Journal of Plant Biology, June 2006, 49(3) : 257-260 **SHORT COMMUNICATION**

Gamma Radiation and Hormone Treatment as Tools to Reduce Salt Stress in Rice (*Oryza sativa* L.)

Myung-Hwa Baek[1], Byung Yeoup Chung[1]*, Jin-Hong Kim[1], Seung Gon Wi[1], Jae-Sung Kim[1], and In-Jung Lee[2]
[1]Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup 580-185, Korea
[2]Department of Agronomy, Kyungpook National University, Daegu 702-701, Korea

We investigated the effects of jasmonic acid (JA) and gamma irradiation on the growth and metabolic responses to salt stress in rice (*Oryza sativa* L.) plants. The relative growth rate (RGR), relative water content (RWC), and chlorophyll (Chl) content were lower in NaCl-treated plants than in the control, whereas the malondialdehyde content (MDA), electrolyte leakage (EL), and contents of proline and abscisic acid (ABA) were higher in the treated plants. When induced by the salt stress, those effects, however, were somewhat alleviated by the application of JA or gamma irradiation. The most significant response was manifested by the proline content, with relatively lower values for alleviation being recorded for the contents of RGR, RWC, Chl, and MDA, as well as EL. Moreover, although total Chl content was not significantly influenced by JA or gamma irradiation in plants under salt stress, an increase in the level of Chl a resulted in a markedly changed ratio of Chl a/b. The degree of alleviation, in terms of growth and metabolic responses, was more extensive for JA-treated plants than for those exposed to gamma irradiation.

Keywords: gamma irradiation, jasmonic acid, rice, salt stress

When subjected to salt stress, many plant species develop mechanisms to either exclude that salt from their cells or else make those cells resistant to its presence. These responses can alter various physiological and biochemical processes, including a decrease in water potential and disturbances to ion homeostasis, as well as secondary stresses, e.g., oxidative damage, reduced photosynthesis capacity, or an increase in the levels of plant hormones such as abscisic acid (ABA) (Kaiser et al., 1983; Hagemann and Erdmann, 1997; Gadallah, 1999; Vaidyanathan et al., 1999; Meloni et al., 2003). These complex responses to salt stress depend on various factors. For instance, phytohormones ABA and jasmonic acid (JA) may act as stress modulators by reducing plant damage. Exogenously applied JA counteracts the physiological and biochemical changes induced by high salt or chilling (Popova et al., 1995; Velitchkova and Fedina, 1998; Fedina and Benderliev, 2000; Fung et al., 2004). In addition, gamma irradiation can improve stress resistance by influencing antioxidant enzyme activity and photosynthetic capacity (Zaka et al., 2002; Lee et al., 2003; Kim et al., 2004). In this study, we evaluated how treatment with either JA or gamma irradiation might alleviate the negative effects of salt stress in rice.

MATERIALS AND METHODS

Rice (*Oryza sativa* L. cv. Ilpoombyeo) seedlings were hydroponically cultivated in a half-strength Murashige and Skoog (1962) solution prepared with distilled water. All experiments were conducted in the greenhouse of Chungnam National University, Daejeon, Korea. Two weeks after sowing, the plants were divided into six treatment groups: 1) control, 2) 30 μM JA, 3) 4 Gy of irradiation from a gamma irradiator (^{60}Co, ca. 150 TBq of capacity; AECL, Canada), 4) 40 mM NaCl, 5) 40 mM NaCl combined with 30 μM JA, and 6) 40 mM NaCl combined with 4 Gy γ-irradiation. The concentration of NaCl was checked and re-adjusted every 2 d. All plants were harvested, measured, and analyzed after 7 d of treatment.

To estimate their growth, the plants were randomly selected and gently uprooted to obtain their shoot dry weights after being oven-dried at 80°C for 72 h. The relative growth rate (RGR) was defined as the increase in dry weight between the beginning and the end of the salt-treatment period, calculated as: $RGR = (\ln W_i - \ln W_j)/(t_i - t_j)$, where W was the dry weight; t, time; and subscripts, denoting the number of days after treatment sampling, i.e., initial (i, 0) and final (j, 7). We used the method of Barr and Weatherley (1962) to measure leaf relative water content (RWC).

Electrolyte leakage (EL) was determined by the procedure described by Dionisio-Sese and Tobita (1998). The contents of malondialdehyde (MDA), chlorophyll (Chl), and proline were determined according to the methods of Dhindsa et al. (1981), Lichtenthaler (1987), and Troll and Lindsley (1955), respectively. ABA content was measured using the techniques of Browning and Wignall (1987), Qi et al. (1998), and Kamboj et al. (1999), with some modifications. All data were examined with

*Corresponding author; fax +82-63-570-3339
e-mail bychung@kaeri.re.kr

ANOVA, and the mean differences were compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Our preliminary studies had shown that 4 Gy of irradiation alone caused no significant phenotypic changes to seedlings and had no negative effects on the growth and yield of the filial generation. Moreover, although JA treatment and gamma irradiation themselves did not elicit any significant alterations in the parameters examined here, the induction of salt stress did somewhat change RGR, RWC, MDA, and EL, as well as Chl, proline, and ABA contents in our control plants. In contrast, RGR, based on dry weights, was sharply decreased, as much as 20%, in the 40 mM NaCl-treated group (Fig. 1A), with a similar tendency found for RWC (Fig. 1B). These observations match those previously reported (Dionisio-Sese and Tobita, 1998; Ashraf and Akhtar, 2004). The opposite trend was noted when salt-stressed plants were treated with JA or irradiation: there, RGR and RWC were significantly alleviated, by up to 11-17% and 3-10%, respectively (Fig. 1A, 1B). Our results demonstrate the necessity for further, detailed research into the interactions among JA, gamma irradiation, and salt stress in the plant response.

Free radical-induced lipid peroxidation generally can cause an increase in membrane permeability or a loss of membrane integrity, which then results in greater levels of MDA and solute leakage (Dionisio-Sese and Tobita, 1998). Changes in those two parameters might serve as an index for determining such permeability and integrity. Therefore, although MDA and EL typically rise when plants are subjected to salt stress, we showed here that both levels improved by up to 10-26% (JA) and 3-7% (gamma irradiation) (Fig. 1C, D).

Under salt stress, the total Chl content decreases (Kumar et al., 1999), a phenomenon also seen with our NaCl-treated plants, where total Chl declined by as much as 51% compared with the control. Although this response was not significantly alleviated by either JA or irradiation (Fig. 2A), the Chl a/b ratio, an indicator of oxidative damage, was relatively and significantly changed. Here, Chl a/b was remarkably increased by JA treatment (ratio of 2.18) compared with the control (1.94), while only a negligible rise was calculated as a result of gamma irradiation (1.94) (Fig. 2B).

Proline, which contributes to the balance in osmotic potential, commonly accumulates in salt-stressed seedlings (Pollard and Wyn Jones, 1979). Likewise, in our study, the increase in proline content was greatest in the NaCl-treated groups. Those that were also supplemented with JA, under salt stress, showed a further, remarkable rise in proline content, while that increase in content also was appreciable in the gamma-irradiated plants (Fig. 2C). These results suggest that JA and gamma irradiation contribute to an effect on osmotic

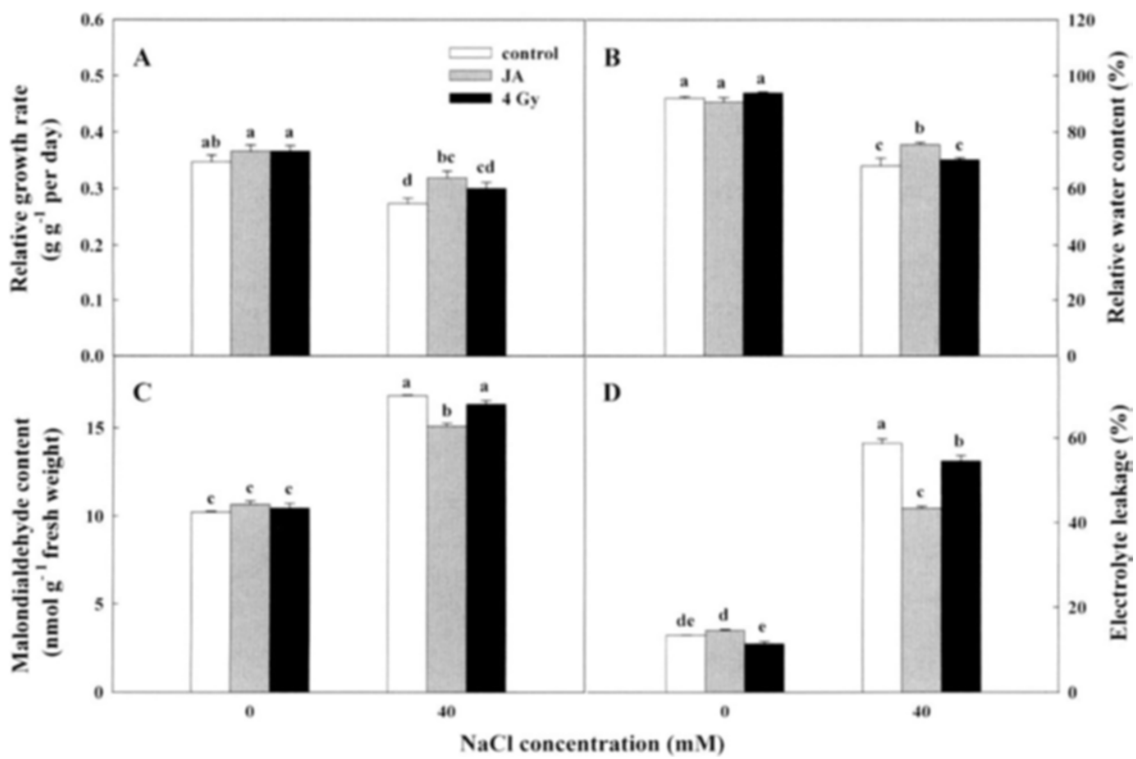

Figure 1. Relative growth rate (**A**), relative water content (**B**), level of malondialdehyde (**C**), and electrolyte leakage (**D**) in salt-stressed (40 mM NaCl) rice when treated with jasmonic acid (JA; 30 μM) or gamma-irradiation (4 Gy). All values are means ± SE (n = 6), except for RGR (n = 30). Bars labeled with same letter are not significantly different at 5% level, by Duncan's Multiple Range Test.

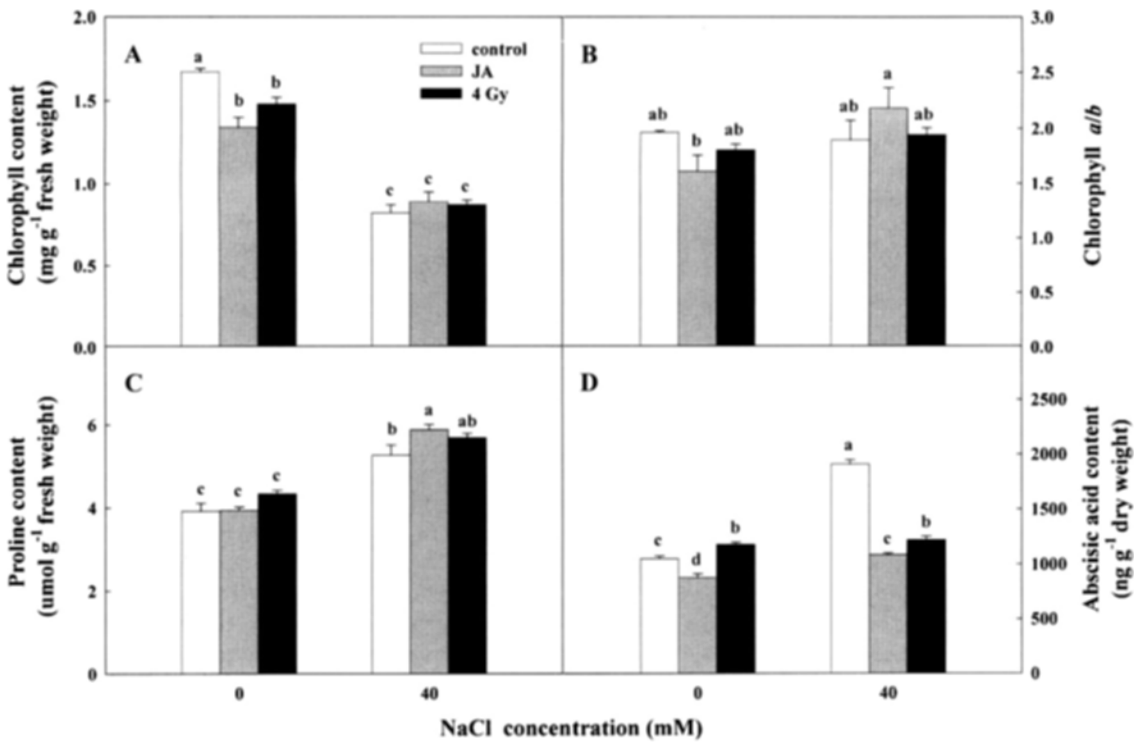

Figure 2. Chlorophyll content (**A**), chlorophyll a/b ratio (**B**), proline content (**C**), and abscisic acid content (**D**) in salt-stressed rice (40 mM NaCl) when treated with jasmonic acid (JA; 30 μM) or gamma-irradiation (4 Gy). All values are means ± SE (n = 6). Bars labeled with same letter are not significantly different at 5% level, by Duncan's Multiple Range Test.

potential due to the greater proline content. Salt stress triggers an increase in the levels of plant hormones, e.g., ABA and cytokinin, the former promoting stomatal closure under such conditions (Vaidyanathan et al., 1999). Likewise, in our study, ABA content was significantly elevated by NaCl treatment. However, that salt stress-induced increase in ABA was reduced up to 37 and 43% by gamma irradiation and JA, respectively (Fig. 2D). Although some salt-resistant cultivars have a higher ABA content under salt stress than do salt-sensitive plants, other research has shown no relationship between salt resistance and the endogenous ABA level when plants are stressed (Yurekli et al., 2004; Kang et al., 2005). Therefore, the relationship between salt resistance and ABA content remains controversial.

In conclusion, although JA and gamma irradiation did not show any positive effect in our control group, both types of treatment acted as modulators under salt stress by altering plant responses, as manifested primarily by a high accumulation of proline. Although JA showed a much greater alleviating effect on all parameters examined here, we can suggest that both factors partially contribute to the increased resistance of rice plants against salt stress.

ACKNOWLEDGEMENT

This project was supported by a grant from the Nuclear R&D Program, Ministry of Science and Technology, Korea.

Received January 9, 2006; accepted March 7, 2006.

LITERATURE CITED

Ashraf M, Akhtar N (2004) Influence of salt stress on growth, ion accumulation and seed oil content in sweet fennel. Biol Plant 48: 461-464

Barr HD, Weatherley PE (1962) A re-examination of the relative turgidity technique for estimating water deficit in leaves. Aust J Biol Sci 15: 413-428

Browning G, Wignall TA (1987) Identification and quantification of indole-3-acetic and abscisic acids in the cambial region of *Quercus robur* by combined gas chromatography-mass spectrometry. Tree Physiol 3: 235-246

Dhindsa RS, Plumb-Dhindsa P, Thorpe TA (1981) Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot 32: 93-101

Dionisio-Sese ML, Tobita S (1998) Antioxidant response of rice seedlings to salinity stress. Plant Sci 135: 1-9

Fedina IS, Benderliev KM (2000) Response of *Scenedesmus incrassatulus* to salt stress as affected by methyl jasmonate. Biol Plant 43: 625-627

Fung RWM, Wang CY, Smith DL, Gross KC, Tian M (2004) MeSA and MeJA increase steady-state transcript levels of alternative oxidase and resistance against chilling injury in sweet peppers (*Capsicum annuum* L.). Plant Sci 166: 711-719

Gadallah MAA (1999) Effects of proline and glycinebetain on *Vicia faba* responses to salt stress. Biol Plant 42: 249-257

Hagemann M, Erdmann N (1997) Environmental stresses, *In* AK Rai, ed, Cyanobacterial Nitrogen Metabolism and Environmental Biotechnology. Springer, New Delhi, pp 156-221

Kaiser WM, Weber H, Sauer M (1983) Photosynthetic capacity, osmotic response and solute content of leaves and chloroplasts from *Spinacia oleracea* under salt stress. Z Pflanzenphysiol 113: 15-27

Kamboj JS, Browning G, Blake PS, Quinlan JD, Baker DA (1999) GC-MS-SIM analysis of abscisic acid and indole-3-acetic acid in shoot bark of apple rootstocks. Plant Growth Regul 28: 21-27

Kang DJ, Seo YJ, Lee JD, Ishii R, Kim KU, Shin DH, Park SK, Jang SW, Lee IJ (2005) Jasmonic acid differentially affects growth, ion uptake and abscisic acid concentration in salt-tolerant and salt-sensitive rice cultivars. J Agron Crop Sci 191: 273-282

Kim JH, Baek MH, Chung BY, Wi SG, Kim JS (2004) Alternations in the photosynthetic pigment and antioxidant machineries of red pepper (*Capsicum annuum* L.) seedlings from gamma-irradiated seeds. J Plant Biol 47: 314-321

Kumar SG, Lakshmi A, Madhusudhan KV, Ramanjulu S, Sudhakar C (1999) Photosynthesis parameters in two cultivars of mulberry differing in salt tolerance. Photosynthetica 36: 611-616

Lee HY, Kim JS, Baek MH, Yoo JC, Kwon ST (2003) Effects of low dose γ irradiation on the physiological activities of radish (*Raphanus sativus* L.) during early growth and the reduction of ultraviolet-B stress. Kor Soc Hort Sci 44: 314-320

Lichtenthaler HK (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Method Enzymol 148: 350-382

Meloni DA, Oliva MA, Martunez CA, Cambraia J (2003) Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ Exp Bot 49: 69-76

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15: 473-497

Pollard A, Wyn Jones RG (1979) Enzyme activities in concentrated solutions of glycinebetain and other solutes. Planta 144: 291-298

Popova LP, Stoinoca ZG, Maslenkova LT (1995) Involvement of abscisic acid in photosynthetic process in *Hordeum vulgare* L. during salinity stress. J Plant Growth Regul 14: 211-218

Qi QG, Rose PA, Abrams GD, Taylor DC, Abrams SR, Cutler AJ (1998) (+)-Abscisic acid metabolism, 3-ketoacyl-coenzyme A synthase gene expression, and very-long-chain monounsaturated fatty acid biosynthesis in *Brassica napus* embryos. Plant Physiol 117: 979-987

Troll W, Lindsley J (1955) A photometric method for the determination of proline. J Biol Chem 215: 655-660

Vaidyanathan R, Kuruvilla S, Thomas G (1999) Characterization and expression pattern of an abscisic acid and osmotic stress responsive gene from rice. Plant Sci 140: 21-30

Velitchkova M, Fedina I (1998) Response of photosynthesis of *Pisum sativum* to salt stress as affected by methyl jasmonate. Photosynthetica 35: 89-97

Yurekli F, Porgali ZB, Turkan I (2004) Variations in abscisic acid, indole-3-acetic acid, gibberellic acid and zeatin concentrations in two bean species subjected to salt stress. Acta Biolog Cracov Ser Botan 46: 201-212

Zaka R, Vandecasteele CM, Misset MT (2002) Effects of low chronic doses of ionizing radiation on antioxidant enzymes and G_6PDH activities in *Stipa capillata* (Poaceae). J Exp Bot 53: 1979-1987