

Influences of gibberellin and auxin on endogenous plant hormone and starch mobilization during rice seed germination under salt stress

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Abstract : The soaking effects of two plant hormones, gibberellic acid (GA₃) and indole-3-acetic acid (IAA) on dehulled rice seeds exposed to low NaCl stress were investigated. Total soluble and reducing sugars in endosperms of rice seeds soaked with GA₃ (10 μM) increased gradually as germination progressed under low NaCl stress. This increase was more pronounced in IAA-soaked rice seeds than in GA₃-soaked rice seeds. Similarity of α-amylase activity in IAA-soaked germinating rice seeds was much more stimulated than that soaked with GA₃. IAA- and GA₃-soaked rice seeds showed relatively high amounts of endogenous IAA under salt stress. In particular, IAA content increased more in GA₃-soaked rice seeds than in IAA-soaked rice seeds. Furthermore, gibberellin content in IAA-soaked rice seeds showed the highest concentrations among all the treatments. It suggests that recovery effect in starch mobilization and root elongation is in some part regulated by soaking treatment of plant hormones under lower salt stress in rice seed germination.

Key words : Plant hormone, Gibberellic acid, IAA, Salinity, *Oryza sativa* L., Starch.

Introduction

Crops are often exposed to environmental factors such as high and low soil salinity (Kaur *et al.*, 1998). Starch mobilization of seed reserves, which occurs during early seed germination, is crucial because it supplies substrates for the proper functioning of different metabolic process that are essential to growth of the embryonic axis (Mayer and Poljakoff-Mayer, 1975). It is well known that rice is a salt sensitive crop (Flowers and Yeo, 1981). The mechanism of NaCl inhibition of rice seedling growth is unclear, but NaCl inhibits mobilization of seed reserves (Prakash and Prathapasesnam, 1988). Plant hormones are considered as key regulators to seed germination and development (Davies, 1987). Gibberellic acid (GA₃) is known to induce the synthesis of α-amylase in embryoless rice seeds (Palmiano and Juliano, 1972). Gibberellic acid reduces NaCl inhibition of α-amylase activity under salt stress (Lin and Kao, 1995).

It is not known whether GA₃ can reduce NaCl inhibition of rice-seedling growth, but GA₃ 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 GA₃ counteracted the influence of NaCl on the carbohydrate metabolism in leaves of *Pennisetum typhoides*. Addition of exogenous gibberellic acid causes an increase in germination and seedling growth by enhancing the availability of endogenous gibberellic acid (Kaur *et al.*, 1998). In addition, indole acetic acid (IAA) reduces amylase activity in cotyledons of chickpea seedlings and also enhances sucrose synthase (SS) and sucrose phosphate synthase (SPS) activities under salt stress (Kaur *et al.*, 2003).

The present work was conducted to examine whether NaCl-inhibited growth in rice seed germination is mediated through diminishing mobilization of starch in endosperm, and to determine the influences of GA₃ and IAA on rice seed germination exposed to NaCl stress.

Materials and Methods

Plant material and growth conditions: Dehulled rice (*Oryza sativa* L. cv. Hwangyoungbyeon) seeds were sterilized with 2% sodium hypochlorite for 5 min and washed repeatedly with deionized water. Rice seeds were then soaked in conical tubes (50 ml) containing gibberellic acid (GA₃), indole-3-acetic acid (IAA), or distilled water as control, respectively for 12 h at 35°C in the darkness. Concentrations of applied plant hormones were 10 μM for GA₃ and 20 μM for IAA, respectively. From our preliminary experiment, it revealed that the specific concentration on each plant hormone responding to lower salt stress (NaCl 20 mM) showed relatively higher germination rate than that of salt exposed rice seeds. Germination rate of rice seeds soaked with gibberellic acid and IAA under lower salt stress was 85 and 74%, respectively. After finishing incubation, dehulled rice seeds soaked with GA₃ and IAA were rinsed with deionized water thoroughly. Rice seeds were transferred to petri dishes (15 cm in diameter) containing three sheets of Whatman No. 1 filter paper moistened with 10 ml of saline solution (20 mM NaCl). Fifty seeds of each treatment were placed on filter paper soaked in NaCl 20 mM and laden on 15 cm petri dish making four or five replicates. Fifty seeds were also soaked in distilled as control making four replicates. Rice seeds in petri dishes were placed in an incubator with continuous light condition with a mixture of cool-white fluorescent and halogen lamps provided a light

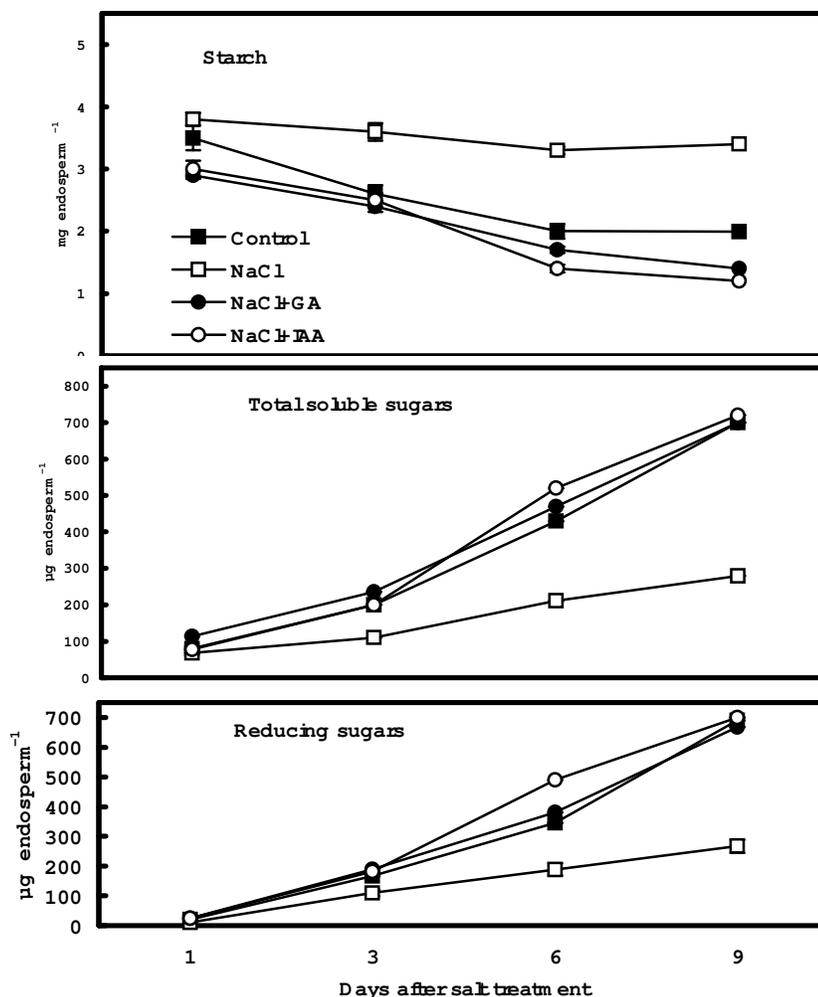


Fig. 1: Differences in the levels of starch, total soluble and reducing sugars in endosperm of germinating rice seeds soaked with GA₃ and IAA under salt stress (20 mM NaCl). Vertical bars represent the mean \pm SE(n=4). If not shown, error bars are smaller than the symbol size.

intensity of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the surface of petri dishes.

Extraction and analysis of starch and sugars: Starch and sugars were extracted from the endosperm of germinated rice seeds three times with boiling ethanol (80%). The extract was evaporated to dryness and the residue was dissolved in 2 ml of deionized water. A part of the extract was determined for total soluble sugars and reducing sugars described by the literature of Yoshida *et al.*, (1972) and Lindsay (1973), respectively. The tissue residues were suspended in 2 ml of 20 mM sodium phosphate (pH 6.9) and 6 mM NaCl, and then boiled for 20 min to gelatinize the starch. Total soluble and reducing sugars were expressed as glucose equivalents per endosperm. Crude boiled homogenates were used to determine the starch as described by the method (Hurng and Kao, 1993). Starch value was expressed as mg maltose equivalents per endosperm.

Measurement of α -amylase activity: Endosperms were homogenized and extracted in 0.2 M sodium acetate (pH 5.4) with 3 mM CaCl₂. After filtration, the extract was allowed to

determine α -amylase activity by the method developed by Riderknecht *et al.*, (1967). One unit in enzyme activity observed at OD of A₅₉₅ was defined as an increment of one A₅₉₅ min⁻¹.

Extraction and analysis of gibberellins and IAA: Endogenous contents for gibberellins (GA₁ and GA₄) and IAA were determined by the modification of those described by Bollmark *et al.*, (1988) and He (1993). Intact rice seeds were extracted in cold 10 ml 80% methanol. The extract was incubated at 4°C for 4 hr and centrifuged at 4,000 rpm for 15 min at 4°C. The supernatants were passed through Chromosep C₁₈ columns (C₁₈ Sep-Park Cartridge, Millford, MA), pre-washed with 10 ml of 100% and 5 ml of 80% methanol, respectively. The hormone fractions eluted with 10 ml of 100% methanol and 10 ml ether from the columns were dried under N₂, and then dissolved in 2 ml phosphate buffer saline (PBS) containing 0.1% Tween 20 and 0.1% gelatin (pH 7.5) for analysis by ELISA method.

Quantification of gibberellins and IAA by ELISA: ELISA (Enzyme Linked Immuno-Sorbent Assay) was performed on a 96-well microtitration plate. Each well on the plate was coated with 100 μ l coating buffer (pH 9.6, 1.5 g l⁻¹ Na₂CO₃, 2.93 g l⁻¹ NaHCO₃, and 0.02 g l⁻¹ NaN₃) containing 0.25 μ g ml⁻¹ antigens against the gibberellins and IAA. The coated plates were incubated for 4 hr at 37°C for gibberellins, and overnight at 4°C for IAA, and then kept at room temperature for 40 to 50 min. After washing with PBS and 0.1% Tween-20 buffer (pH 7.4) four times, each well was filled with 50 μ l of either the extracts or gibberellins and IAA standards (0 ~ 2,000 ng ml⁻¹ dilution range), and 50 μ l of 20 μ g ml⁻¹ antibodies against gibberellins and IAA, respectively. The plate was incubated for 3 hr at 28°C for gibberellins and overnight at 4°C for IAA. After rinsing, 100 μ l of 1.25 μ g ml⁻¹ IgG-horseradish peroxidase substrate was added to each well and incubated for 1 hr at 30°C. The plate was rinsed five times with Tween-20 buffer containing BBS, and 100 μ l color-appearing solution containing 1.5 mg ml⁻¹ *o*-phenylenediamine and 0.008% H₂O₂ was added to each well. The reaction progress was stopped by adding of 50 μ l 6 N H₂SO₄ per well when the 2,000 ng ml⁻¹ standard had a pale color, and the 0 ng ml⁻¹ standards had a deeply thick color in the wells. Color development in each well was detected using an ELISA Reader (Model EL310, Bio-TEK) at OD A₄₉₀. Contents of gibberellins and IAA were calculated by the method of Weiler and Ziegler (1981). The results are the means \pm SE of four replicates.

Results

Soaking effects of plant hormones on carbohydrates: The soaking effects of gibberellic acid (GA₃) and IAA on the levels of starch, total soluble sugars, and reducing sugars in

endosperm of germinating seeds is shown in Fig. 1. Endosperm of germinating rice seeds soaked with GA₃ and IAA showed lower starch levels than that of rice seeds in NaCl (20 mM) and control. Starch content in GA₃ and IAA-soaked rice seeds was lower than that of control and rice seeds grown in NaCl. The endosperm of rice seeds treated with GA₃ contained lower starch content in comparison with the endosperm of rice seeds treated with IAA at 1 and 3 days under salt stress, in contrast the endosperm of rice seeds soaked with IAA was also lower than that soaked with GA₃ at 6 and 9 days after salt treatment. Fig. 1 also shows the effect of plant hormone-soaking treatments in relation to changes of total soluble and reducing sugars. Total soluble sugars in endosperm of germinating rice seeds exposed to NaCl was slightly increased during germination periods. Otherwise, total soluble sugars in endosperm of rice seeds treated with GA₃ and IAA and control were more linearly enhanced as germination progressed. In particular, total soluble sugar content in IAA-soaked rice seeds were much more increased than that soaked with GA₃ and it always kept a high level compared to the GA₃-soaked rice seeds. In the change of reducing sugars under salt stress, reducing sugars in plant hormone-soaking treatments were similar to the results in that of total soluble sugars. Reducing sugars was markedly enhanced at 6 days after salt exposure.

Soaking effects of plant hormones on α -amylase activity and root growth: Fig. 2 shows changes of α -amylase activity as affected by the soaking treatment with GA₃ and IAA under salt stress.

NaCl stressed seeds showed a lowest α -amylase activity compared to the GA₃-soaked seeds and control. α -activity among these treatments during seed germination

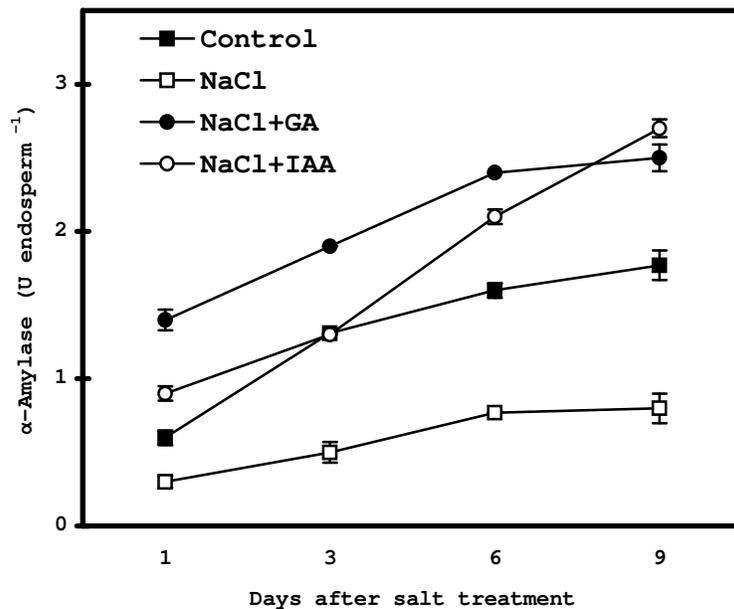


Fig. 2: Changes of α -amylase activity in endosperm of germinating rice seeds soaked with GA₃ and IAA under salt stress (20 mM NaCl). Vertical bars represent the mean \pm SE (n=4). If not shown, error bars are smaller than the symbol size. All vertical bars at only 9 days after treatment are significantly different to control at $p < 0.01$.

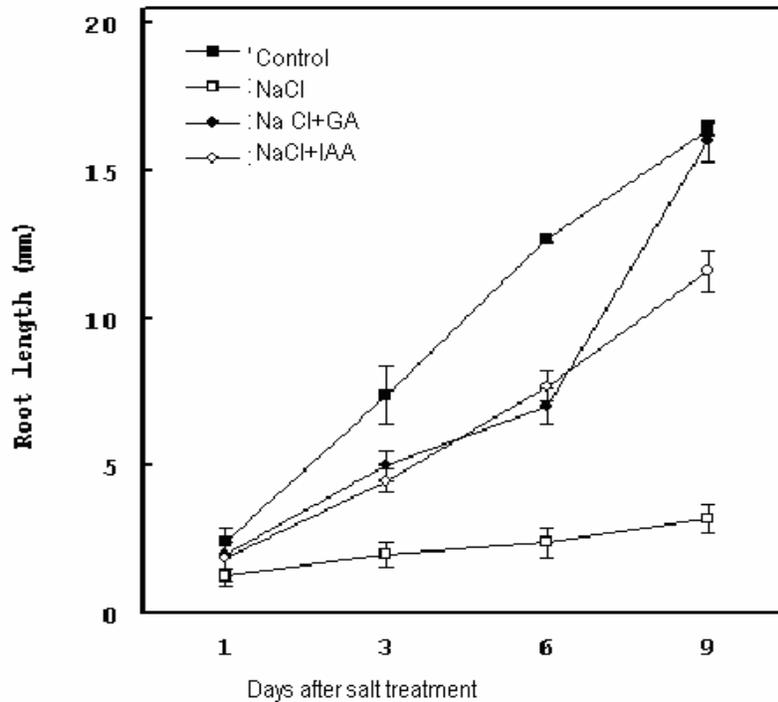


Fig. 3: Root growth in germinating rice seeds soaked with GA₃ and IAA under salt stress (20 mM NaCl). Vertical bars represent the mean±SE (n=4). If not shown, error bars are smaller than the symbol size. Each of vertical bars at only 9 days after treatment except for NaCl + GA are significantly different to control at $p < 0.01$.

amylase activity was sharply increased in endosperm of IAA-soaked seeds and also linearly enhanced in endosperm of GA₃-soaked seeds under salt stress. In addition, α -amylase activity in IAA- and GA₃-soaked rice seeds was higher than that of control and NaCl under salt stress.

Fig. 3 shows the changes of root growth in rice seeds exposed to NaCl and soaked with two plant growth hormones. NaCl induced dramatically a decreased root growth. Root length in GA₃ and IAA-soaked seeds was increased compared to the rice seeds exposed to NaCl. In comparison of two plant hormones, root length was more enhanced in GA₃-soaked seeds than in IAA-soaked seeds. Furthermore, root length in GA₃-soaked seeds was increased at 6 days thereafter.

Changes of endogenous IAA and GAs level under salt stress: Endogenous IAA contents were measured in the rice seeds soaked with GA₃ and IAA under salt stress (Fig. 4). Endogenous IAA content was dramatically increased by GA₃ and IAA-soaking treatment under salt stress. Endogenous IAA content in GA₃-soaked rice seeds was low at 1 day after salt treatment compared to the IAA-soaked rice seeds. However, endogenous IAA level in GA₃-soaked rice seeds were higher than that of IAA-soaked rice seeds at 3, 6 and 9 days after salt treatment during seed germination under salt stress. IAA soaking treatment did not induce an endogenous IAA elevation in IAA-soaked rice seeds.

Fig. 5 shows the active endogenous GA (as GA₁ and GA₄) content on plant hormone-soaking effect of GA₃ and IAA under salt stress. Endogenous GA content was slightly increased in rice seeds exposed to NaCl. GA₃-soaked rice seeds showed a lower endogenous GA content compared to the control as germination progressed. Endogenous GA content in the IAA-soaked rice seeds was quickly increased at 3 days after salt treatment. IAA-soaking treatment led to highest GA content among these treatments under salt stress.

Discussion

The present study reports the soaking effects of gibberellic acid and IAA on starch, root growth and endogenous hormonal change in rice seed germination exposed to salt stress.

It is generally recognized that rice is a salt-sensitive crop species (Flowers and Yeo, 1981) and may inhibit the mobilization of seed reserves (Prakash and Prathapasenam, 1988). Our results were partially consistent with the hypothesis that NaCl-induced inhibition of early seedling growth is mediated through mobilization of endosperm reserves (Prakash and Prathapasenam, 1988). Plant hormones are considered as key regulators to seed germination and development (Davies, 1987). Gibberellic acid (GA₃) is well known to induce the synthesis of α -amylase and hydrolysis of starch in rice seeds (Palmiano and Juliano, 1972). In our study, GA₃ induced the lower starch content and the endosperm of

germinating rice seeds treated with IAA was also promoted the starch degradation under lower salt stress. However, this was

inconsistent with the results of Kaur *et al.*, (1998). It can be

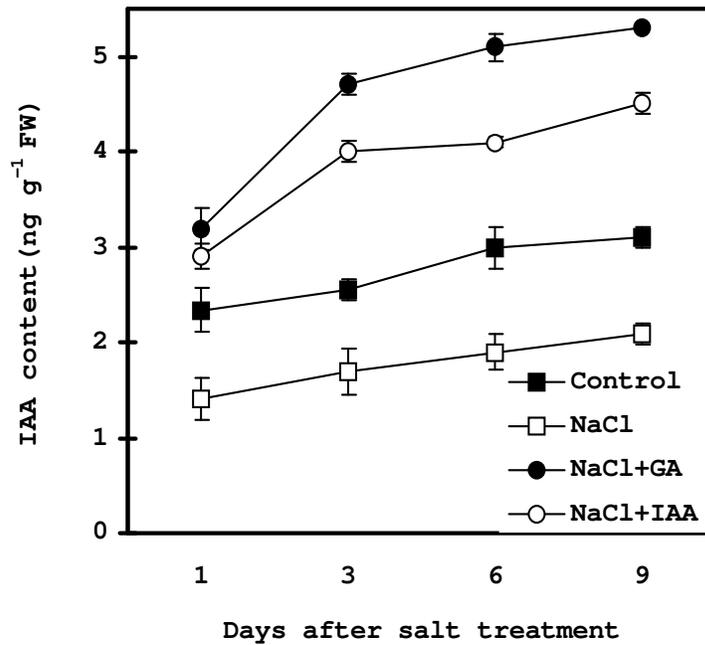


Fig. 4: Difference in endogenous IAA content in endosperm of germinating rice seeds soaked with GA₃ and IAA under salt stress (20 mM NaCl). Vertical bars represent the mean±SE(n=4). If not shown, error bars are smaller than the symbol size.

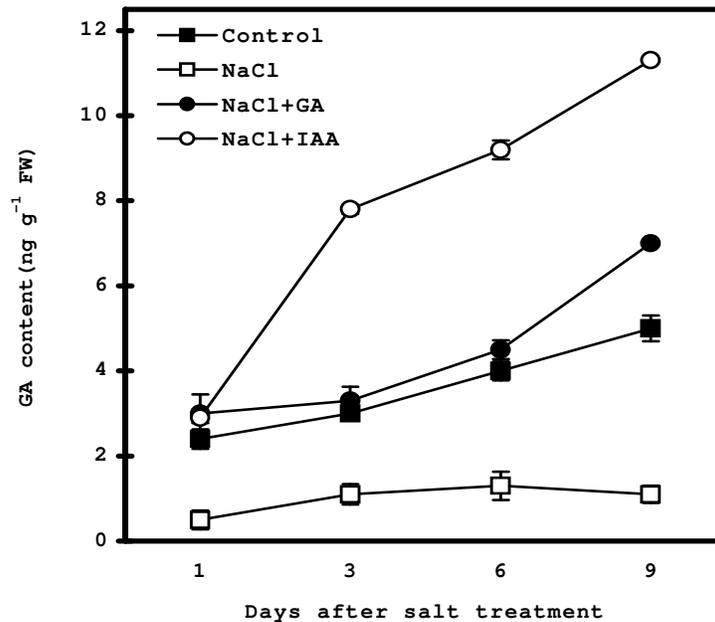


Fig. 5: Difference in endogenous gibberellin content in endosperm of germinating rice seeds soaked with GA₃ and IAA under salt stress (20 mM NaCl). Vertical bars represent the mean±SE (n=4). If not shown, error bars are smaller than the symbol size.

explained possibly that the differences in exogenous effects of GA₃ under salt stress are in fact under different saline conditions such as lower and higher NaCl concentration, growth stages and plant hormone concentration. Content of total soluble and reducing sugars was much more increased in

GA₃- and IAA-soaked rice seeds than in the NaCl-treated rice seeds with increasing α-amylase activity. Lin and Kao (1995) reported that an exogenous application of gibberellic acid in the germinating rice seeds promotes α-amylase activity under NaCl stress. Furthermore, α-amylase activity in IAA-soaked rice

seeds was much more enhanced than that soaked with GA₃. Damian and Ross (2002) reported that indole-3-acetic acid (IAA) promoted the biosynthesis of the active gibberellin (GA₁) in shoots of pea seedlings. Like this phenomenon, we have assumed that IAA will be involved in the biosynthesis of the active gibberellins (GA₁ and possibly GA₄) in rice plants led to increased α -amylase activity.

In the root growth exposed to salt stress, root length in IAA-soaked seeds was more reduced than that of GA₃-soaked seeds. Root length was oppositely decreased in IAA-soaked rice seeds, although it is considered that root elongation in IAA-stimulated increase with gibberellin biosynthesis should be enhanced under salt stress. It can be explained that IAA has positive effect on root growth under salt stress although the total soluble and reducing sugar contents induced by higher α -amylase activity was sufficient to promote the root growth under salt stress.

Endogenous IAA contents were measured from the germinated rice seeds exposed to lower salt stress. NaCl-stressed rice seeds showed lower concentration in IAA content, whereas IAA- and GA₃-soaked rice seeds have produced higher amounts under salt stress. In particular, IAA contents was more stimulated in GA₃-soaked rice seeds than in IAA-soaked rice seeds. In the change of endogenous gibberellin under salt stress, its content was slightly increased in the rice seeds exposed to NaCl stress. Exogenous IAA in rice seeds did not affect the increased endogenous IAA concentration under salt stress. In addition, gibberellin in IAA-soaked rice seeds had highest contents among all treatments.

Meanwhile, gibberellin content in GA₃-soaked rice seeds was more decreased than that of control. It means that an exogenous application of gibberellic acid to rice seeds did not induce the increase of endogenous gibberellin under salt stress. In respect to the hormonal balances in both gibberellin and IAA, these endogenous hormonal changes are always under control by salt stress. As a result, it suggests that reduction in starch mobilization and root length against NaCl stress will be recovered by application of plant hormone. However, in the recovery effect of plant hormones, it might be dependent on their concentrations, soaking time and the exposed salt concentrations.

It is considered that reverse increase in gibberellin and IAA in NaCl with GA and IAA treated seedlings may be specific event such as poor environmental condition including salt stress. In this point, we need a further study for better understanding of this phenomenon.

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