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BRIEF COMMUNICATION

Alleviation of salt stress by low dose γ-irradiation in rice

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Abstract

The effects of salt stress on the growth, photosynthesis, and antioxidative ability of the rice (*Oryza sativa* L.) plants raising from γ-irradiated seeds were investigated using two cultivars, Ilpumbyeo and Sanghaehyanghyella. The 50 and 100 mM NaCl solutions caused a remarkable decrease of the early germination rate and seedling growth. However, the salt stress-induced inhibition of the growth was significantly alleviated in the γ-irradiated plants. The chlorophyll contents and the effective quantum yield of photosystem 2 ($\Phi_{PS\,2}$) were lower in the NaCl-treated plants than in the control ones, while the non-photochemical quenching was higher in the former ones. Activities of the antioxidant enzymes such as superoxide dismutase (SOD) and ascorbate peroxidase (APX) increased with increasing NaCl concentrations, and the irradiated groups had even higher SOD and APX activities than the non-irradiated ones. These alleviation effects were observed similarly in both the cultivars tested.

Additional key words: ascorbate peroxidase, fluorescence parameters, NaCl, *Oryza sativa*, photosynthesis, superoxide dismutase.

Reduction in the crop productivity under the salt stress has been revealed in various plant species and often associated with a decrease in the photosynthetic capacity. Meloni *et al*. (2003) reported that the decrease of the photosynthesis could be induced by the stomata closure or the direct effect of the salt stress on the photosynthetic apparatus. The salt stress induces decreases in both the chlorophyll (Chl) contents and the photosystem (PS) 2 activity and it also elevates the production of superoxide radical and hydrogen peroxide (Hernández *et al.* 1995). It has been well documented that the antioxidant enzymes such as superoxide dismutase and ascorbate peroxidase are largely responsible for scavenging the reactive oxygen species (ROS) (Halliwell 1982, Fridovich 1991, Allen 1995, Asada 1999, Rout and Shaw 2001).

In plants, the antioxidant enzyme activity and the photosynthetic capacity are known to be positively affected by the low dose γ-irradiation (Lee *et al*. 2002, 2003). These effects of the low dose γ-irradiation can improve the stress-tolerance in plants subjected to the salt stress. To clarify this possibility, we attempted to investigate the growth, Chl contents, Chl fluorescence parameters, and antioxidant enzyme activities in the γ-irradiated rice plants under the salt stress using two cultivars differing in the phenotype and other characteristics.

Seeds of two rice (*Oryza sativa* L.) cultivars, Ilpumbyeo and Sanghaehyanghyella, were irradiated with 8 Gy by a gamma irradiator (^{60}Co, *ca*. 150 TBq of capacity, *AECL,* Canada) at a dose rate of 2 Gy h^{-1}. After the irradiation, the sterilized seeds were sown at two seeds per hole on a styrofoam sheet with 100 holes and nylon net bottom. The styroform sheets were floated in a plastic tray provided with distilled water (control), 50 and 100 mM NaCl solutions. The concentrations of NaCl solutions were checked and re-adjusted every 2 d for 14 d. The experiment was conducted in the greenhouse of Chungnam National University, Daejeon, Korea. Temperature was ranged from 17 to 24 °C during day time and 13 to 20 °C during night time. The germination

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Abbreviations: APX - ascorbate peroxidase; Chl - chlorophyll; F_v/F_m - variable to maximum fluiorescence ratio; $\Phi_{PS\,2}$ - effective quantum yield of photosystem 2; NPQ - non-photochemical quenching; PS - photosystem; SOD - superoxide dismutase.

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rate was determined from 3 to 9 d after sowing. All plants were harvested, measured, and analyzed after 14 d of exposure to the salt stress. The shoot length and the Chl fluorescence were measured immediately after harvesting. For determination of the total Chl contents and the antioxidant enzyme activities, fresh leaf samples were immediately frozen in liquid nitrogen and stored at -70 °C until used.

The Chl fluorescence was measured using a Chl fluorometer (*IMAGING-PAM*; Walz, Effeltrich, Germany). The maximal PS 2 quantum yield was calculated as the ratio of variable fluorescence (F_v) to the maximum of fluorescence (F_m) according to Genty *et al*. (1989). Variable fluorescence was obtained by subtracting the initial Chl fluorescence (F_0) from the maximum of fluorescence. Readings were taken after the samples were dark-adapted for 10 min at room temperature. The parameters of quantum yield ($\Phi_{PS\ 2}$) and NPQ were calculated by the equations described in van Kooten and Snel (1990) as follows; quantum yield $\Phi_{PS\ 2} = (F_m'-F)/F_m'$ and NPQ = $(F_m-F_m')/F_m'$, where F and Fm' is steady-state level and the maximum yield of fluorescence in light-acclimated samples reached by application of a saturation pulse. The total Chl contents were determined using a spectrophotometer (*Kontron Instruments*, Zurich, Switzerland) according to Arnon (1949).

The determination of SOD (EC 1.15.1.1) activity was performed by using SOD-dependent inhibition of the reduction of nitroblue tetrazolium (NBT) to purple formazan by superoxide as described by Beyer and Fridovich (1987). APX (EC 1.11.1.11) activity was determined from the decrease in absorbance at 290 nm during the oxidation of ascorbate using an absorbance coefficient of 2.8 mM^{-1} cm^{-1} as described by Nakano and Asada (1981). Enzyme activity was expressed on the basis of protein contents. Protein contents were determined according to Bradford (1976).

All data were subjected to *ANOVA* test and the mean differences were compared by Duncan's Multiple Range Test (DMRT).

The salt stress significantly decreased the early germination rate and seedling growth in both cultivars (Table 1). Especially the seedling growth was sharply decreased, showing a decrease of up to 33 - 58 % and 151 - 209 % at 50 and 100 mM NaCl, respectively as compared with that of control. These results are in good agreement with the negative influence of the salt stress on the growth of rice as reported by Prisco and O'Leary (1970), Ahmed *et al*. (1987), and Promila and Kumar (2000). Interestingly, the salt stress-induced decrease in the seedling growth was significantly alleviated by the low dose γ-irradiation in both cultivars. The seedling growth in the irradiated groups was elevated about 5 - 6 % and 10 - 13 % at 50 and 100 mM NaCl, respectively.

The relatively low F_v/F_m ratio was observed only in 100 mM NaCl-treated groups of Ilpumbyeo (Table 2), indicating that the high NaCl concentration affects the leaf photochemistry. The effective quantum yield of PS 2 ($\Phi_{PS\ 2}$) was lower in the NaCl-treated groups than in control ones in both cultivars as reported by Delfine *et al.* (1999). The NPQ, which is one of the protective mechanisms against damages in the photosynthetic apparatus by excess light under various stress conditions (Demmig-Adams and Adams 1996), was increased in the

Table 1. The effects of the γ-irradiation on the germination rate (n = 10) and seedling growth (n = 100) in two rice cultivars under the salt stress (d - days after sowing). The seedling growth was evaluated as the shoot length. The concentrations of NaCl solutions were 50 and 100 mM. Mean ± SE. Values with the same letters are not significantly different within the same cultivar at 5 % level by DMRT.

Parameter		Cultivar	Gy	0 mM	50 mM	100 mM
Germination [%]	3 d	Ilpumbyeo	0	45.0 ± 3.33b	32.5 ± 0.83c	6.1 ± 0.04e
			8	52.5 ± 0.01a	36.6 ± 3.20c	13.8 ± 1.25d
	4 d	Sanghaehyanghyella	0	40.0 ± 0.83a	17.5 ± 0.83d	1.0 ± 0.33e
			8	37.5 ± 0.01b	20.5 ± 0.68c	1.0 ± 0.33e
	6 d	Ilpumbyeo	0	97.5 ± 0.83ab	100.0 ± 0.00a	91.7 ± 1.38c
			8	98.8 ± 0.42ab	100.0 ± 0.00a	96.3 ± 1.25b
		Sanghaehyanghyella	0	80.0 ± 0.00a	65.0 ± 1.67cd	61.4 ± 2.13d
			8	71.3 ± 1.25b	69.3 ± 2.25bc	52.5 ± 0.00e
	9 d	Ilpumbyeo	0	97.5 ± 0.83bc	100.0 ± 0.00a	95.9 ± 1.38c
			8	98.8 ± 0.42ab	100.0 ± 0.00a	98.8 ± 0.42ab
		Sanghaehyanghyella	0	87.5 ± 0.83a	75.0 ± 0.83b	76.3 ± 0.42b
			8	73.8 ± 2.08b	77.2 ± 4.00b	73.8 ± 2.92b
Shoot length [cm]		Ilpumbyeo	0	16.4 ± 0.16b	10.4 ± 0.14d	5.3 ± 0.09f
			8	16.9 ± 0.12a	11.0 ± 0.14c	6.0 ± 0.10e
		Sanghaehyanghyella	0	20.9 ± 0.22b	15.5 ± 0.15d	7.8 ± 0.08f
			8	21.6 ± 0.21a	16.2 ± 0.18c	8.6 ± 0.13e

Table 2. The effects of the γ-irradiation on the photosynthetic parameters and antioxidant enzyme activities in two rice cultivars under the salt stress. The concentrations of NaCl solutions were 50 and 100 mM. Mean ± SE (n = 3). Values with same letters are not significantly different within the same cultivar at 5 % level by DMRT.

Parameter	Cultivar	Gy	0 mM	50 mM	100 mM
Fv/Fm	Ilpumbyeo	0	0.86 ± 0.00a	0.85 ± 0.00a	0.76 ± 0.01b
		8	0.86 ± 0.00a	0.85 ± 0.00a	0.74 ± 0.02b
	Sanghaehyanghyella	0	0.86 ± 0.00a	0.85 ± 0.00ab	0.84 ± 0.00b
		8	0.86 ± 0.00a	0.85 ± 0.00ab	0.85 ± 0.00ab
$\Phi_{PS\,2}$	Ilpumbyeo	0	0.41 ± 0.01a	0.35 ± 0.03a	0.36 ± 0.02a
		8	0.41 ± 0.04a	0.38 ± 0.02a	0.39 ± 0.01a
	Sanghaehyanghyella	0	0.41 ± 0.04ab	0.37 ± 0.01bc	0.33 ± 0.02bc
		8	0.46 ± 0.01a	0.38 ± 0.03bc	0.39 ± 0.01abc
NPQ	Ilpumbyeo	0	0.61 ± 0.19b	0.93 ± 0.11a	0.96 ± 0.02a
		8	0.49 ± 0.09b	0.90 ± 0.14a	0.90 ± 0.02a
	Sanghaehyanghyella	0	0.91 ± 0.23bc	1.28 ± 0.04ab	1.60 ± 0.17a
		8	0.56 ± 0.06c	1.20 ± 0.28ab	1.12 ± 0.10abc
Total Chl [mg g^{-1}(f.m.)]	Ilpumbyeo	0	1.80 ± 0.03b	1.26 ± 0.01d	0.36 ± 0.00e
		8	2.15 ± 0.01a	1.56 ± 0.01c	0.38 ± 0.00e
	Sanghaehyanghyella	0	2.04 ± 0.01b	1.44 ± 0.02d	0.74 ± 0.00e
		8	2.25 ± 0.03a	1.62 ± 0.02c	0.76 ± 0.00e
SOD [U mg^{-1}(protein)]	Ilpumbyeo	0	31.61 ± 4.31bc	41.56 ± 1.97b	37.26 ± 1.36bc
		8	29.77 ± 1.21c	42.16 ± 3.78b	56.95 ± 6.06a
	Sanghaehyanghyella	0	30.30 ± 0.74c	36.10 ± 3.17c	46.45 ± 1.94b
		8	34.35 ± 2.13c	43.37 ± 0.85b	64.35 ± 0.30a
APX [U mg^{-1}(protein)]	Ilpumbyeo	0	0.30 ± 0.01c	0.33 ± 0.03bc	0.38 ± 0.01ab
		8	0.28 ± 0.01c	0.37 ± 0.03ab	0.42 ± 0.01a
	Sanghaehyanghyella	0	0.32 ± 0.00c	0.36 ± 0.02c	0.53 ± 0.02b
		8	0.35 ± 0.02c	0.36 ± 0.01c	0.64 ± 0.03a

NaCl-treated groups. The total Chl contents were decreased with increasing NaCl concentrations in both cultivars as reported by Kumar *et al*. (1999) and Sultana *et al*. (1999). This decrease in the total Chl content may indicate a possible damage in the photosynthetic capacity of chloroplasts (Malanga *et al.* 1997). The decrease of the total Chl contents at 50 mM NaCl-treated groups was significantly alleviated by the γ-irradiation. In contrast, the Chl fluorescence parameters were not significantly changed by the γ-irradiation under the salt stress.

The activities of SOD and APX were correlated with increasing NaCl concentrations (Table 2) as reported in a previous study (Vaidyanathan *et al*. 2003). The SOD activities under the salt stress were more increased in the irradiated groups than in the non-irradiated ones in both cultivars. Especially the 100 mM NaCl solution caused a drastic increase of the SOD activities up to 53 and 39 % in the irradiated groups of Ilpumbyeo and Sanghaehyanghyella, respectively. The activities of the APX were also higher in irradiated groups than in non-irradiated ones. This result is in good agreement with the report that the low dose γ-irradiation enhanced activities of antioxidant enzymes, especially SOD (Zaka *et al*. 2002).

In conclusion, the results obtained suggest that the low dose γ-irradiation could alleviate the oxidative damages triggered by the salt stress, increasing the antioxidant enzyme activities. These effects of the low dose γ-irradiation under the salt stress were very similar to those of brassinosteroids (Núñez *et al*. 2003/4) or paclobutrazol (Özmen *et al*. 2003). Therefore, a further study needs to be performed to elucidate possible involvements of these hormones in the signaling pathway induced by the low dose γ-irradiation under the salt stress.

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