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BRIEF COMMUNICATION

Effect of jasmonic acid on endogenous gibberellins and abscisic acid in rice under NaCl stress

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Abstract

Content of endogenous abscisic acid (ABA) increased in rice plants under salt stress. Pre- or post-treatment by jasmonic acid (JA) mostly further increased ABA content. In the presence of salt stress also content of gibberellins (GAs) mostly increased more after treatment by JA. Endogenous content of bioactive GA_1 was higher in post-treatment by JA than in pre-treatment by JA.

Additional key words: *Oryza sativa*, pre- and post-treatment.

Plant hormones play crucial role in regulating plant responses to stress (Pandey *et al*. 2003/4). It is well known that abscisic acid and jasmonic acid generally increase, whereas indole-3-acetic acid and salicylic acid decline in response to salt stress (*e.g.* Wang *et al*. 2001). Jasmonic acid (JA) and its methyl ester (JA-Me) are endogenous growth regulators identified in many plant species, and induce a wide variety of physiological, morphological and developmental responses (Creelman *et al*. 1992, Kovač *et al*. 2003/4, Engelberth *et al*. 2001). Gibberellins (GAs) are essential phytohormones that control many aspects of plant development, including seed germination, leaf expansion, stem elongation, flowering, and seed development (Davies 1995). Pre-treatment by foliar spray of gibberellic acid (GA) was able to overcome to variable extents the adverse effects of stress imposed by NaCl (Chakrabarti and Mukherji 2003). GAs increased germination rate and seedling growth under salt stress (Kaur *et al*. 1998)

In the present study, we aimed to evaluate the influences of JA on contents of endogenous GAs and ABA in rice seedlings exposed to NaCl stress.

Rice (*Oryza sativa* L. cv. Donjinbyeo) seeds were germinated in Petri dishes containing distilled water for 6 d. Germinating seeds were transferred to plastic pots (25 × 20 cm) containing a modified Hoagland solution (Hoagland and Arnon 1950) supplemented with 1 mM KNO_3, 1 mM NH_4NO_3, 0.25 mM KH_2PO_4, 0.625 mM K_2SO_4, 0.5 mM $CaSO_4 \cdot$ 1/2 H_2O, 0.5 mM $MgSO_4 \cdot 7\ H_2O$, 9.5 μM Fe-EDTA and micro-nutrient elements. 21-d-old seedlings were grown under 12-h photoperiod (a mixture of cool-white fluorescent and halogen lamps provided an irradiance of 1000 μmol m^{-2} s^{-1}) and day/night temperature 28 ± 0.5/20 ± 0.5 °C. Jasmonic acid (*Sigma Chemicals*, St. Louis, USA; 30 μM) was applied at 48 or 24 h before NaCl exposure and 24 or 48 h after NaCl (40 mM) exposure, and finally jasmonic acid was applied with NaCl solution simultaneously. In order to determine contents of endogenous ABA and GAs, rice seedlings were sampled at 4-d after each treatment, respectively.

In a further experiment, 11-d-old rice seedlings were used to investigate time course changes of endogenous

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Abbreviations: GAs - gibberellins; JA - jasmonic acid; ABA - abscisic acid.
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plant hormones in relation to effect of jasmonic acid under salt stress. JA (30 μM) was applied 24 h after 40 mM NaCl exposure. Endogenous ABA and GAs were also measured at 3, 6, 12, or 24 h after JA treatment.

Endogenous ABA was quantified following the method of Browning and Wignall (1987) and Kamboj *et al*. (1999). Dry mass (0.5 g) was extracted with 30 cm^3 of 95 % isopropanol, 5 % glacial acetic acid, and 100 ng of [(±)-3,5,5,7,7,7-d6]-ABA standard. After extraction and filtration, residue was dissolved in 4 cm^3 of 1 M NaOH solution, and then washed three times with 3 cm^3 of methylene chloride to remove lipophilic materials. The extracts were dried and methylated by adding diazomethane for GC-MS analysis. For quantification, the Lab-Base (*ThermoQuset*, Manchester, UK) data system software was used to monitor the response of ions, which were at m/E (mass versus charge) 162 and 190 for Me-ABA and 166 and 194 for Me-[$^2H_6$]-ABA.

For the analysis of endogenous gibberellins (Lee *et al*. 1998), lyophilized tissue samples were ground to a fine powder in a mortar and pestle with the aid of acid-washed sea sand. The powdered tissue was extracted with 80 % methanol (MeOH). The 80 % MeOH was removed by filtration, and the tissue was then extracted with 100 % MeOH until the extract was clear. The volume of the 80 % and 100 % extracts were recorded, the two extracts were combined, and water was added to bring the combined MeOH extract concentration to 60 %. This solution was chilled for 1-h at -70 °C, and precipitated chlorophyll was removed by filtration through a GF/A filter. The extract was adjusted to pH 8.0 to 8.3 using 2 M NH_4OH and passed through a 3 g of *Davisil* C_{18} column (90-130 μm, *Alltech*, Deerfield, USA). The eluant was reduced to near dryness at 40 °C *in vacuo*. The sample was dried onto 1 g celite and then loaded onto 4 g SiO_2 partitioning column (deactivated with 20 % water) to separate the gibberellins as a group from more polar impurities. Gibberellins were eluted with 80 cm^3 of 95:5 ethyl acetate (EtOAc): hexane saturated with formic acid. This solution was dried at 40 °C *in vacuo*, redissolved in 4 cm^3 of EtOAc and partitioned 3 times against 4 cm^3 of 0.1 M phosphate buffer (pH 8.0). Dropwise addition of 2 M NaOH was required during the first partitioning to neutralize residual formic acid. Polyvinylpolypyrrolidone (PVPP) 1 g was added to the combined aqueous phases, and this mixture was slurried for 1 h. Following the removal of the PVPP by filtration, 6 M HCl was added to reduce the pH 2.5. The extract was partitioned 3 times against equal volumes of EtOAc. The combined EtOAc fraction was dried *in vacuo*, and the residue was dissolved in 3 cm^3 of 100 % MeOH. This solution was dried on a *Savant Automatic Environmental Speedvac* (*AES 2000*, Madrid, Spain). The dried sample was subjected to reverse-phase C_{18}-HPLC. Radioactivity for each concentrated GA fraction was counted for 3 min in liquid scintillation counter. Each GA fraction was re-dissolved in 100 % methanol, transferred to a 1 cm^3 vial and dried under N_2 at 40 °C. The sample was dissolved in 35 mm^3 of methanol, and the GA methyl ester was prepared with ethereal diazomethane. The sample was dried under N_2, re-dissolved in methanol and methylated one more time. The sample was dissolved in 35 μm^3 pyridine, and silylated for 30 min at 65 °C with the same amount of *N*, *O*-Bis (trimethylsilyl)-trifluoroacetamide (BSTFA) with 1 % trimethylchlorosilane (TMCS) (*Pierce Chemical Co*., Rockford, USA). The sample was then reduced to dryness with N_2 and solublized in anhydrous dichloromethane. 1 μm^3 of each sample was injected on-column on a 30 m × 0.25 mm (i.d.), 0.25 μm film thickness *DB-1 capillary* column (*J & W Co*., Folson, USA). The GC (*Finnigan Mat GCQ*, Hemel Hempstead, UK) oven temperature was programmed for a 1 min hold at 60 °C, then to rise at 15 °C min^{-1} to 200 °C followed by 5 °C min^{-1} to 285 °C. Helium carrier gas was maintained at a head pressure of 30 kPa. The GC was directly interfaced to a Mass Selective Detector with an interface and source temperature of 280 °C, an ionising voltage of 70 eV and a dwell time of 100 ms. Full scan mode (the first trial) and three major ions of the supplemented [$^2H_2$]GA internal standards (the second trial) and the endogenous gibberellins were monitored simultaneously. Retention time was determined by using the hydrocarbon standards (C_{23}, C_{24}, C_{25}, C_{26}, C_{27} and C_{28}) to calculate the KRI (Kovats retention indices) value.

It is well known that endogenous ABA content is commonly increased in response to salt stress (Wang *et al*. 2001). Similar result was observed in our study, but the changes of endogenous ABA content in rice plants under salt stress were affected by application time of JA. Endogenous ABA content in salt stressed plants (40 mM NaCl) was 1.8 times higher than that of the control. When JA acid was applied before salt stress to plants, endogenous ABA content was higher than in the plants exposed to salt stress alone. In particular, when jasmonic acid was applied 24-h before salt stress, endogenous ABA content was 1.3 times more enhanced than in plants exposed to salt stress alone. However, when JA acid and salt solution were applied at the same time, endogenous ABA content was lower compared to the plants exposed to salt stress alone. Endogenous ABA content in plants treated with jasmonic acid after salt stress (post-treatment 24 and 48 h) was also lower compared to plants exposed to salt stress alone (Table 1).

It was reported that exogenously applied gibberellic acid (GA_3) ameliorate the NaCl-induced reduction of rice growth (Kaur *et al*. 1998). Lin *et al*. (1995) reported that GA_3 reduces NaCl-induced inhibition of shoot growth, but not of root growth in rice seedlings. It is inferred that the contents of endogenous gibberellins in rice plants might be changed under salt stress. Endogenous GA_{12} content under salt stress was lower compared to the control, and when JA was applied with NaCl at the same

Table 1. Change of endogenous ABA and GA contents [ng g^{-1} (d.m.)] in rice seedlings as affected by different application times of jasmonic acid (30 µM) under NaCl stress (40 mM). JA was treated 48 or 24 h before NaCl exposure, at the same time with NaCl, or 24 and 48 h after NaCl exposure. Endogenous ABA or GAs were measured at 4-d after all treatments. Means of 4 replicates ± SE.

	ABA	GA_{12}	GA_{53}	GA_{19}	GA_{20}	GA_1	GA_8
Control	78.3 ± 5.7	7.68 ± 0.02	4.30 ± 0.04	90.2 ± 0.03	4.57 ± 0.08	2.30 ± 0.07	1.59 ± 0.03
NaCl	137.3 ± 7.4	7.01 ± 0.11	8.83 ± 0.09	92.8 ± 0.13	3.33 ± 0.49	1.84 ± 0.06	0.77 ± 0.02
Pre-treatment 48 h	145.2 ± 9.1	6.54 ± 0.01	2.06 ± 0.06	80.5 ± 0.05	5.00 ± 0.05	2.57 ± 0.38	2.97 ± 0.08
Pre-treatment 24 h	170.5 ± 1.3	4.91 ± 0.05	1.84 ± 0.02	55.4 ± 0.04	3.89 ± 0.07	1.98 ± 0.09	3.63 ± 0.04
Treatment 0 h	127.4 ± 9.8	2.50 ± 0.01	2.54 ± 0.02	69.7 ± 0.05	7.86 ± 1.11	5.48 ± 0.05	3.28 ± 0.35
Post-treatment 24 h	118.9 ± 16.9	6.64 ± 0.03	0.91 ± 0.04	92.2 ± 0.02	4.85 ± 0.23	5.67 ± 0.19	3.02 ± 0.16
Post-treatment 48 h	135.7 ± 4.6	5.77 ± 0.04	5.99 ± 0.01	88.2 ± 0.03	2.29 ± 0.05	2.96 ± 0.03	2.38 ± 0.05

Table 2. Effect of JA (30 µM) on time course of endogenous ABA and GA contents [ng g^{-1} (d.m.)] in rice seedlings exposed to NaCl stress (40 mM).

Phytohormones	Treatments	3 h	6 h	12 h	24 h
ABA	control	101.5 ± 2.1	103.1 ± 0.7	102.2 ± 0.6	100.3 ± 0.4
	NaCl	166.5 ± 3.2	101.8 ± 0.9	126.6 ± 1.1	147.8 ± 0.4
	NaCl + JA	130.6 ± 1.8	75.8 ± 0.3	68.2 ± 0.3	68.0 ± 0.2
GA_{20}	control	0.55 ± 0.07	0.54 ± 0.03	0.53 ± 0.02	0.52 ± 0.03
	NaCl	0.62 ± 0.02	0.56 ± 0.01	0.37 ± 0.02	1.56 ± 0.01
	NaCl + JA	1.02 ± 0.00	1.18 ± 0.01	1.56 ± 0.00	1.12 ± 0.02
GA_1	control	0.31 ± 0.01	0.35 ± 0.01	0.33 ± 0.02	0.29 ± 0.01
	NaCl	0.40 ± 0.02	0.44 ± 0.01	0.45 ± 0.03	0.32 ± 0.00
	NaCl + JA	0.70 ± 0.03	0.65 ± 0.03	0.64 ± 0.00	0.60 ± 0.02

time GA_{12} content was the lowest among all treatments. Endogenous GA_{53} content under salt stress was 2 timesmore enhanced than that of control, whereas its content in the JA treatments (with the exception of post-treatment after 48 h) was declined. Salt stress alone did not reduce endogenous GA_{19} content compared to the control while it was reduced at simultaneous application of NaCl and JA and in the case of JA pre-treatment (24 h).

Endogenous content of GA_{20}, a precursor of bioactive GA_1, was reduced in the presence of NaCl 40 mM compared to the control. Content of GA_{20} was increased in case of JA pre-treatment (48 h) or simultaneous treatment. Endogenous GA_1 content was also slightly reduced under salt stress compared to the control. Endogenous GA_1 content was highest at simutaneous JA and na Cl treatment and 24 h post-treatment. Finally, content of GA_8, a metabolite of bioactive GA_1, was reduced by salt stress. GA_8 content was higher in all JA-treated plants (Table 1).

Endogenous ABA content did not changed with time in the control. The highest ABA content was observed 3 h after NaCl treatment. ABA content under salt stress was lowest at 6-h and thereafter enhanced slightly. When JA acid was treated 24 h after NaCl, ABA content was increased only 3 h after JA application and then decreased (Table 2). These results confirmed previous reports that ABA content increased in salt stressed plants (Itai *et al*. 1968, Mizrahi *et al*. 1971, Boucaud and Unger 1976).

Reduced GA_{20} content under salt stress was found only 12 h after application but increased content 24 h after application. When JA acid was treated 24 h after NaCl, endogenous GA_{20} content was enhanced in comparison to the control and NaCl treated plants. Endogenous GA_1 content in rice plants exposed to NaCl slightly increased. When JA was applied to rice plants exposed to NaCl, endogenous GA_1 content was further increased (Table 2).

The present study shows that level of endogenous gibberellin under salt stress is generally reduced and in particular, endogenous bioactive GA_1 level is enhanced by exogenous jasmonic acid under salt stress.

Further investigations are needed, however, to enhance our better understanding in possible roles of endogenous gibberellins and jasmonic acid under salt stress.

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