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Individual Differences of Ozone Resistance for Seed Germination and Seedling Development of *Pinus thunbergii*

Du-Hyun Kim* and Sim Hee Han
Department of Forest Resources Development, Korea Forest Research Institute, Suwon 441-350, Korea
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해송의 종자 발아 및 유묘 생장에 대한 오존저항성의 개체간 차이

김두현* · 한심희
국립산림과학원 산림자원육성부
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ABSTRACT

Individual differences of ozone (O_3) resistance for seed production, seed germination and seedling development were examined in this study. Five in each healthy and damaged trees of *Pinus thunbergii* growing in air polluted area for 12 years were chosen based on visible foliar injury and growth. The cones of *P. thunbergii*, which were collected from healthy and damaged trees, were analyzed for physical characteristics and seeds from the cones were used to test germination percentage under O_3 treatment. The germinated seeds were continuously exposed to O_3 treatment and the lipid peroxidation and activities of antioxidative enzymes were determined for both seeds and seedlings. The O_3 treatment for seed germination and seedling development were conducted at three conditions: control, 150 ppb and 300 ppb of O_3. The non-treated seeds from the damaged trees showed 21.6% lower germination than those from the healthy ones. On the O_3 treatment of 300 ppb, seed germination decreased approximately 10% for the healthy trees and 19% for the damaged trees compared to that on the control. The seeds from the healthy trees showed significantly higher activities of superoxide dismutase (SOD), glutathione reductase (GR), and catalase (CAT) than those from the damaged trees. The activities of GR, ascorbate peroxidase (APX), and CAT decreased along with the increasing O_3 concentration in two tree grades. Malondialdehyde (MDA) content of seeds was not influenced by O_3 treatment for two tree grades. In seedling development, there were no significant differences for length and biomass of needle and root of two tree grades at both the control and 150 ppb of O_3. At 300 ppb of O_3 treatment, however, the length and biomass of needle and stem decreased for two tree grades but no significant differences was detected in root. The seedlings from the damaged trees were more sensitive to the O_3 treatment, showing higher activities of SOD, APX, and CAT and content of MDA compared to those from the healthy tree seedlings. Our results indicate that seed germination and seedling development are vulnerable to increasing O_3 concentrations and that attention must be paid to the individual selection of tree species for reforestation.

Key words **: Black pine, Ozone, Germination, Antioxidative enzyme, Lipid peroxidation**

I. INTRODUCTION

Ozone (O_3) is the most important phytotoxic air pollutant in many areas of Asia, Europe, North and Central America (Ashmore, 2005). Tropospheric O_3 concentrations have increased largely since pre-industrial times and they are predicted to continue rising simultaneously in the future (IPCC, 2007).

* Corresponding Author: Du-Hyun Kim (dhkim@forest.go.kr)

Exposure to O_3 affects both vegetative and reproductive growth in plants, although consequences for seed yield depend on the efficacy of compensatory reproductive processes. There were several reports indicating improvements of seed germination by O_3 gas or aqueous O_3 treatments in agricultural crops (Yvuin and Coste, 1995; Violleau *et al*, 2008). However, negative effect of ozone on seed germination and seedling growth in pine, spruce, and wheat were also reported (Wu *et al*., 2006; Prosherina *et al*., 2009).

O_3 induces an increase in production of reactive oxygen species (ROS) and results in the oxidative damage of cell constitutes such as proteins, lipids and pigments in leaves. Similarly, plant growth and productivity may be negatively affected (Iriti and Faoro, 2008). Antioxidative systems are considered to play a fundamental role in mediating O_3 resistance by scavenging ROS (Tausz *et al*., 2007). The antioxidative systems consist of small molecular antioxidants and antioxidative enzymes. Superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11), glutation reductase (EC 1.8.1.7) and catalase (CAT, EC 1.11.1.6) are crucial for ROS-scavenging enzymes in plant cells. SOD catalyzes the dismutation of O_2^- to molecular oxygen and H_2O_2, which can be subsequently scavenged by APX and CAT. They work together to control the concentrations of O_2^- and H_2O_2, which then limits the generation of $^{\cdot}OH$, the most dangerous species of all the ROS (Mittler, 2002). Malondialdehyde (MDA) concentration, which estimates the state and integrity of membrane through the degree of lipid peroxidation, has been shown to correlate with the level of O_3 exposure. It was reported that O_3 treated plants showed an increase in MDA content (Ranieri *et al*., 1996; Iglesias *et al*., 2006). Lipid peroxidation is the ultimate effect of O_3 attack to membranes, once the antioxidant defenses of membranes fail to cope with O_3 and oxygen radicals (Calatayud *et al*., 2002; Iglesias *et al*., 2006). Consequently, all plants contain these antioxidant metabolites, but it is not clear whether sufficient quantities are present to maintain metabolic functions and participate in O_3 detoxification reactions. Genetic differences in the antioxidant content of imbibed seeds and seedlings could account for differences in O_3 sensitivity.

Black pine, *Pinus thunbergii* Parl., is a native tree species to coastal areas of Korea and Japan. Because of its resistance to pollution and salt tolerance, it is a popular horticultural tree and recommended for rehabilitation of reclaimed coastal lands with extreme environmental conditions. In our research, we selected healthy and damaged trees of *P. thunbergii* as a seed source that were grown an experimental plantation in the middle of Yeocheon industrial complexes since 1996. The experimental forest constructed for the breeding of tolerant tree species or varieties is deemed necessary to produce suitable tree species for the restoration of disturbed forest ecosystem on this kind of environmental conditions.

O_3 experiments have been mainly conducted with seedlings, and their physiology, growth, needle structure, phytochemistry, and signaling. Molecular changes have been extensively studied in *Quercus* species (Kim *et al*., 2008), hybrid aspen (Häikiö *et al*., 2009), citrus (Iglesias *et al*., 2006), *Betular* species (Oksanen *et al*., 2009; Pääkkönen *et al*., 1997), and Russian pine and spruce (Prozherina *et al*, 2009). However, there is little information about O_3 sensitivity of *P. thunbergii*, and the impact of O_3 on early plant development processes is still poorly understood. Therefore, in this experiment, we study changes in seed germination and early growth of *P. thunbergii* seedlings exposed to elevated O_3 levels. We hypothesized that O_3 will have negative effects on seed production, germination and seedling development. The first objective was to measure the effect of O_3 on seed germination and seedling growth for two different tree grades (i.e., healthy and damaged trees) that may differently response to O_3 treatment. The second objective was to compare the activities of antioxidative enzymes in seeds and seedling of the tree grades and attempt to identify response patterns that distinguish O_3 sensitive and tolerant individuals.

II. MATERIALS AND METHODS

2.1. Plant materials and treatments

Two different grades of *P. thunbergii* tree based on growth responses were chosen for this study. Each of five healthy and damaged trees of *P. thunbergii,* that were growing in Yeocheon industrial complex area for 12 years (1996-2008), were selected by visible foliar injury and growth according to Alexander and Shelley(1987)'s manual. Cones were collected from each of five healthy and damaged trees of *P. thunbergii* that were grown under high O_3 condition and air polluted industrial area for 12 years. The average O_3 concentration for 12 years in Yeocheon site (27 ppb) was higher than national average (20 ppb) according to Report of Korea Ministry of Environment (2008). Cones were collected from those trees to perform cone analysis fol-

lowing Lee *et al*. (1984)'s method. After cone analysis, seeds and seedlings were transferred and set up in the growth chambers (3 m × 3 m × 1.8 m (L × W × H, 16.2 m^3) that were used to establish either a charcoal filtered (CF) air control (5 ± 1 ppb O_3) or a treatment where CF air was supplemented with O_3 from 8:00 to 20:00 h with a daily 12 h mean of 150 ppb ± 10 ppb and 300 ppb ± 10 ppb (fumigation), respectively. O_3 treatment levels were based on Korea Air Pollution Warning system. O_3 warnings are issued when exceed 120 ppb, and highest warning level that are considered very unhealthy for people are above 300 ppb averaged over one hour. As of 2008, the highest concentration per hour was 203 ppb in Yeocheon (Ministry of Environment, 2008). Air temperature and relative humidity were controlled at 22-24°…and 60-80% RH. The mixed humid and ozonated air entered through the bottom of each chamber and exited the chamber top via two exhaust filters. Airflow was maintained at about 1 ml s^{-1}. The O_3 concentrations were continuously monitored with a photometric O_3 analyzer (Model 400, API, Inc., USA).

2.2. Seed germination and seedling development

Healthy and damaged trees were compared for O_3 tolerance in seed germinability and seedling growth by measurements of cone analysis, germination, and antioxidant levels. Germination tests were performed twice after cone and seed character analysis. The first germination test was done in a soil inside green house for 50 days while the second germination test was conducted inside a chamber for O_3 treatments. For the second test, seeds were germinated inside a Petri dish with two layers of moist filter paper. Four 50-seed replicates for each treatment were used. Germination counts were performed daily for 28 days and germination was considered to have occurred if the radicle protruded was about 2 mm or longer from the seed coat according to the ISTA rules (ISTA, 1999). The total germination percentage was expressed as the average of the four replicates. The parameters gathered include: germination percentage, mean germination time (MGT) and germination value (GV) according to Kim *et al*. (2010). Seedlings were transferred into plug tray in the same growth chamber after germination test in Petri dish. A commercial soil for tree seedling composes of mixture of perlite, peat moss and vermiculite in the ratio of 1:1:1 including nitrogen (150±50 mg/L) and P_2O_5 (100±100 mg/L). Growth, MDA content and activities of antioxidative enzymes were measured for 10-week-old seedling grown under O_3 fumigation. Biomass of seedlings was determined after harvesting and oven drying the shoots and roots at 70°C for 48 h.

2.3. Lipid peroxidation

Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) produced by thiobarbituric acid reaction as described by Heath and Packer (1968). O_3-treated seeds (weighed 0.1 g prior to imbibition) imbibed for three days and seedlings (weighted 0.1 g) were homogenized in 5 ml of 62.5 mM phosphate buffer (pH 7.8). The crude extract was mixed with the same volume of 0.5% (w/v) thiobarbituric acid solution containing 20% (w/v) trichloroacetic acid. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice-bath. The mixture was centrifuged at 3000 × g for 10 min and the absorbance of the supernatant was monitored at 532 and 600 nm. After subtracting the non-specific absorbance (600 nm), MDA concentration was determined by its molar extinction coefficient (155 mM^{-1} cm^{-1}) and the results expressed as ìmol MDA g^{-1}FW.

2.4. Antioxidative enzyme activities

Activities of antioxidative enzyme determined to assess whether antioxidant levels in seeds and seedlings were related to O_3 tolerance. Imbibed seeds for 3 days (0.1 g) and 10 weeks old seedling (0.1 g) under O_3 fumigation conditions that were weighted and frozen with liquid nitrogen for analysis were homogenized under ice-cold condition with 5 mL of 50 mM phosphate buffer (pH 7.0), 10 mM ascorbic acid (AsA) and 1.0% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 20,000×g for 30 min, and the supernatant was collected for enzyme assays. Superoxide dismutase (SOD) was assayed based on the inhibition of reduction of nitro-blue tetrazolium in the presence of xanthine at 530 nm according to the method of Beauchamp and Fridovich (1971). Ascorbate peroxidase (APX) activity was determined by the method of Nakano and Asada (1981). Activity of glutathione reductase (GR) was assayed as described in Carlberg and Mannervik (1985). Catalase (CAT) activity was determined by following a two-step procedure as described by Fossati *et al*., (1980). All the activities of enzyme were measured using UV-120 (Shimadzu, Japan).

2.5. Statistical analysis

The data were statistically analyzed using SAS Sys-

tem for Windows, Version 8.01 (SAS Institute, USA). Mean values per treatment were compared by generalized linear model (GLM). When significant differences ($p < 0.05$) were indicated, Duncan's multiple range tests were performed.

III. RESULTS AND DISCUSSIONS

3.1. Seed production and germination

Cone analysis results were compared between healthy and damaged trees in Table 1. Cone length and weight of healthy trees were significantly higher than damaged ones at $p < 0.05$. The cone length and weight from healthy trees were higher by 17% and 56%, respectively based on their counterpart from damaged trees. However, there were no significant differences in other seed characteristics except seed efficiency of 42.6 % for the healthy trees and 29.0% for the damaged trees.

Meanwhile, seeds from healthy trees germinated under control and elevated O_3 conditions showed significantly higher germination rate (>80%) than those from damaged trees (<69%). In addition, the germination percentages were decreased in both the healthy and damaged trees under O_3 treatment. The germination reduction rate at 300 ppb of O_3 treatment was 10% and 19% in healthy and damaged tree seeds, respectively. Mean germination time (MGT) showed significant difference in both tree grades and O_3 treatments, but no significant interaction effect between tree grade and O_3 treatment was

Table 1. Cone and seed characteristics of two different grades (i.e., healthy and damaged trees) of *P. thunbergii* trees

Parameter	Healthy (H)		Damaged (D)		Pr>F
	Number of seed	%	Number of seed	%	H × D
Cone length (mm)	39.4		33.64		*
Cone width (mm)	32.49		25.13		ns
Cone dry weight (g)	6.71		4.26		*
Total no. of seeds	274.60		202.40		ns
Seed mass/100 seeds (mg)	1.38		0.91		ns
Seed potential	87.7		79.5		ns
First-year aborted ovules	11.0	12.5	9.7	12.2	ns
Second-year aborted ovules	7.6	8.7	6.4	8.0	ns
Developed seeds	40.7	46.3	37.2	46.8	ns
Filled seeds	37.4	92.0	23.1	62.0	ns
Empty seeds	3.3	8.0	14.1	38.0	ns
Germinated seeds	68.1	77.6	42.0	52.8	ns
Seed efficiency		42.6		29.0	*

All the values are means of five replicates ± SD. * $p < 0.05$ and ns: non-significance.

Table 2. Effect of ozone fumigation on germination, mean germination time (MGT), and germination value (GV) in seed from healthy and damaged *P. thunbergii* trees

Grade	O_3 (ppb)	Germination (%)	MGT (day)	GV
Healthy	0	88±9.1a	7.7±1.11cd	24.4±5.22a
	150	88±11.0a	9.4±1.30b	21.9±5.72a
	300	80±7.1ab	7.2±0.33d	20.1±2.66a
Damaged	0	69±9.6bc	8.0±0.55cd	13.4±3.28b
	150	61±9.6c	11.1±0.74a	9.8±3.05b
	300	58±9.1c	8.95±1.38bc	9.8±3.14b
Pr>F	Grade (G)	***	**	***
	Ozone (O)	ns	***	ns
	G × O	ns	ns	ns

All the values are means of five replicates ± SD: Values with the different letter indicate significant differences ($p < 0.05$) according to Duncan's test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and ns: non-significance.

observed. The seeds from damaged trees showed higher MGT than the seeds from healthy trees, regardless of the O_3 treatment, and 150 ppb of O_3 treatment increased the MGT of the two seed source. High GV value means high germinability of seed. Healthy tree seeds showed significantly higher GV than damaged ones and O_3 treatment resulted in lower GV than control (Table 2). Our study showed that the elevated O_3 levels tend to impair germination processes. Recent experiments with Russian pine, spruce and corn (*Zea mays*) also showed that long-term O_3 exposure was unfavorable for seed growth, although very short O_3 pulses for corn resulted in earlier start of germination (Prozherina *et al*. 2009; Violleay *et al*., 2008). It is likely that O_3 disturbed cell division and the enlargement process during the germination phase. The negative impact of O_3 on seed development was observed as reduced seed weight and germination rate in paper birch (*Betula papyrifera*) (Darbah *et al*, 2008). Our results also suggest that the seed from damaged trees under the elevated O_3 level are more sensitive to increasing O_3 concentrations than those of seeds from healthy trees. This finding was supported by the reports from North America, where natural selection for O_3-tolerant genotypes has been reported for *Populus tremuloides* (Berrang *et al*., 1989). On the other hand, positive effect of O_3 on seed germination has been described that germination and growth properties of corn, haricot, barley and sunflowers seeds have been increase after treatment by Yvin and Coste (1995) and Violleau *et al*. (2008).

Overall, O_3 sensitive and tolerant tree seeds were distinguished by differences in seed antioxidative activity. Seeds from healthy trees showed significantly higher activities of SOD, GR, and CAT, while the activities of SOD in seeds from damaged tree were significantly reduced with increased O_3 concentration. There was no significant difference in APX activities for tree grade alone. However, GR, APX, and CAT activities were decreased with the increased O_3 concentration. There was no significant differences due to grade of trees in the malondialdehyde (MDA) content ($p > 0.05$). In healthy trees, exposure of seeds to increasing amount of O_3 concentration resulted in an increase in MDA content by as much as 56% at 300 ppb of O_3 treatment but no observable change in damaged tree seeds (Table 3). Sasaki *et al*., (2005) reported that an increase of germination and seedling growth was related with expression of gene encoding ascorbate peroxidase (APX) and catalase (CAT) in rice seed. The increase of SOD, GR and CAT activities and the decrease of lipid peroxidation was also related to the high seed germinability of *P. thunbergii* seed (Kim *et al*., 2010). However, the changes of MDA content of seeds did not reflect O_3 sensitivity between tree grades in our results. It suggested that the increase in activity of antioxidative enzymes was an acclimation effect, but three days of O_3 treatment for seed was not sufficient to result in the increase in lipid peroxidation as shown by MDA content.

3.2. Seedling quality

Growth and biomass of seedlings germinated from healthy and damaged tree seeds were compared in Table 4. There were no significant differences for length and biomass of needle and root between healthy and damaged tree seedlings. However, significant dif-

Table 3. Effect of ozone fumigation on antioxidative enzyme activities and MDA contents in seed from healthy and damaged *P. thunbergii* trees

Grade	O_3 (ppb)	SOD (unit g^{-1})	GR (nM g^{-1})	APX (umol min^{-1} g^{-1})	CAT (unit g^{-1})	MDA (uM g^{-1})
Healthy	0	606±52bc	6228±51a	74±15a	255±61a	0.71±0.10c
	150	746±76a	5602±81c	24±26b	226±50ab	1.32±0.42ab
	300	633±28bc	5963±258b	25±9b	205±36abc	1.60±0.62a
Damaged	0	667±76b	5270±212d	56±12ab	155±72bc	1.31±0.52ab
	150	502±46d	5007±211e	23±17b	132±60c	0.99±0.25bc
	300	567±37cd	5007±107e	94±54a	127±60c	1.41±0.60ab
Pr>F	Grade (G)	**	***	ns	**	ns
	Ozone (O)	ns	***	**	*	**
	G × O	***	ns	ns	ns	*

All the values are means of five replicates ± SD; Values of each plant part among in healthy and damaged significant different ($p < 0.05$) according to Duncan's test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and ns: non-significance.

Table 4. Effect of ozone fumigation on growth and biomass in seedling from healthy and damaged *P. thunbergii* trees Length (cm)

Grade	O_3 (ppm)	Needle	Stem	Root
Healthy	0	3.48±0.50a	3.64±0.78ab	9.76±1.86ns
	150	3.50±0.61a	3.95±0.57a	13.50±5.87
	300	2.52±0.61b	2.92±0.48bc	8.82±2.40
Damaged	0	2.98±0.58ab	3.22±0.43abc	12.86±7.26
	150	3.44±0.34a	3.36±0.57ab	15.08±6.26
	300	2.34±0.18a	2.54±0.49c	12.40±7.99
Pr>F	Grade (G)	ns	*	ns
	Ozone (O)	***	**	ns
	G × O	ns	ns	ns
Biomass (g)				
Grade	O_3 (ppm)	Needle	Stem	Root
Healthy	0	0.18±0.08ab	0.027±0.006ab	0.047±0.086ns
	150	0.20±0.08ab	0.029±0.007a	0.087±0.070
	300	0.12±0.07b	0.018±0.007c	0.035±0.033
Damaged	0	0.18±0.05ab	0.019±0.005bc	0.042±0.025
	150	0.27±0.09a	0.028±0.006a	0.065±0.027
	300	0.14±0.05b	0.015±0.007c	0.031±0.023
Pr>F	Grade (G)	ns	ns	ns
	Ozone (O)	**	**	ns
	G × O	ns	ns	ns

All the values are means of five replicates ± SD; Values of each plant part among in healthy and damaged significant different ($p < 0.05$) according to Duncan's test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and ns: non-significance.

ferences due to tree grades or O_3 treatment alone on stem length were observed and that the stem biomass was only affected by O_3 treatment but not the tree grade. In general, the stem length of seedlings originated from healthy tress was longer than those from damage trees regardless of the O_3 treatment. On the other hand, a reduction of stem length was observed with increasing O_3 concentration regardless of the source of seedlings. Specifically, 300 ppb of O_3 fumigation decreased in length and biomass of needle and stem at $p < 0.05$ but it showed no significant differences in root, which is in accordance with Prozherina *et al.* (2009)' study, in which cumulative O_3 stress induced a reduction in height and shoot dry mass growth of Scots pine seedlings but not in root growth. This trend is contradictory in respect to other studies that found a greater reduction in root biomass as compared to shoots (Paludan-Müller *et al.*, 1999; Rebbeck and Scherzer, 2002). Novak *et al.* (2008) reported that *Viburnum* root biomass affected negatively as result of O_3 stress, whereas root biomass in *Platanus occidentalis* tended to be no stimulated under O_3 stress (Kim *et al.*, 2008). As previous studies, growth reductions induced by O_3 were accompanied by injuries of photosynthetic machinery and impaired decrease of carbohydrate gain, which negatively affected the early growth of foliage first (Utriainen and Holopainen, 2000; Oksanen, 2003). Species, O_3 concentration, or duration of treatment could be reason for different response of O_3. Our data show that O_3 treatment decrease seed germination and seedling growth. The results on seedling growth parameters indicated that there are differences in O_3 responses within same species, as previously reported for *Pinus sylvestris, Picea* species (Prozherina *et al*., 2009*), Betula pendula* (Pääkkönen *et al*., 1997), and *Populus* species (Häikiö *et al*., 2009), but more extensive studies are needed to correlate O_3 responses with growth rate. It is well shown in model systems that O_3 sensitivity/tolerance is a complex outcome of physiologic characters (e.g., stomatal conductance, regulation), antioxidants, anatomic properties, signaling pathways, stress hormones, and action of several genes and not directly linked to growth rate (Oksanen *et al*, 2009; Overmyer *et al*, 2008). In addition, in birch and aspen studies, fast-growing and slow-growing genotypes have been found in both O_3-sensitive and O_3-tol-

Table 5. Effect of ozone fumigation on antioxidative enzyme activities and MDA content in seedling from healthy and damaged *P. thunbergii* trees

Grade	O_3 (ppb)	SOD (unit g^{-1})	GR (nM g^{-1})	APX (umol min^{-1} g^{-1})	CAT (unit g^{-1})	MDA (uM g^{-1})
(a) Shoot						
Healthy	0	500±56c	8578±388c	164±29c	1749±113cd	3.27±0.25a
	150	541±69bc	8097±316c	38±18d	1595±45de	3.00±0.17ab
	300	591±38ab	13607±749b	218±18a	1424±36e	3.19±0.30a
Damaged	0	505±94c	8407±375c	137±21c	1836±30bc	3.12±0.25a
	150	608±44ab	2869±175d	141±9c	1988±263ab	2.72±0.21b
	300	670±23a	15456±573a	192±17b	2066±275a	3.23±0.37a
Pr>F	Grade (G)	*	***	*	***	ns
	Ozone (O)	**	***	***	ns	**
	G × O	ns	***	***	***	ns
(b) root						
Healthy	0	328±61b	4947±130b	119±58e	469±175b	2.70±0.14a
	150	363±64b	1821±42e	354±19c	368±44b	2.26±0.17cd
	300	353±35b	5802±295a	230±94d	451±55b	2.16±0.13d
Damaged	0	457±56a	3960±163c	794±43a	685±264a	2.44±0.12bc
	150	376±86b	3192±166d	694±31b	468±66b	2.64±0.13ab
	300	353±44b	4091±155c	143±17e	424±39b	2.52±0.20ab
Pr>F	Grade (G)	*	***	***	ns	**
	Ozone (O)	ns	***	***	*	**
	G × O	*	***	***	ns	***

All the values are means of five replicates ± SD; Values of each plant part among in healthy and damaged significant different ($p < 0.05$) according to Duncan's test.

erant plant groups (Häikiö *et al*., 2009).

The shoot seedlings originated from healthy trees had significantly lower activity of SOD and CAT than the shoots of seedlings originated from damaged trees (Table 5). On the other hand, GR and APX showed interaction effect between the tree grades and the O_3 treatments, while the MDA content in shoots did not show remarkable differences due to tree grades. O_3 treatment from the control to 300 ppb increased the activities of SOD, GR, APX, and CAT in shoot in both seedlings from different sources but the increase observed in seedlings from damaged tree were higher compared to their counterpart. The SOD, GR, APX, and CAT in shoot of seedlings from damaged trees increased by 24%, 46%, 29%, and 11%, respectively relative to the activities of the control. The MDA content was significantly reduced due to 150 ppb O_3 treatment especially in the shoots of seedlings from damaged trees. However, changes of MDA content in shoot did not observe in both seedling source and O_3 treatment.

In the non-treated seedlings, the SOD activities in the root of the damaged trees seedlings were higher than those of healthy trees. O3 treatment significantly decreased SOD activity in the root of damaged tree seedlings, not in healthy tree seedlings ($p > 0.05$). The GR activity in the root was significantly affected by the combination of tree grades and O_3 treatment. The GR activity decreased under the 150 ppb O_3 treatment but it increased by 14% and 3% in 300 ppb of O_3 treatment for seedlings from healthy and damaged trees, respectively. On the other hand, the activity of APX in the roots was increased from 119 to 230 μmol min^{-1} g^{-1} in healthy tree seedling, whereas it was dramatically decreased from 794 to 143 μmol min^{-1} g^{-1} in damaged tree seedling by O_3 treatment from 150 ppb to 300 ppb. The CAT activity was generally higher in damaged tree seedling than in healthy one, and that the activity was reduced due to O_3 treatment. The root MDA content of seedlings from the control (no- O_3 fumigation) showed higher level in seedlings from damaged tree than those from in the healthy one. However, O_3 treatment

resulted in significant decrease of root MDA by 20% in seedlings of healthy trees and increased by 30% in seedlings from damaged trees. Plant responds to O_3 induced oxidative stress by activation of a number of antioxidative stress-related defense mechanisms. Karnosky *et al*. (1998) reported that antioxidant enzyme analysis showed elevated SOD levels in the tolerant clone but not in the sensitive clone following O_3 exposure in *Populus tremuloides*. Northern blot analysis indicated that the chloroplastic and cytosolic Cu/Zn SODs were significantly increased in response to O_3 in the tolerant but not the sensitive clone. *Q. acutissima* that showed sensitivity for O_3 than tolerance *Quercus* species (*Q. aliena* and *Q. palustris*) resulted higher increase of APX, SOD, and GR activities and MDA content in O_3-treated plants (Kim *et al*. 2008). Similar to those results, O_3 treatments for healthy tree seedlings alleviate the oxidative stress or those enzymes already used detoxification as defensive response in our results. We could assume that APX, SOD and GR activities were highly connected with O_3 induced stress in sensitive species as much as their activities were highly increased. Therefore, our data indicate that the exposure of seedlings to high O_3 levels triggered the alteration of several physiological parameters, and antioxidant mechanisms in plants especially in sensitive species.

Overall, our experiment demonstrated that the seeds from damaged *P. thunbergii* tree proved to be more sensitive to O_3 than those of seeds originated from healthy ones, which is consistent with the recent meta-analyses and reviews on forest trees (Wittig, *et al*., 2007; Wittig, *et al*., 2009). Although gymnosperms are generally regarded as more O_3 tolerant compared with angiosperms (Wittig, *et al*., 2007; Wittig, *et al*., 2009), we have now indicated that increasing O_3 concentration can disturb seed germination and growth of pine seedlings, which may have a large negative impact on reforestation in the future (Duryea and Brown, 1984). Since sexual development is an important stage in the life cycle of plants, any change in the involved processes might have significant implications for the productivity of the plants and their survival (Black *et al*., 2000). Seed and seedling responses play a key role in tree regeneration and succession, as germination and initial seedling growth set the pattern for future growth (Miao, 1995).

적 요

본 연구에서는 종자생산, 발아와 유묘 발달에 대한 오존 저항성의 개체간 차이를 비교하였다. 대기오염지역에서 12년간 자란 해송 중에서 가시적 피해와 생장을 기준으로 건전목과 피해목을 선정한 후, 구과분석, 종자발아, 지질과산화, 항산화효소 활성을 측정하였다. 구과분석 후, 종자와 유묘를 대상으로 대조구, 150, 300ppb 농도의 오존 처리를 실시하였다. 피해목 종자의 발아율은 건전목 종자보다 발아율이 21.6% 낮았다. 건전목과 피해목 종자의 발아율은 300ppb 오존처리 시 대조구에 비해 각각 10, 19% 감소하였다. 종자의 SOD, GR, CAT 활성도 건전목 종자가 높았다. 오존처리 시 GR, APX와 CAT 활성은 두 종류의 종자 모두에서 감소하였고 MDA 함량은은 종자에 따른 차이가 없었다. 유묘 생육은 침엽 길이, 줄기와 뿌리의 길이 및 무게에서 종자에 따른 차이는 없었으나 300ppb의 오존 처리 시 침엽과 줄기의 길이 및 무게는 감소되었다. 피해목 유묘의 SOD, APX, CAT 활성과 MDA 함량은 건전목 유묘보다 높았고, 오존처리 시 피해목 유묘에서 유의하게 감소하였다. 결론적으로, 오염지역의 건전목과 피해목은 종자 발아 특성과 유묘의 항산화 능력 차이가 뚜렷하며, 그들의 종자의 발아 특성과 유묘 생장은 오존농도 증가에 민감하게 반응하므로 조림을 위한 개체 선정 시 고려해야 한다.

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