Jour. Korean For. Soc. Vol. 95, No. 2, pp. 209~215 (2006)

JOURNAL OF KOREAN FOREST SOCIETY

Antioxidant Characteristics in the Leaves of 14 Coniferous Trees under Field Conditions

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Abstract : We investigated antioxidant capacity in leaves of 14 coniferous trees under field conditions. We focused on understanding the species characteristics on antioxidant systems and screening the coniferous tree species with the best antioxidant systems using their characteristics. The antioxidant capacity of 14 coniferous trees was divided into three groups. First group was *Thuja orientalis* and *Chamaecyparis obtusa* and those species had the highest content of β-carotene and xanthophyll. Second group, *C. obtusa* and *Juniperus chinensis*, used antioxidant enzymes to mitigate stress. *C. obtusa* represented high activity at superoxide dismutase (SOD), glutathione reductase (GR), and peroxidase (POD), and *J. chinensis* exhibited high activity at SOD, POD, catalase (CAT). Third group employed antioxidant such as ascorbic acid and α-tocopherol. The antioxidant content of *T. orientalis* was the highest while that of *Pinus parviflora* and *C. obtusa* were the lowest. Few species belonged in three groups simultaneously, and most species belonged in at least one or two groups. In summary, we proposed that *C. obtusa* and *T. orientalis* had the highest antioxidant capacity while *P. parviflora* and *P. desiflora* for. *multicalus* had the lowest antioxidant capacity.

Key words **:** *coniferous, β-carotene, xanthophyll, antioxidant enzyme, ascorbic acid, α-tocopherol*

Introduction

Under natural conditions of growth and development, plants are inevitably exposed to different types of stress such as high irradiance, heat, chilling, drought, salinity, and mineral deficiency, which may cause increased production of active oxygen species (AOS) (Smirnoff, 1993). These AOS include superoxide radical (O_2^-•), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH•) and singlet oxygen ($^1O_2$) (Foyer *et al.*, 1994). The balance between the production of AOS and the quenching activity of the antioxidants is upset and this often results in oxidative damage (Larson, 1995).

Plants have evolved various protective mechanisms to eliminate or reduce AOS (Foyer *et al.*, 1994). In plant cells, one of such protective mechanism is an antioxidant system which is composed of both non-enzymatic and enzymatic antioxidants (Foyer *et al.*, 1994). The capacity of the antioxidant defense system is often increased under stress conditions (Gressel and Galun, 1994), but efficiencies of those enzymes declined when stress became more severe (Baisak *et al.*, 1994; Sgherri and Navari-Izzo, 1995; Schwanz *et al.*, 1996).

AOS are highly reactive in the absence of any protective mechanism. They can seriously disrupt normal metabolism through oxidative damage to membrane lipids, proteins, pigments and nucleic acids. These AOS are detoxified by the sequential and simultaneous action of a number of enzymes, including GR, SOD, POD, CAT and glutathione-S-transferase (GST). SOD, located in various cell compartments, is believed to play a crucial role in antioxidant defense because it catalyzes the dismutation of O_2^-• into H_2O_2 whereas CAT and POD destroy H_2O_2 (Smirnoff, 1993; Scandalios, 1993). SOD, together with ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and GR, constitutes the major defense system against AOS in chloroplasts (Dalton *et al.*, 1993).

In addition, plants contain substantial amounts of carotenoids that serve as non-enzymatic scavengers of AOS (Young and Britton, 1990). It is now well documented that carotenoids (Car) are involved in the protection of photosynthetic apparatus against photoinhibitory damage by singlet oxygen ($^1O_2$) that is produced by the excited triplet state of chlorophyll (^{3}Chl*). Carotenoids can directly deactivate $^1O_2$ and also quench the ^{3}Chl*, thus indirectly reducing the formation of $^1O_2$ species (Siefermann-Harms, 1987; Minkov *et al.*, 1999). Metabolites such as ascorbate, glutathione and α-tocopherol also contribute to control the levels of AOS in plant tissues (Noctor and Foyer, 1998)

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Antioxidant capacity to stress varies from species to species and varieties even among cultivars of a single species (Oncell *et al.*, 2004; Calatayud and Barreno, 2004). Several mechanisms have been proposed to explain this variability. Kolb *et al.*, (1997) suggested that differing O_3 sensitivity in plants might occur because of differences in O_3 uptake (avoidance), compensation for injured tissues, internal properties that oppose the production of tissue injury (defense), and repair of tissue injury. In general, higher activities of scavenger antioxidant enzymes or substances may protect from oxidative stress (Asada, 1997; Pasqualini *et al.*, 2001). However there are many conflicting reports about the effects of O_3 on antioxidant systems (Tanaka *et al.*, 1985). In addition, plants may not need all the components of antioxidant defense. Instead a plant may contain one or two of these components at very high concentrations. Therefore we tried to understand the species characteristics on antioxidant systems and to screen the coniferous tree species with the best antioxidant systems using their characteristics.

Materials and Methods

1. Plant materials

In this study, 14 coniferous tree species were collected from Department of Forest Genetic Resources, Korea Forest Research Institute located in Suwon, Korea (Table 1). Annual average air temperature, mean relative humidity and precipitation of Suwon in 2005 were 12°C, 61%, and 1428 mm respectively. Plant samples were collected during June, 2005. Collected leaf samples were carried to the laboratory in portable freezers (-20°C). Samples were collected from at least five different individuals. Half of each material was kept in nylon bags at -70°C and another half was freeze dried.

2. Chlorophyll

Chlorophyll extraction from fresh leaf material was carried out with dimethyl sulfoxide (DMSO) (Hiscox and Israelstam, 1979). The chlorophyll *a*, chlorophyll *b* and total chlorophyll measurements were done by using a spectrophotometer at 470, 645, 663 nm. Chlorophyll contents were calculated according to Lichtenthaler (1987) and chlorophyll *a*/*b* ratios were determined.

3. β-carotene and xanthophyll

Fresh leaf material (0.5 g) was ground in pre-chilled mortar, in 5 mL acetone containing 200 mg Na_2SO_4 and then filtered through glass fiber disks (Whatman GF/A). The volume of the acetone extracts was reduced in a rotary evaporator and then resuspended in 1 mL chloroform. Twenty micro-liters of the extracts and standards were applied to silica gel TLC plates (Sigma Type GF, 10-40 μm, 20×20 cm, 0.5 mm thickness). The chromatograms were developed with hexane/diethyl ether/acetone (60:30:20, v/v/v) (Moore, 1974). β-carotene and xanthophyll spots were scraped from the TLC plates and centrifuged in 5 mL acetone for 5 min at a wavelength of 450 nm by spectrophotometer. β-carotene and xanthophyll (Sigma) were used as standards.

4. DPPH radical scavenging assay

Three grams of the ground power was mixed with 50 mL of methanol and placed in a shaking incubator for 24 h at 25°C. The macerated mixture was filtered and centrifuged (5 min, 395×g). The remaining residue was extracted again with water sequentially following the above extraction procedure.

This assay was based on the scavenging of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals by the radical scavenging components in water extracts. Modified method of Brand-Williams (1995) was used to investigate the free radical scavenging activity. DPPH solution in DMSO was prepared at the concentration of 300 mM. A 2 mL fraction of extract and 2 mL of fresh prepared DPPH solution were throughly mixed. the reaction mixture was incubated for 1h and centrifuged (5 min, 222×g). Absorbance of the supernatant was measured at 517 nm using UV-VIS spectrophotometer (SHIMADZU, Japan).

5. Enzyme activity analyses

Fresh leaves (0.1 g) 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. 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). Activity of GR was assayed as described in Carlberg and Mannervik (1985). The assay was carried out in a reaction mixture containing 50 mM phosphate buffer (pH 7.8), 0.1 mM NADPH, 0.5 mM GSSH and 0.1 mL enzyme extract, The change in A_{340} was recorded for 5 min after the addition of enzyme extract. POD activities were determined specifically with guaiacol at 470 nm following the method of Polle *et al* (1994). The reaction mixture contained 100 mM guaiacol, and 10 μL of 10% H_2O_2. The reaction was initiated by adding plant extract and following for 10 min. CAT activity was determined by following a two-step procedure (Fossati *et al.*, 1980). The rate of dismutation of H_2O_2 to water and molecular oxygen is proportional to the concentration of catalase. Therefore, the sample containing catalase is incubated in the presence of a known concentration of H_2O_2. After incubation for

Table 1. The list of 14 coniferous trees investigated in this study and height and DBH.

Scientific name	Abbreviation	Height (m)	DBH (cm)
– *Abies holophylla* Maximowicz	Ah	4.2±0.9	8.1±1.6
– *Pieca abies* (L.) Karst.	Pa	7.0±0.9	13.0±1.6
– *Larix leptolepis* (Sieb. et Zucc.) Gordon	Ll	17.3±0.4	20.4±1.2
– *Pinus densiflora* Sieb. et Zucc.	Pd	3.4±0.7	9.0±1.9
– *P. densiflora* for. *multicalus* Uyeki	Pdm	6.2±2.7	24.8±14.3
– *P. koraiensis* Sieb. et Zucc.	Pk	11.6±1.0	27.1±3.4
– *P. parviflora* Sieb. et Zucc.	Pp	4.1±1.6	16.8±7.9
– *P. strobus* Linné	Ps	3.8±0.3	13.4±1.9
– *Thuja orientalis* Linné	To	4.3±0.4	10.6±2.1
– *Chamaecyparis obtusa* (Sieb. et Zucc.)	Co	16.8±1.3	30.4±14.0
– *C. pisifera* (Sieb. et Zucc.)	Cp	11.5±0.6	15.7±1.9
– *C. pisifera* var. *filifera* B. et H.	Cpf	6.8±3.1	16.3±4.7
– *Juniperus chinensis* Linné	Jc	4.2±0.6	14.3±3.5
– *J. chinensis* var. *kaizuka*	Jck	6.7±3.7	26.5±13.8

exactly one minute, the reaction is quenched with sodium azide. The amount of H_2O_2 remaining in the reaction mixture is then determined by the oxidative coupling reaction of 4-aminophenazone (4-aminoantipyrene, AAP) and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) in the presence of H_2O_2 and catalyzed by horseradish peroxidase (HRP). The resulting quinoneimine dye is measured at 520 nm. All the activities of enzyme were measured using UV-120 (SHIMADZU, Japan).

6. Total ascorbic acid

Frozen leaf material (0.5 g) was homogenized for 30 s in 6 ml of 0.1 M cooled sodium acetate buffer, pH 3. The homogenate was centrifuged for 5 min at 4°C and 7000×g. The supernatant was filtered through a nylon filter (Whatman, pore size 0.45 μm) and stored at -70°C. Total ascorbic acid was determined through a reduction of dehydroascobate to ascorbate by dithiothreitol. For the chromatographic separation, a TSP HPLC system (Thermo Separation Products Inc., California) controled by a personal computer including PC1000 software was used. TAA was separated on a Luna C_{18} column (Phenomenex, 250×4.6 mm, 5 μm) using a solvent of 0.1 M sodium acetate buffer, pH 5 with an isocratic flow of 0.6 mL min^{-1}. The elutes were monitored by an UV detector at 264 nm for 16 min. (Schmieden and Wild, 1994).

7. α-Tocopherol

Frozen leaf material (0.5 g) was homogenized at 4°C for 30 s in 8 ml of ethanol containing 0.1 g insoluble polyvinylpyrolidone (PVP) and 0.2 g Na_2SO_4. The homogenate was centrifuged for 5 min at 4°C and 5000×g. The supernatant was filtered through a nylon filter (Whatman, pore size 0.45 μm), and stores at -70°C (Schmieden and Wild, 1994). α-Tocopherol was separated at room temperature on a μ-Bondapack C_{18} column (Waters, 250×4.6 mm) using solvents A [95% methanol +5% water (v/v)] and B [95% methanol+5% ethyl acetate (v/v)] with a flow rate of 1.2 mL min^{-1}. The gradient elution started at 100% A and 0% B, changed to 90% A and 10% B within 1 min and finished with 100% A and 0% B in 15 min. Eluted were monitored by an UV detector at 292 nm. For this chromatographic separation, a TSP HPLC system (Thermo Separation Products Inc., California) controled by a personal computer including PC1000 software was used. Amounts of α-tocopherol were calculated from a standard curve prepared with tocopheryl acetate.

Results

The chlorophyll content in the needles of 14 coniferous trees was highly variable (Table 2). The range of chlorophyll a content was 0.17 to 2.25 mg/g FW and that of chlorophyll b content was 0.05 to 0.54 mg/g FW. The highest chlorophyll a contents were measured in the needles of *Larix leptolepis* (2.25 mg/g FW) and then *Pieca abies* (0.75 mg/g FW) and the lowest values were found in *Pinus parviflora* (0.17 mg/g FW) and *Chamaecyparis pisifera* var *filifera* (0.19 mg/g FW). The highest chlorophyll b content was also observed in *L. leptolepis* (0.54 mg/g FW) but the lowest values was observed in *Thuja orientalis* (0.04 mg/g FW) and *Juniperus chinesis* (0.05 mg/g FW). The highest chlorophyll content was also found in *L. leptolepis* (2.79 mg/g) and then *P. abies* (0.92 mg/g FW). The lowest values were observed in the needles of trees such as *P. parviflora* (0.23 mg/g FW) and *C. pisifera* var *filifera* (0.24 mg/g FW). The chlorophyll a/b ratio of coniferous trees ranged from 2.69 (*P. parviflora*) to 6.26 (*J. chinensis* var *kaizuka*). The chlorophyll a, b, total chlorophyll and chlorophyll a/b ratio showed significant variation between species (P=0.0001).

The β-carotene content of coniferous trees ranged

Table 2. Differences in chlorophyll (Chl) content among 14 coniferous trees.

Species	Chl *a*	Chl *b*	Total Chl	Chl a/b
	mg/g			
Abies holophylla	0.59 ± 0.09^{bc}	0.14 ± 0.03^{bc}	0.73 ± 0.12^{bc}	4.33 ± 0.36^{bc}
Pieca abies	0.75 ± 0.32^{b}	0.18 ± 0.07^{b}	0.92 ± 0.06^{b}	4.21 ± 0.35^{bc}
Larix leptolepis	2.25 ± 0.52^{a}	0.54 ± 0.12^{a}	2.79 ± 0.65^{a}	4.12 ± 0.12^{bc}
Pinus densiflora	0.60 ± 0.05^{bc}	0.14 ± 0.01^{bcd}	0.73 ± 0.06^{bc}	4.42 ± 0.27^{bc}
P. densiflora for. *multicalus*	0.40 ± 0.08^{bcd}	0.10 ± 0.02^{bcde}	0.50 ± 0.10^{bcd}	3.84 ± 0.06^{bc}
P. koraiensis	0.50 ± 0.15^{bcd}	0.13 ± 0.03^{bcde}	0.63 ± 0.18^{bcd}	3.94 ± 0.22^{bc}
P. parviflora	0.17 ± 0.02^{d}	0.06 ± 0.01^{cde}	0.23 ± 0.03^{d}	2.69 ± 0.21^{d}
P. strobus	0.27 ± 0.06^{cd}	0.08 ± 0.01^{cde}	0.35 ± 0.07^{cd}	3.56 ± 0.56^{c}
Thuja orientalis	0.20 ± 0.04^{d}	0.04 ± 0.01^{e}	0.25 ± 0.04^{d}	4.67 ± 1.16^{b}
Chamaecyparis obtusa	0.27 ± 0.09^{cd}	0.06 ± 0.01^{cde}	0.32 ± 0.10^{cd}	4.53 ± 0.40^{b}
C. pisifera	0.32 ± 0.11^{cd}	0.07 ± 0.01^{cde}	0.39 ± 0.12^{cd}	4.69 ± 1.19^{b}
C. pisifera var. *filifera*	0.19 ± 0.01^{d}	0.05 ± 0.01^{de}	0.24 ± 0.01^{d}	3.85 ± 0.21^{bc}
Juniperus chinensis	0.27 ± 0.05^{cd}	0.05 ± 0.01^{e}	0.32 ± 0.05^{cd}	5.92 ± 0.51^{a}
J. chinensis var. *kaizuka*	0.42 ± 0.18^{bcd}	0.06 ± 0.02^{cde}	0.48 ± 0.20^{cd}	6.26 ± 0.76^{a}
Pr > F	0.0001***	0.0001***	0.0001***	0.0001***

Each data represents mean values and standard deviations of five individuals. The same letters are not significantly different at 5% level in Duncan's multiple range test. ***Significant at $P \leq 0.001$

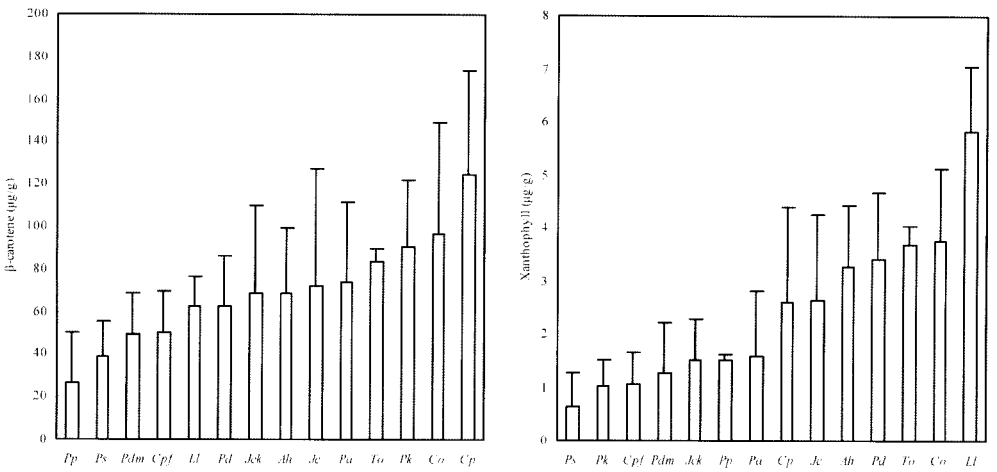

Figure 1. Differences of β-carotene (left) and xanthophyll (right) content in the leaves of 14 coniferous trees. Each bar represents mean values and standard deviations of five individuals (β-carotene P=0.0554, xanthophyll P= 0.0001*, ***significant at P≤0.001).**

from 26.3 to 124.3 μg/g and xanthophyll content in the needles of 14 trees showed the range of 0.62 to 5.84 μg/g (Figure 1). The highest β-carotene contents were observed in *C. pisifera* (124.3 μg/g), *C. obtusa* (96.2 μg/g), *P. koraiensis* (90.6 μg/g) and *T. orientalis* (83.6 μg/g), and the lowest values were found in the needles of *P. parviflora* (26.3 μg/g), *P. strobus* (38.5 μg/g), *P. densiflora* for. *multicalus* (49.2 μg/g), and *C. pisifera* var. *filifera* (49.6 μg/g). However, no significant difference between trees was observed with regard to the β-carotene values (P=0.0554). The range of xanthophyll content in the needles of 14 trees was from 0.62 μg/g to 5.84 μg/g, and showed significant difference between trees (P=0.0001). The highest xanthophyll contents were found in *L. leptolepis*, *C. obtusa*, *T. orientalis*, and *P. densiflora* were the trees with the highest xanthophyll value with 5.84 μg/g, 3.76 μg/g, 3.69 μg/g and 3.41 μg/g respectively. The lowest values were observed in *P. strobus* (0.62 μg/g), *P. koraiensis* (1.01 μg/g), *C. pisifera* var. *filifera* (1.04 μg/g) and *P. densiflora* for. *multicalus* (1.28 μg/g).

Antioxidant capacity, represented by DPPH radical scavenging assay, ranged from 62.6% to 92.3% (Figure 2). Radical scavenging capacity of *P. strobus*, *Abies holophylla* and *C. pisifera* var. *filifera* was more than 90%. On the other hand *L. leptolepis* represented the lowest antioxidant capacity with 62.6%. The statistical tests revealed significant difference with regard to antioxidant capacity between coniferous trees (P=0.0217).

Antioxidant enzyme activity in the needles of 14 tree species was depicted in Table 3. SOD activity was shown the range of 1058 unit/g to 17396 unit/g (P<0.001). Among the

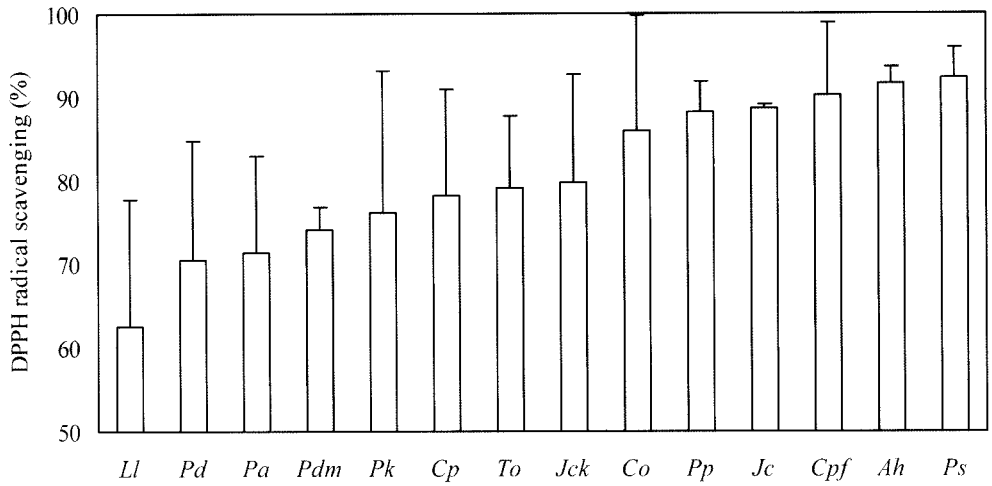

Figure 2. Difference of DPPH radical scavenging ability in the leaves of 14 coniferous trees. Each bar represents mean values and standard deviations of five individuals (P=0.0217*, *significant at P≤0.05).

Table 3. Differences of antioxidant enzyme activities in the leaves of 14 coniferous trees.

Species	SOD	GR	POD	CAT
Abies holophylla	3027 ± 664^{cd}	313 ± 57^{ab}	1.30 ± 1.17^{d}	519 ± 27^{bcde}
Pieca abies	2894 ± 294^{cd}	387 ± 136^{ab}	1.49 ± 0.49^{d}	526 ± 16^{bcd}
Larix leptolepis	4512 ± 398^{c}	356 ± 49^{ab}	0.67 ± 0.25^{d}	468 ± 26^{cde}
Pinus densiflora	3944 ± 55^{cd}	417 ± 51^{a}	2.01 ± 1.19^{d}	457 ± 24^{cde}
P. densiflora for. *multicalus*	4016 ± 98^{cd}	393 ± 84^{ab}	0.92 ± 0.22^{d}	587 ± 121^{ab}
P. koraiensis	4313 ± 1008^{cd}	363 ± 60^{ab}	4.56 ± 0.86^{c}	439 ± 46^{de}
P. parviflora	2297 ± 1030^{cd}	269 ± 148^{b}	0.49 ± 0.01^{d}	442 ± 16^{de}
P. strobus	1165 ± 598^{cd}	421 ± 102^{a}	1.53 ± 0.66^{d}	432 ± 77^{c}
Thuja orientalis	2063 ± 952^{cd}	310 ± 8^{ab}	8.32 ± 0.96^{a}	438 ± 8^{de}
Chamaecyparis obtusa	17396 ± 4416^{a}	389 ± 11^{ab}	7.70 ± 2.08^{ab}	510 ± 40^{bcde}
C. pisifera	8726 ± 435^{b}	323 ± 101^{ab}	7.15 ± 1.98^{ab}	510 ± 224^{bcde}
C. pisifera var. *filifera*	1665 ± 714^{cd}	346 ± 13^{ab}	5.93 ± 0.55^{bc}	515 ± 25^{bcde}
Juniperus chinensis	4557 ± 584^{c}	270 ± 64^{b}	8.61 ± 1.79^{a}	539 ± 32^{abc}
J. chinensis var. *kaizuka*	1058 ± 175^{d}	292 ± 171^{ab}	9.29 ± 1.83^{a}	609 ± 104^{a}
Pr > F	0.0001***	0.1951	0.0001***	0.0001***

Each data represents mean values and standard deviations of five individuals. The same letters are not significantly different at 5% level in Duncan's multiple range test. ***Significant at $P \leq 0.001$.

coniferous trees *C. obtusa* had the highest SOD activity with 17396 unit/g. On the other hand *J. chinensis* var. *kaizuka* showed the lowest activity with 1058 unit/g and then *P. strobus* (1165 unit/g).

The highest GR activity was exhibited by *P. strobus* and *P. densiflora* with 421 μmol/g and 417 μmol/g respectively. *P. parviflora* and *J. chinensis* had the lowest activity (269 nmol/g and 270 nmol/g). However no significant difference between the coniferous trees was observed with regards to GR activity (P=0.1951).

POD activity was highly variable among species ($P < 0.001$). The highest POD activity was observed in *J. chinensis* var. *kaizuka* (9.29 unit/g) and *J. chinensis* (8.61 unit/g) and the lowest values were measured in the needles of *P. parviflora* (0.49 unit/g) and *L. leptolepis* (0.67 unit/g).

CAT activity was significantly different between tree species ($P<0.001$). *J. chinensis* var. *kaizuka* and *P. densiflora* for. *multicalus* were represented the highest activity with 609 unit/g and 587 unit/g, and *P. strobus* was exhibited the lowest activity (432 unit/g).

The antioxidant contents of the coniferous trees were shown in Figure 3 and were significantly different between tree species (P=0.0001). Among 14 tree species, *C. pisifera* had the highest total ascorbic acid (1840 μg/g) and then *P. densiflora* (927 μg/g). The lowest values were observed in the needles of *C. obtusa* (256 μg/g) and *P. densiflora* for. *multicalus* (288 μg/g). The α-tocopherol contents of trees were also significantly different (P=0.0001). The highest content was observed in the needles of *L. leptolepis* (867 μg/g), and there were significantly lower α-tocopherol contents in trees such as *C. pisifera* var. *filifera* (99 μg/g), *P. parviflora* (100 μg/g), *J. chinensis* var. *kaizuka* (105 μg/g), and *C. obtusa* (117 μg/g).

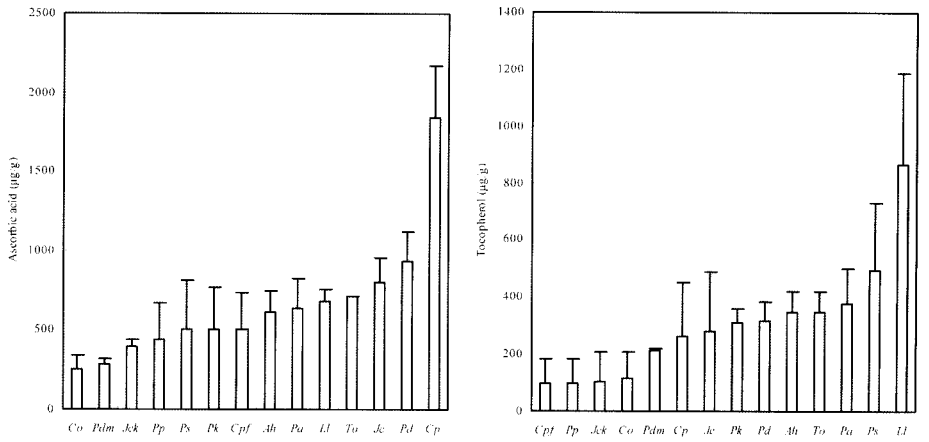

Figure 3. Differences of ascorbic acid (left) and α-tocopherol (right) content in the leaves of 14 coniferous trees. Each bar represents mean values and standard deviations of five individuals (ascorbic acid *P*=0.0001*, α-tocopherol *P*=0.0001***, ***significant at $P \leq 0.001$).**

Discussion

The antioxidant capacity in plants represented significant differences among species and their capacity can be characterized based on tree species. The antioxidant characteristics of each species cause the different sensitivity among species under stress condition. In general, tree species with high antioxidant capacity have higher tolerance against stress when they exposed to stress (Asada, 1997; Pasqualini *et al.*, 2001). However, trees don't express all components related antioxidant systems and show different antioxidant mechanism among species. In our study, the correlation among antioxidative components was not clear (data not shown). For example, some steppe plants have high antioxidant concentrations while others have very low concentrations of antioxidant compounds as shown here. These results supported the existence of two different strategies: antioxidant protection and avoidance from stress (Streb *et al.*, 1997).

Antioxidant defence mechanisms can be vitally important in the survival of plants. However, the present study clearly shows that plants may not need all the components of antioxidant protection (Öncel *et al.*, 2004). Plants, which contain all three components of the antioxidant defense, namely carotenoids, ascorbate and α-tocopherol at high concentrations are very rare. Instead, a plant may contain one or two of these components at very high concentrations. This observation was supported by the findings of Polle *et al.*, (1999 a, b). They found that ascorbate and dehydroascorbate content increased while glutathione content decreased in antioxidant and SOD activity increased while GR activity decreased in antioxidative enzymes in spruce threes grown at high altitudes.

Based on our results, the antioxidant capacity of 14 coniferous trees can be divided into three groups. First group was *T. orientalis* and *C. obtusa* that were shown high antioxidant capacity in carotenoid system. Both species had the highest content of carotenoid (β-carotene and xanthophyll) while *P. densiflora* for. *multicalus* and *C. pisifera* var. *filifera* represented lower dependance on carotenoid. Second group, *C. obtusa* and *J. chinensis*, used antioxidative enzymes to mitigate stress. *C. obtusa* represented high activity at SOD, GR and POD, and *J. chinensis* exhibited high activity at SOD, POD and CAT. On the contrary, *T. orientalis* had the lowest SOD, GR and CAT activity, and *P. parviflora* had the lowest GR, POD and CAT activity. Third group employed antioxidants such as ascorbic acid and α-tocopherol. The antioxidant content of *T. orientalis* was the highest while those of *P. parviflora* and *C. obtusa* were the lowest. Few species belonged in three groups, and most species belonged in at least one or two groups. Specially *C. obtusa* depended on carotenoid and antioxidative enzyme, but lowed the dependance on antioxidant. Meanwhile *T. orientalis* was highly dependant on carotenoid and antioxidant, but the function of antioxidant enzymes was highly low. In summary, we proposed that *C. obtusa* and *T. orientalis* had the highest antioxidant capacity while *P. parviflora* and *P. desiflora* for. *multicalus* had the lowest antioxidant capacity.

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(Received February 14, 2006; Accepted April 5, 2006)