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Indicative Responses of Rice Plant to Atmospheric Ozone

Jae-Seoun Hur*, Pan Gi Kim[1], Sung-Chul Yun[2] and Eun Woo Park[2]

**Department of Environmental Education, Sunchon National University, Sunchon 540-742, Korea*

[1]National Instrumentation Center for Environmental Management, Seoul National University, Suwon 441-744, Korea

[2]School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea

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Differences in physiological and biochemical responses between sensitive and tolerant rice cultivars to ozone were investigated to develop reliable indications of early ozone damage. Three Korean local rice cultivars – sensitive cultivar Dongjin (DJ), moderately tolerant cultivar Hwayeong (HY) and tolerant cultivar Ilmee (IM) were exposed to ozone at the concentrations of 100 nl l^{-1} or 200 nl l^{-1}, 8 h per day for 10 days in a controlled-environment fumigation chamber. The rice cultivars seemed to be endurable to ozone stress at the concentration of 100 nl l^{-1} which is frequently monitored during the growing season in summer. However, severe damage was induced and differential sensitivity was clearly noted among the rice cultivars at the higher ozone concentration. Activation of the glutathion (GR) - ascorbate peroxidase (APX) cycle was likely to be responsible for protection of rice plants against ozone exposure, relating difference in sensitivity of rice cultivars to ozone. Photosynthetic activity appeared to be one of sensitive responses, for which chlorophyll fluorescence and leaf greenness can together provide a very reliable index, a degree of photosynthetic damage by ozone. Formation of malondialdehyde (MDA) was also considered as an indication that can differentiate cultivars sensitivity to ozone. However, the changes in polyamines and total phenolics were not consistent with exposed ozone concentrations and/or ozone sensitivity of the cultivars. The behavior of polyamines and phenolics in the damaged plants at high ozone levels could be interpreted as an indication of ozone injury rather than activation of additional protection mechanisms scavenging active oxygen species formed by ozone. Several responses triggered by ozone could explain the differential sensitivity of the rice cultivars and be used as reliable indications of relative ozone damage to rice plant.

Keywords : glutathion-ascorbate cycle, malondialdehyde, photosynthesis, chlorophyll fluorescence, leaf greenness.

*Corresponding author.
Phone) +82-61-750-3383, Fax) +82-61-750-3308
E-mail) jshur1@sunchon.ac.kr

The potential damage of ozone to vegetation has been known for over 30 years, but it is only over the last decade that its impact has become of major concern in many areas. Concentrations of tropospheric O_3 are expected to increase because of worldwide increases in urbanization and industrialization (Krupa and Kickert, 1989). Studies conducted in National Crop Loss Assessment Network (NCLAN) in the USA have clearly shown that ambient O_3 levels over a range 0.04-0.07 ppm (7 h per day seasonal mean) can significantly reduce yield in many crops (Heck et al., 1988). It is now established that ozone can cause a range of effects including visible leaf injury, growth and yield reductions, and altered sensitivity to biotic and abiotic stresses (Further and Achermann, 1994).

The assessment of possible harmful effects of atmospheric O_3 on vegetation has shown that several O_3-sensitive plants displayed characteristic visible symptoms of O_3 injury at ambient levels just above the current guideline or limit values now used for air quality regulation. Controlled-environment studies using lower concentrations of O_3 have also shown that the O_3-sensitive plants may exhibit several signs of invisible injury while the O_3-tolerant plants show evidence of acclimation (Langerbartels et al., 1991; Mehlhorn et al., 1991; Rao et al., 1996; Wellburn and Wellburn, 1996). The differential responses between acclimated and non-acclimated plants to ozone injury can account for the mechanisms responsible for differential sensitivity of plants to ozone. Therefore, the difference in subtle changes occurring in plant cells before the emergence of visible symptoms can be used as a reliable indication of early damage of ozone to plants. Such an indicative response of ozone injury can contribute to the development of monitoring system to protect crops from ozone in the field.

Rice is the single most important grain crop in Korea. Several attempts have been made to select O_3-tolerant rice cultivars in Korea (Sohn and Lee, 1997; Sohn et al., 1998). In their studies, relatively high level of ozone (0.3 ppm) was employed and tolerance of rice cultivars against ozone damage was screened only by the degree of visible injury. However, very limited research on Korean local cultivars of rice has been carried out to evaluate the relative sensitivity

to atmospheric ozone by proper assessment of invisible injury induced by lower levels of ozone at early stage of injury. Considering a potential for the rapid increase in atmospheric O_3 levels in Korea as well as the importance of rice in the region, it is important that tolerant rice cultivars should be selected by the proper assessment of invisible injury that is usually caused under the realistic concentrations of ambient ozone.

This research was purposed to investigate the difference between sensitive and tolerant rice cultivar to ozone injury for (1) the development of reliable indications of early ozone injury and (2) the proper assessment of invisible injury associated with rice sensitivity to ozone. For the purpose, common differences among Korean local cultivars of rice plant with differential sensitivity to ozone were found out in (1) physiological and biochemical responses such as photosynthetic activity and antioxidative enzyme activity, and (2) changes in the levels of antioxidants (polyamines and total phenols) and ozone-induced oxidative products (malondialdehyde: MDA).

Material and Methods

Plant material. Seeds of three commercial rice (*Oryza sativa* L.) cultivars, Dongjin (DJ), Hwayeong (HY) and Ilmee (IM), were obtained from National Crop Experiment Station (Suwon, Korea). All three cultivars are japonica-type rice. Relative sensitivity of the cultivars was determined by the extend of visible injury at concentration of 300 nl l^{-1} O_3 for 8 h per day over 7 days. According to severity of visible injury, cultivar IM was tolerant, cultivar HW was moderately tolerant and cultivar DJ was sensitive to ozone.

Rice seeds were immersed in tap water for three days at 25°C and the germinated seeds were raised in seedling plates for 10 days and ten plants of each cultivar were then transplanted into a large plastic pot (30 cm in diameter). Two replicate pots of each cultivar were placed at 25±3°C, 60 to 80% RH and at a PPFD of 400 $\mu molm^{-2}s^{-1}$ with a 14 h photoperiod in a growth chamber for 3 weeks. One-month-old plants were transferred into controlled-environment fumigation chambers (CEFC) enriched with ozone. Control plants were maintained in the CEFC with no additional ozone. Plants were acclimated in the CEFCs for 3 days before initiation of ozone exposure.

Fumigation. Ozone was generated by a high-voltage discharge generator and monitored with a photometric ozone analyzer (Model 400, API Co., USA). Ozone concentrations were controlled with two mass flow meters (100 cc min^{-1} and 1000 cc min^{-1}). Plants were exposed to 100 or 200 nl l^{-1} ozone for 8 h per day (from 08:00 to 16:00) for 10 days. Environmental conditions in the CEFCs were maintained at 25±0.5°C, 70% RH, and a PPFD of 1,000 $\mu molm^{-2}s^{-1}$, with a 14 h photoperiod. High-pressure sodium lamps were used as the PPFD source.

Physiological and biochemical assays.

Leaf gas exchange. Gas exchange, chlorophyll fluorescence and chlorophyll contents were measured one day before the termination of ozone exposure. Instantaneous gas-exchange measurements were made with the LI-6400 open-flow system using a saturating red light source at a PPFD of 1,500 $\mu molm^{-2}s^{-1}$. The cuvette temperature was held at the air temperature of CEFCs and the relative humidity within the cuvette was maintained at 40%. A series of five consecutive measurements on the same leaf was averaged for each individual plant and the mean values of 2 plants for each pot were used as the experimental unit. Afterwards, net photosynthesis and internal CO_2 concentration (C_i) were calculated by the built-in microprocessor.

Chlorophyll fluorescence. Chlorophyll fluorescence was used to assess the photochemical efficiency (Fv/Fm) of the photosystem II (PS II). Terminal leaflets of 10 fully expanded leaves for each pot were adapted to darkness by holding horizontally the leaflets in a leaf-clip holder (Morgan, USA) for 30 min and then the fluorescence was measured for 60 s with a steady-state modulated fluorimeter (Model 300, Morgan, USA). The measuring beam of the fluorimeter was provided by ultrabright red LEDs (650 nm, 1,200 $\mu molm^{-2}s^{-1}$). Values for maximum fluorescence (Fm) and ground fluorescence (Fo) were used for the calculation of variable fluorescence (Fv=Fm-Fo). The efficiency of excitation energy capture by open PSII reaction centers was estimated by the fluorescence ratio (Fv/Fm=(Fm-Fo)/Fm). During the fluorescence measurement, the leaf temperature was not controlled.

Chlorophyll absorbance. Chlorophyll absorbance was measured using a SPAD 501 meter (Minolta Instrument, Inc., Japan) on 40 leaflets of the fully expanded leaves of 10 plants in each pot. This measurement directly estimates the chlorophyll concentration in the leaf.

Polyamines. The liquid N_2-frozon leaf samples (1.0 g) were used to determine polyamine concentrations. Free and conjugated polyamines were extract, dansylated, solvent-purified, separated by TLC and quantified using a spectrophotofluorimeter (model RF-1501, Shimadzu, Japan) as detailed previously (Tiburico et al., 1985).

Phenols. Total phenols were determined using a modified method described by Wellburn & Wellburn (1996). Briefly, liquid N_2-frozen leaves (500 mg) were powdered and ground in ice-cold 99% (v/v) ethanol with use of mortar and pestle. After centrifugation for 10 min at 15,000 g, 800 µl of the supernatant was added to 3 ml of distilled water. Folin & Ciocalteu's reagent (300 µl) was added and thoroughly mixed using a Voltex mixer, and then 1 ml of Na_2CO_3 solution (20 g anhydrous per 100 ml) was added. The volume was adjusted to 10 ml with distilled water and the absorbance at 675 nm measured after 1 h at 25°C. Chlorogenic acid was used as a standard.

Soluble protein. Total soluble protein contents were determined by the method of Bradford (1976) using protein assay reagent from Bio-Rad (Hemel Hempstead, UK). Bovine serum albumin (BSA) was used as a standard.

Malondialdehyde (MDA). MDA was measured by a colorimetric method (Heath and Packer, 1968). Briefly, liquid N_2-frozen leaves (500 mg) were powdered and ground in 2.5 ml of distilled water with use of mortar and pestle. An equal volume of 0.5% TBA (2-thio-barbituric acid) in 20% trichloroacetic acid solution

was added and the sample was incubated at 95°C for 30 min. The reaction was stopped by placing the reaction tubes in an ice bath. The samples were then centrifuged at 15,000 g for 30 min. The supernatant was removed and was read at 532 nm, and the value for nonspecific absorption at 600 nm was read and subtracted from this. The amount of MDA present was calculated from the extinction coefficient of 155 $mM^{-1}cm^{-1}$ (Kwon et al., 1965).

Enzyme Assay.

Extraction. *Liquid N_2-frozen leaves (3 g) were powdered and* ground in 10 ml of 100 mM potassium phosphate buffer (pH 7.5) containing 2 mM EDTA and 1% PVP-40 at 4°C. The homogenate was centrifuged at 15,000 g for 30 min. The collected supernatants were maintained in ice and then directly used for enzyme assay of glutathion reductase (GR). For determination of ascorbate peroxidase (APX) activity, leaves were homogenized in 100 mM potassium phosphate buffer (pH 7.5) containing 1 mM EDTA and 5 mM ascorbate at 4°C.

GR (EC 1.6.4.2) assay. GR activity was determined by following the oxidation of NADPH at 340 nm (extinction coefficient 6.2 $mM\ cm^{-1}$) as described by Rao (1992). The 1 ml assay mixture contained 100 mM potassium phosphate buffer (pH 7.8), 2 mM EDTA, 0.2 mM NADPH, 0.5 mM GSSG, and the leaf extract. The assay were initiated by the addition of NADPH at 25°C.

APX (EC1.11.1.11) assay. APX activity was determined by following the decrease in the absorbance at 290 nm (extinction coefficient 2.8 $mM\ cm^{-1}$) as described by Chen and Asada (1989). *One ml of the reaction volume contained 100 mM potassium* phosphate buffer (pH 7.5), 0.5 mM ascorbate, and 0.2 mM H_2O_2 at 25°C. The reaction was initiated by adding the plant extract and followed for 5 min.

Results

Photosynthetic responses to ozone. Inhibitory effect of ozone on net photosynthesis of all the rice cultivars tested appeared to be dependent on the levels of ozone (Table 1). No significant reduction in net photosynthetic rates was caused by the low level (100 nl l^{-1}) of ozone, but large reduction in the rates was induced by the high level (200 nl l^{-1}) of ozone. Reduction in net photosynthetic rates was more pronounced in the sensitive cultivar DJ than in the tolerant cultivar IM or the moderately tolerant cultivar HY, compared with control. The low level of ozone also showed no difference in internal CO_2 concentrations of the cultivars from filtered-air. However, internal CO_2 concentrations were significantly increased by the high level of ozone. The sensitive cultivar DJ showed much higher increase in the concentrations by almost 40% than the less sensitive cultivars of HY and IM. It was likely that internal CO_2 concentrations were negatively correlated with net photosynthetic rates in the rice plants damaged at the high level of ozone.

The low level of ozone also had no effect on chlorophyll contents (leaf greenness) of the rice cultivars (Table 1). However, significant decrease in chlorophyll contents was observed in the plants exposed to the high level of ozone. Relatively smaller reduction in chlorophyll contents was found in the tolerant cultivar IM by 12% and the moderately tolerant cultivar HY by 11.9% than in the sensitive cultivar DJ by 22.1%.

Chlorophyll fluorescence was also significantly decreased by the ozone exposure at the high level in the all rice cultivars (Table 1). The sensitive cultivar DJ again showed the largest reduction in Fv/Fm ratios by more than 15%. The less sensitive cultivars of HY and IM showed smaller decrease in Fv/Fm ratios than the sensitive cultivar DJ.

Changes in soluble protein, MDA, total phenols and putrescine contents. Soluble protein contents were slightly decreased in the rice plants exposed to ozone at both concentrations, but the change was not significant in all the cultivars (Table 2).

Ozone-exposed rice plants showed elevated levels of MDA and the contents were increased with increasing

Table 1. Light-saturated net photosynthesis (Asat), internal CO_2 concentration (C_i), chlorophyll content (leaf greenness) and chlorophyll fluorescence of Korean local rice cultivars exposed to ozone[a]

	O_3 level ($nl\ l^{-1}$)	Ilmee (IM)		Hwayeoun (HY)		Dongjin (DJ)	
		Ozone	Control	Ozone	Control	Ozone	Control
Asat ($\mu mol\ m^{-2}\ s^{-1}$)	100	15.6±1.2	16.0±2.2	15.7±2.0	17.1±3.1	13.8±1.5	13.3±0.7
	200	9.8±1.0	13.9±2.4***[b]	6.6±0.2	12.5±1.6***	3.2±0.2	12.3±0.4***
C_i ($\mu mol\ mol^{-1}$)	100	301±17.7	287±3.5	269±15.0	279±11.3	289± 6.4	288±2.6
	200	302± 1.8	269±8.1***	302±1.8	284± 3.9***	353± 2.7	254±2.6***
Leaf greenness (SPAD value)	100	36.7±4.8	37.6±4.4	35.4±4.5	34.8±4.8	38.3±3.1	37.8±2.6
	200	30.9±5.3	35.1±4.3*	35.7±7.3	40.5±6.1*	31.4±6.8	40.3±4.6***
Chlorophyll fluorescence (Fv/Fm)	100	0.867±0.02	0.874±0.02	0.864±0.07	0.872±0.07	0.825±0.03	0.836±0.02
	200	0.842±0.01	0.870±0.01**	0.840±0.01	0.876±0.01***	0.730±0.03	0.867±0.05**

[a] Rice plants were exposed to 100 or 200 nl l^{-1} ozone 8 h per day for 10 days. The data represent the means and standard deviations of n replicates ($n = 10$ for Asat and C_i, $n = 20$ for chlorophyll fluorescence and $n = 80$ for leaf greenness).

[b] The asterisks indicate significant difference between control and ozone exposure at $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

Table 2. Sobule protein, malondialdehyde (MDA), total phenolics and putrescine contents of Korean local rice cultivars exposed to ozone[a]

	O_3 level (nl l^{-1})	Ilmee (IM)		Hwayeoun (HY)		Dongjin (DJ)	
		Ozone	Control	Ozone	Control	Ozone	Control
Soluble protein (mg gfw^{-1})	100	185.7±4.2	190.1±1.3	178.5±6.2	182.1±5.1	200.7±14.5	206.3±11.3
	200	181.2±2.0	185.7±2.4	194.8±9.8	197.5±4.1	185.3± 3.6	194.0± 4.7
MDA (μmol gfw^{-1})	100	5.53±0.32	5.15±0.25	5.50±0.26	5.12±0.35	6.94±0.08	5.35±0.10***
	200	6.72±1.58	5.60±0.30	7.36±0.72	5.56±0.60***	8.48±0.65	5.68±0.20***
Phenolics (mg gfw^{-1})	100	3.60±0.50	3.37±0.36	3.88±0.63	3.43±0.44	3.54±0.08	3.55±0.12
	200	3.70±0.35	3.52±0.16	2.74±0.08	3.58±0.20***	2.58±0.50	3.71±0.13***
Putrescine (mmol gfw^{-1})	100	7.04±0.77	4.63±0.46*[b]	8.59±0.92	4.44±0.92*	10.11±0.73	4.16±0.39***
	200	5.60±0.72	4.40±0.16**	8.58±1.25	4.74±0.61**	6.51±1.09	4.56±0.61*

[a] Rice plants were exposed to 100 or 200 nl l^{-1} ozone 8 h per day for 10 days. The data represent the means and standard deviations of 10 replicates.
[b] The asterisks indicate significant difference between control and ozone exposure at $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

ozone level in all the cultivars (Table 2). The sensitive cultivar DJ exhibited highly significant difference in MDA contents between polluted air and control by nearly 30% and 50% at the level of 100 nl l^{-1} and 200 nl l^{-1}, respectively. The moderately tolerant cultivar HY showed significant increase in MDA contents by almost 30% only after 200 nl l^{-1} ozone exposure. Ozone-induced increase in MDA contents was also observed in the tolerant cultivar IM, but the difference was not significant between ozone treatment and control.

Ozone-induced changes in total phenols were dependent on ozone levels and the rice cultivars (Table 2). Ozone exposure increased the content of total phenols in the tolerant cultivar IM by 5 to 7%, compared with control, but the increase was not significant at the both levels of ozone. However, the sensitive cultivar DJ showed significant reduction in total phenols by nearly 24% at 200 nl l^{-1} ozone treatment. Interestingly, the moderately tolerant cultivar HY exhibited increase in total phenols by 13% at low concentration of ozone, but significant decrease by almost 24% at high ozone exposure.

Significant increase in putrescine was also caused by ozone exposure at both concentrations in all the rice cultivars (Table 2). The enhancement of putrescine levels was more pronounced at the low level than at the high level of ozone. Especially, in the sensitive cultivar DJ, the exposed plants exhibited high amounts of putrescine as much as 2.5 times, compared with control plants. The elevated levels of putrescine were likely related with sensitivity of the cultivars to ozone.

Antioxidant enzyme activities. Antioxidant enzyme activities of glutathion reductase (GR) and ascorbate peroxidase (APX) were enhanced by the ozone exposure (Table 3). Much higher elevation of the enzyme activities was found in the tolerant IM cultivar than in the less tolerant HY and DJ cultivars. APX activity was more highly enhanced than GR activity. More pronounced increase in the enzyme activities was observed at the low concentration than the high concentration of ozone. Unlike the less sensitive cultivars of IM and HY, the sensitive cultivar DJ showed no activation of the enzymes at the high ozone concentration.

Discussion

In this study, the highest levels of GR and APX activities were induced by the ozone exposures in tolerant IM culti-

Table 3. Activity of antioxidative enzyme, glutathion reductase (GR) and ascorbate peroxidase (APX) in sensitive and tolerant rice cultivars exposed to ozone[a]

	O_3 level (nl l^{-1})	Ilmee (IM)		Hwayeoun (HY)		Dongjin (DJ)	
		Ozone	Control	Ozone	Control	Ozone	Control
GR (mmol g $protein^{-1}$ min^{-1})	100	8.66±0.13	5.48±0.36***[b]	6.93±0.26	5.25±0.14**	6.48±0.25	5.81±0.16**
	200	7.46±0.80	4.71±0.39*	6.41±0.16	5.11±0.42*	5.41±0.16	5.33±0.14
APX (mmol g $protein^{-1}$ min^{-1})	100	7.96±0.44	4.74±0.36***	5.64±0.44	4.16±0.34***	6.84±0.44	4.91±0.59***
	200	7.15±0.47	4.05±0.21***	5.32±0.36	4.11±0.39*	4.65±0.67	5.68±0.31

[a] Rice plants were exposed to 100 or 200 nl l^{-1} ozone 8 h per day for 10 days. The data represent the means and standard deviations of 10 replicates.
[b] The asterisks indicate significant difference between control and ozone exposure at $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

var. This suggests that activation of the GR-APX cycle may be responsible for protection of rice plants against ozone damage and determine differential sensitivity of the rice cultivars to ozone. Photosynthetic activity appeared to be a sensitive response to ozone and combined measurement of chlorophyll fluorescence and leaf greenness can provide very reliable indication of ozone-induced damage to photosynthesis of rice plants. Formation of MDA was also considered as a clear indication of relative ozone damage. However, the changes in polyamines and total phenolics were not consistent with exposed ozone concentrations and/or ozone sensitivity of the cultivars. The behavior of polyamines and phenolics could be interpreted in this study as an indication of ozone injury rather than activation of additional protection mechanisms scavenging active oxygen species formed by ozone.

It is likely that the rice cultivars were endurable to ozone stress at 100 nl l^{-1} ozone concentration which is frequently monitored during ozone episodes in summer (Yun et al., 1999). It was already demonstrated that the rice plants of cultivar DJ exposed to ambient ozone during the whole growing season under the field condition were not affected with no yield reduction, which was not different from the rice plants exposed to activated charcoal filtered-air (Hur and Lee, 1998). Insensitivity of rice plants to low ozone concentrations is also confirmed by previous reports (Kats et al., 1985; Kobayashi and Okada, 1995; Nakamura et al., 1975; Nouchi et al., 1995). However, severe damages were induced and sensitivity to ozone was clearly differentiated among the rice plants by the high concentration of 200 nl l^{-1} ozone. Several responses triggered at the high concentration could explain the differential sensitivity of the rice cultivars and be used as reliable indications of relative ozone damages.

Net photosynthesis, chlorophyll contents and chlorophyll fluorescence were simultaneously decreased with increasing ozone concentrations in all the cultivars. The magnitude of ozone damage to photosynthetic activity was closely proportional to the sensitivity of the rice plants to high ozone concentration. This is believed that differential photosynthetic responses to ozone can be a clear indication of relative ozone damage to rice plant. As previously reported in several plants (Reich, 1983; Reichenauer et al., 1997), the exposure of 200 nl l^{-1} ozone caused a significant reduction in the light-saturated photosynthesis (Asat) of the rice cultivars. This decrease in CO_2 uptake was not mainly due to closure of stomata, since intercellular CO_2 concentration rose under the elevated ozone concentrations (Schenone et al., 1994). Positive correlation between Asat and C_i would be only expected if CO_2 uptake was limited by stomatal closure (Farquhar and Sharkey, 1982). In contrast, negative correlation between them was found at the high ozone level in this study. This result implies that the rice leaves damaged by the high ozone level could not actively use the intercellular CO_2 and thus the restriction of CO_2 uptake could be attributed to an impairment of carboxylation efficiency (Farage et al., 1991) and/or the electron transport chain (Reichenauer et al., 1997). A simultaneous decrease in the current photochemical capacity of PSII (chlorophyll fluorescence; Fv/Fo ratio) measured after dark-adaptation strongly supports that the reduction of Asat in ozone-exposed rice leaves was due to an impairment of PSII photochemistry. The decrease in leaf greenness (chlorophyll content) also supports that the functionality of PSII in rice plants was affected under ozone exposure.

It was suggested that an impairment of carboxylation efficiency was the initial effect of ozone on photosynthesis (Farage et al., 1991). They found a decrease in carboxylation efficiency, but no change in the current quantum yield of CO_2 uptake or O_2 evolution in *Triticum aestivum* (after 16 h of treatment with 200 nl l^{-1} ozone). A decrease in current photochemical capacity and quantum yield was only detectable following treatment with a high concentration of 400 nl l^{-1} ozone. Pell et al. (1992) also reported that ozone stress induces degradation and/or inhibition of Rubicose synthesis in *Populus maximowizii trichocarpa* NE388. They argued that oxidation of the enzymes by ozone or ozone-induced free radicals could render it more vulnerable to proteolysis. However, this study clearly demonstrated that the current photochemical capacity of PSII was severely impaired by ozone in the rice plants and the degree of PSII mal-functionality was likely to be correlated with the sensitivity of rice plants to ozone stress. Therefore, chlorophyll fluorescence can be applied as an easy and non-destructive (repeatable) measurement to estimate ozone-induced damage to plants in the field, compared with laborious and destructive (disposable) measurement of carboxylation efficiency such as Rubicose activity.

SPAD units express leaf greenness related to chlorophyll contents. Although the SPAD measurements have arbitrary units and can be affected by leaf thickness, previous studies demonstrated that they provide a reliable indication of relative O_3 injury (Olszyk and Wise, 1997; Tenga et al., 1989). The injury response of the rice cultivars with different sensitivity to high ozone concentration as measured in SPAD units was similar to the reduction in chlorophyll fluorescence. Therefore, measurement of leaf greenness can also be applied as a nondestructive indication of relative O_3 damage to plants along with chlorophyll fluorescence measurement.

It is well known that ozone is potent catalyst of the peroxidation of membrane lipids (Menzel, 1976) and the presence of oxidation products such as malondialdehyde (MDA) is directly related to the beginning of peroxidation

of unsaturated fatty acids in biological system (Mehelman and Borek, 1987). Since every type of membrane is sensitive to oxidation processes generated by ozone-induced free radicals, elevated levels of MDA indicate that degradation of cell membrane has been progressed in ozone-exposed rice plants. Adverse effect of ozone on cell membrane integrity was well agreeable with the difference in sensitivity of the rice cultivars to high concentration of ozone. This result suggests that MDA measurement is also very reliable indication of relative O_3 damage to plants.

Phenolic compounds were suggested to be possibly involved in protecting plant cells from ozone injury (Jordan et al., 1991; Wellburn and Wellburn, 1996). Unlike previous results, large reduction in total phenols at the high ozone concentration suggests that changes of total phenols in rice plants could be an indication of cellular damage induced by ozone exposure. Retarded synthesis of secondary metabolites such as phenolic compounds may be due to the extra costs to repair cellular and metabolic damages (Runeckles and Chevone, 1991).

Polyamines have been known to play important roles in preventing cells from oxidative stress by eliminating active oxidants induced by ozone (Bors et al., 1989; Smith, 1985). Ozone-exposed cells appeared to gain protection from ozone by increasing pools of polyamines or by synthesizing quickly protective amounts of them (Langerbartels et al., 1991). A previous report showed that a tolerant cultivar had more putrescine than sensitive one (Wellburn and Wellburn, 1996). It was also suggested that there was O_3-sensitive inputs and outputs to and from the polyamine pool (Kangasjavi et al., 1994). However, in this study, putrescine contents were decreased with increased tolerance of rice plants to ozone. No significant changes in spermidine and spermine levels were also found in the ozone-exposed rice plants (data not shown). This implies that enhanced level of putrescine in the sensitive cultivar might be due to the inhibition of conversion of putrescine to other polyamines such as spermidine (Wellburn and Wellburn, 1996). Although no precise researches on polyamines behavior in rice plants exposed to ozone have been attempted, changes in polyamines following ozone treatment may be the consequence of ozone injury rather than the activation of defense system against ozone damage.

Ozone is believed to be converted into active oxygen species such as superoxide anion (O_2^-), hydroxyl radicals (OH), and H_2O_2 in the mesophyll cells (Mehlhorn et al., 1990). The antioxidant defense system consists of low molecular weight antioxidants such as ascorbate, glutathione and carotenoids, as well as several enzymes such as superoxide dismutase, catalase, peroxidase, glutathion reductase (GR) and ascorbate peroxidase (APX) (reviewed by Bowler et al., 1994; Creissen et al., 1994). APX catalyzes the first step of H_2O_2 scavenging pathway by oxidizing reduced ascorbate (ASA). GR and APX are believed to act in conjunction to metabolize H_2O_2 to H_2O. This study also confirmed that the ascorbate-glutathion cycle was activated in the rice plants to metabolize ozone-induced activated O_2 species. Tolerance of the rice cultivars to ozone was paralleled to the efficacy of the cycle. This finding agrees with previous results suggesting that high redox state of the ascorbate-glutathion cycle develops plant tolerance to oxidative stress (Creissen et al., 1994; Edwards et al., 1994; Foyer et al., 1994).

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