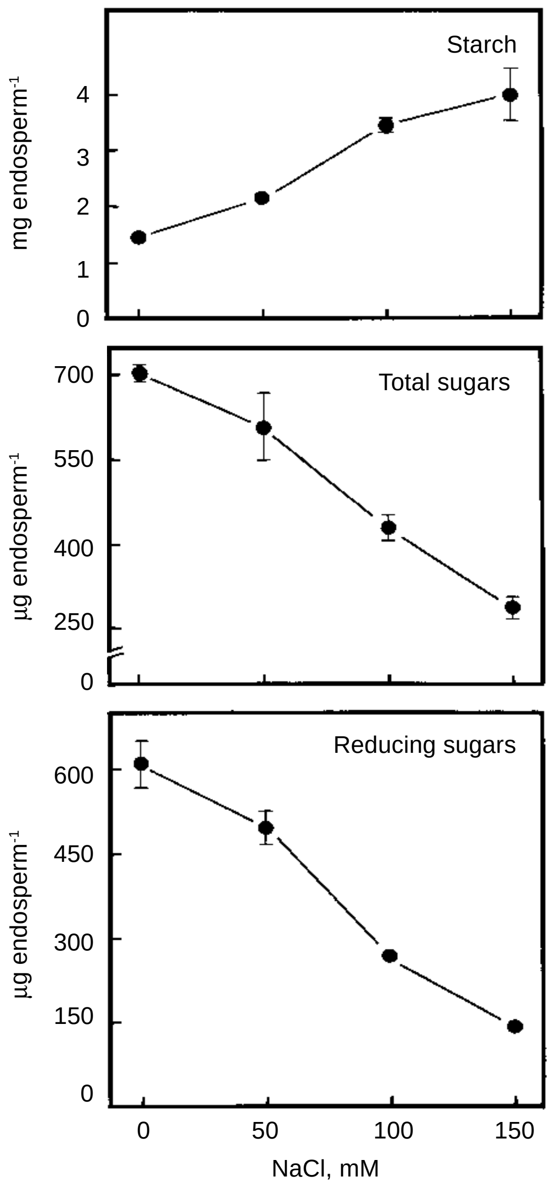

Figure 1. Influence of NaCl on the levels of starch, total soluble sugars, and reducing sugars in endosperm of germinating rice seeds. Starch, total soluble sugars, and reducing sugars were determined after 5 days of treatment. Vertical bars represent standard errors. Only those standard errors larger than the symbol size are shown.

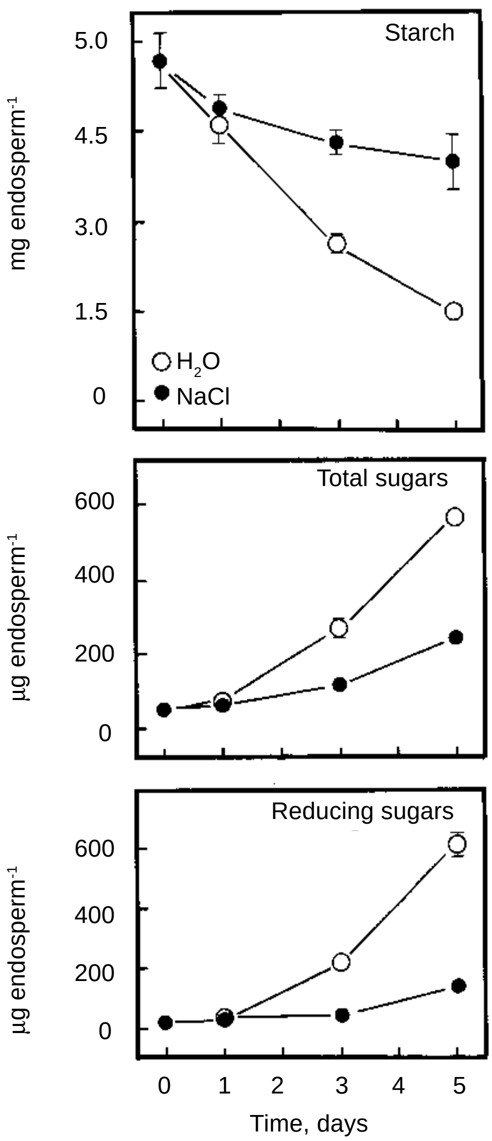

Figure 2. Changes in the levels of starch, total soluble sugars, and reducing sugars in endosperm of germinating rice seeds treated with 150-mM NaCl. Vertical bars represent standard errors. Only those standard errors larger than the symbol size are shown.

per endosperm (23 mg) treated with NaCl (150 mM) for 5 days was found to be higher than that of the control (18 mg). Our results were similar to those reported by Prakash and Prathapasenan (1988).

Comparative studies of α-amylase activity in endosperm revealed a continuous increase in enzyme activity with duration of germination in both the control and NaCl treatments (Figure 3). NaCl inhibited α-amylase activity in endosperm in a concentration-dependent manner (Figure 3). Dubey (1982), however, demonstrated that 150-mM NaCl increased the activities of α-amylase in rice endosperm.

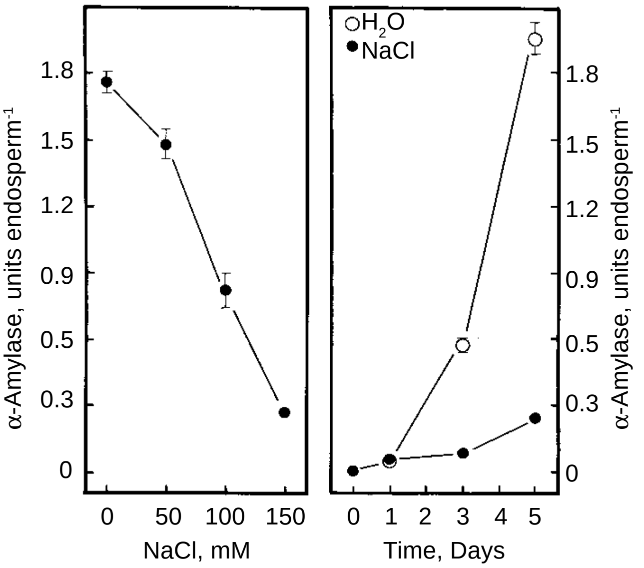

Figure 3. Influence of NaCl on the activity of α-amylase in endosperm of germinating rice seeds. For the dose-response study, α-amylase in the endosperm was assayed after 5 days of treatment. The concentration of NaCl used in the time-course study was 150 mM. Vertical bars represent standard errors. Only those standard errors larger than the symbol size are shown.

The growth of shoots and roots was tracked by measuring their fresh weight. Figure 4 shows the influence of NaCl on the growth of seedlings with and without GA_3 treatment. In the absence of GA_3, NaCl significantly suppressed the growth of shoots and roots. The growth of shoots and roots was reduced to 15 and 30% of the control values (without NaCl), respectively, by 150 mM NaCl. These results are generally consistent with those reported by Flowers and Yeo (1981). NaCl-induced inhibition of shoot growth was significantly reduced by GA_3, but GA_3 did nothing to counteract NaCl inhibition of root growth.

Figure 5 shows the influence of GA_3 on the activities of α-amylase in endosperm of seedlings treated with various concentrations of NaCl. In the absence of GA_3, NaCl inhibited α-amylase activities in endosperm of seedlings. GA_3 effectively reduced NaCl-induced inhibition of α-amylase activities in endosperm.

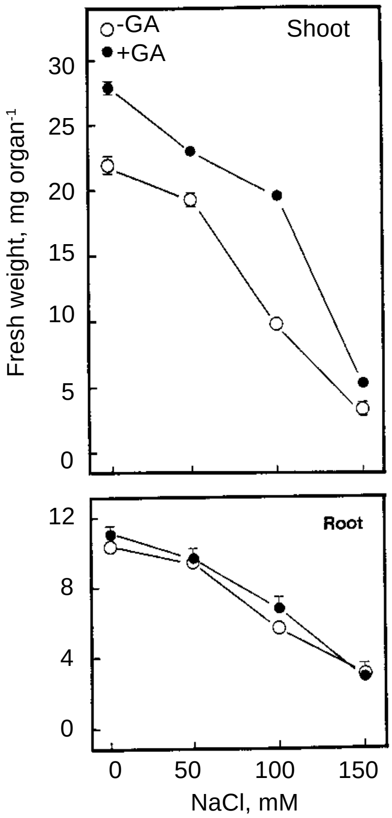

Figure 4. Counteraction by GA_3 of the NaCl-induced inhibition of seedling growth. The concentration of GA_3 was 0.1 μM. Seedling growth was measured after 5 days of treatment. Vertical bars represent standard errors. Only those standard errors larger than the symbol size are shown.

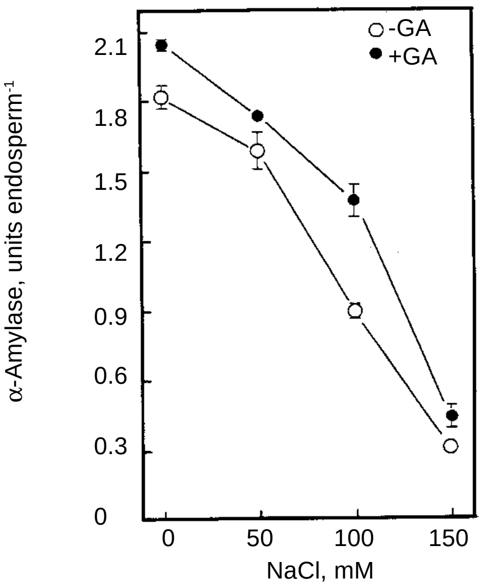

Figure 5. Counteraction by GA_3 of the NaCl-induced inhibition of α-amylase activities (units/endosperm) in endosperm. The concentration of GA_3 was 0.1 μM. α-Amylase was assayed after 5 days of treatment. Vertical bars represent standard errors. Only those standard errors larger than the symbol size are shown.

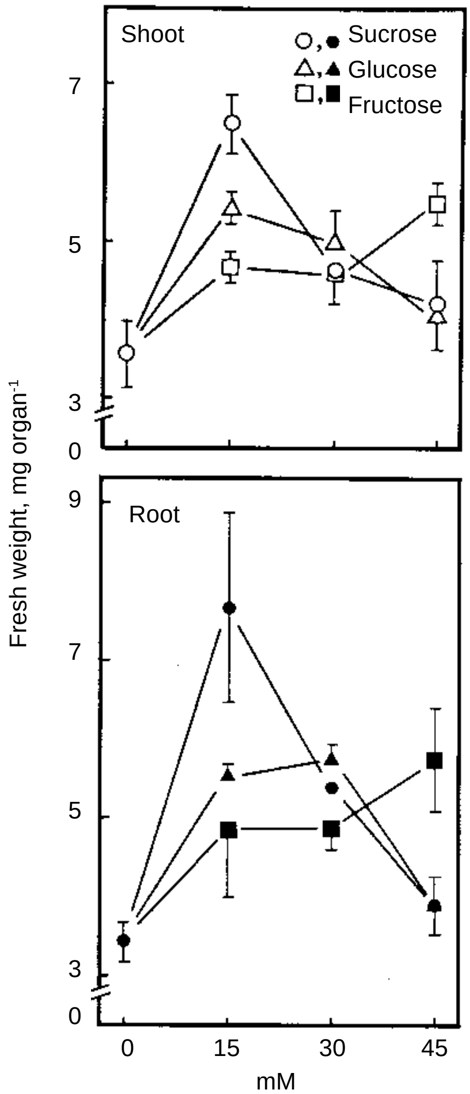

Figure 6. Counteraction by sugars of the NaCl-induced inhibition of seedling growth. The concentration of NaCl was 150 mM. For all treatments, 0.25 mg/l chloramphenicol was added to prevent bacterial growth. Seedling growth was measured 5 days after treatment. Vertical bars represent standard errors. Only those standard errors larger than the symbol size are shown.

Experiments were conducted to determine whether sugars can counter the growth inhibition induced by NaCl. Shoot growth and root growth in NaCl medium were both found to be enhanced by the addition of sucrose, fructose, or glucose (Figure 6).

Discussion

Our results are generally consistent with the hypothesis that NaCl-induced inhibition of early seedling growth is mediated through mobilization of endosperm reserves (Prakash and Prathapasenan, 1988). Since NaCl-inhibited seedling growth was not completely reversed by exogenous sugars, other factors responsible for growth inhibition by NaCl can not be ruled out.

Sucrose is considered to be a sole transport sugar in the majority of plants (Giaquinta, 1980). It seems that the uptake of glucose and fructose by rice seedlings is less effective than that of sucrose. This may explain why glucose and fructose have little influence on the growth of shoots or roots.

GA_3 is an important factor in enhancing the α-amylase activities in germinating rice seeds (Palmiano and Juliano, 1972). It is conceivable that the mechanism by which NaCl-induced inhibition of α-amylase activities is counteracted by GA_3 is related to a deficiency of GA_3 in NaCl-stressed endosperm.

GA_3 has been reported to reduce NaCl-induced growth inhibition in some plant species (Agakishiev, 1964; Boucaud and Ungar, 1976a; 1976b; Zhao et al., 1986). We found, unexpectedly, that in rice seedlings, GA_3 counteracted NaCl-inhibited shoot growth, but not root growth. This is the first evidence that under NaCl stress, shoot growth and root growth respond differently to GA_3.

The results of our analysis of α-amylase activities show that the reduction of NaCl-inhibition of shoot growth by GA_3 results from an enhancement of hydrolysis of starch in endosperm. Because the addition of sugars improved the root growth of seedlings in NaCl medium, and because GA_3 did not reduce NaCl-inhibition of root growth, the translocation of sugars from endosperm into roots is most likely inhibited by NaCl. We cannot, however, exclude the possibility that in the presence of NaCl, rice-seedling shoots draw more sugars from the endosperm. A deeper knowledge of NaCl-inhibition of the export of starch-mobilization products from the endosperm to roots is necessary for a better understanding of the mechanism of NaCl-inhibition of root growth.

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水稻幼苗之氯化鈉逆境：胚乳澱粉分解及 gibberellic acid 處理與幼苗生長之關係

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氯化鈉處理顯著的抑制水稻 (台中在來 1 號) 幼苗地上部與根的生長，同時抑制幼苗胚乳內澱粉之分解與降低胚乳內澱粉水解酵素的活性。 Gibberellic acid (GA_3) 處理可克服氯化鈉所降低的澱粉水解酵素活性。 GA_3 處理可降低氯化鈉所抑制的水稻幼苗地上部生長，但對根的生長則無效。蔗糖、葡萄糖或果糖處理均可恢復氯化鈉所抑制的幼苗地上部與根之生長。氯化鈉處理下水稻幼苗地上部與根對 GA_3 反應不同的可能機制在文中加以討論。

關鍵詞：澱粉分解酵素； Gibberellic acid ；氯化鈉；水稻；澱粉分解。