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Mycorrhizal Activities in *Pinus densiflora*, *P. koraiensis* and *Larix kaempferi* Native to Korea Raised under High CO_2 Concentrations and Water Use Efficiency

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Summary

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The effects of infection by the ectomycorrhiza, *Pisolithus tinctorius* (*Pt*), on the growth and photosynthetic characteristics of *Pinus densiflora*, *P. koraiensis* and *Larix kaempferi* were studied. Seedlings of these species were grown at ambient (36 pa) and elevated (72 pa) CO_2 concentrations [CO_2] with and without *Pt* infection. After 180 days, *Pt* inoculation had given rise to significant increases in dry mass and stem diameter of each species at both [CO_2], compared with non-inoculated (control) seedlings. Moreover, *Pt* inoculation at elevated [CO_2] (72 Pa) significantly increased ectomycorrhizal development. The phosphorus (P) concentration in needles inoculated with *Pt* was significantly higher than in non-inoculated seedlings at both [CO_2] concentrations. The maximum net photosynthetic rate at saturated CO_2 concentration (P_{max}) and the carboxylation efficiency (CE) of each species inoculated with *Pt* were higher than for non-inoculated seedlings at

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both [CO_2] concentrations. The difference was significantly greater in *P. densiflora*. The water use efficiency (WUE) of seedlings inoculated with *Pt* was significantly raised at both [CO_2] concentrations. Moreover, P_{max} and CE of non-inoculated *P. densiflora* and *P. koraiensis* seedlings grown in 72 Pa CO_2 for 180 days showed down-regulation compared with those grown in 36 Pa CO_2, whereas *Pt* inoculated seedlings showed no photosynthetic down-regulation at elevated [CO_2]. The ectomycorrhizae therefore act to enhance physiological functions related to water, carbon and phosphorus absorption at elevated [CO_2].

Introduction

Symbiotic microorganisms are essential to pine trees growing in infertile soil conditions. Forest soil in Korea derives mainly from granite and provides poor nutrient conditions. With increasing atmospheric CO_2 concentrations the pattern and amount of precipitation at middle and high latitudes are now predicted to undergo great changes. The amount of precipitation in mid latitudes is predicted to decrease significantly in the near future. Symbiotic microorganisms such as ectomycorrhizae usually act as an efficient absorbing system for water and essential nutrients such as nitrogen and phosphate (SMITH & READ 1997). The activity of the host plant should be enhanced at high [CO_2], since symbiotic ectomycorrhizae also act as a large carbon sink (SMITH & READ 1997). In fact, plants often show photosynthetic depression when grown under elevated [CO_2] with limited nutrients or water. No down-regulation of photosynthesis is predicted following the inoculation of trees with ectomycorrhiza at high [CO_2].

Pisolithus tinctorius (*Pt*) is the most common ectomycorrhizae species in pine forests worldwide (ALLEN 1991). What functional changes will take place in a host plant infected with *Pt* under elevated [CO_2]?

To answer this question, the most popular pine and larch species in Korea were cultivated and inoculated with *Pt* at high [CO_2]. We then evaluated the effect of ectomycorrhiza infection on the down-regulation of photosynthesis and the growth of pine seedlings at elevated [CO_2]. Water use efficiency (WUE) and the phosphate concentration in needles were also measured.

Material and Methods

Seeds of *Pinus densiflora*, *P. koraiensis* and *Larix kaempferi* were sterilized with 30% H_2O_2 for 20 minutes and rinsed 4-5 times with deionized water. For germination they were then placed on sterilized media in a glasshouse at day/night temperatures of 25/20 °C with a 16-h photoperiod. The germinated seedlings were inoculated with ectomycorrhiza fungi, *Pisolithus tinctorius* (*Pt*), as follows. The *Pt* spores were dissolved in distilled water, then inoculated directly to the root of *P. densiflora*, *P. koraiensis* and *L. kaempferi* seedlings; the rest of the solution was then mixed with prepared soil media. The seedlings inoculated with *Pt* were transferred to a rhizo-box filled with a sterilized medium consisting of vermiculate : black sand : peat moss in the ratio 2:2:1.

The seedlings were grown in a phytotron (Forest and Forest Products Research Institute, Sapporo, Japan) with a day/night temperature range of 26/16°C and a humidity range of 55 - 75% during the study period, which lasted 180 days. The rhizo-boxes were allocated at random such that half of the seedlings experienced ambient [CO_2] (36 Pa), and the other half experienced elevated [CO_2] (72 Pa). At each CO_2 concentration (three phytotron rooms), half of the seedlings were inoculated with ectomycorrhizal fungus (*Pt*); the other half were not inoculated (Control). Each treatment was repeated in three rooms, and the treatments are denoted as follows: (1) 36 Pa, Control, (2) 36 Pa, *Pt*, (3) 72 Pa, Control, (4) 72 Pa, *Pt*.

Photosynthesis measurement

The P_N/C_i (P_N=net photosynthetic rate, C_i=intercellular CO_2 concentration) curves were examined using an open gas exchange system (LI-6400, Li-Cor, Lincoln, NE, USA) between 09:00 and 15:00 local time. The change in P_N was measured at PAR saturation, corresponding to a photosynthetic photon flux density (PPFD) of 1000 - 1200 $\mu mol \cdot m^{-2} s^{-1}$; this was provided by a cool halogen lamp (Walz, Effeltrich, Germany). The leaf temperature was 25 °C, and the relative humidity was 50 - 70 %. Leaves were allowed to acclimatize to their surroundings for 10 min prior to measurement, when P_N was determined at 15 - 150 Pa [CO_2]. The initial slope of the P_N/C_i curve is proportional to the carboxylation activity of Rubisco [i.e., the carboxylation efficiency (CE, $\mu mol \cdot Pa^{-1}$)]. The maximum net photosynthetic rate at the CO_2 saturation concentration (P_{max}, $\mu mol \cdot m^{-2} s^{-1}$) was determined as 120 Pa [CO_2]. The water use efficiency (WUE, $\mu mol \cdot mmol^{-1}$) was taken as the ratio of P_N to the transpiration rate at PAR saturation and constant water vapour pressure deficit (FIELD & al. 1983).

Infection rate of ectomycorrhiza

The infection rate of ectomycorrhiza (IRE) on roots (r < 2mm) was determined according to the method described by BECKJORD & al. 1985, in which IRE (%) = ER/(ER+NR)×100, where ER and NR respectively denote the number of ectomycorrhizal and non-ectomycorrhizal roots.

Measurement of element concentration

To determine the phosphate concentration (P), the samples were digested by a microwave digestion system (O·I analytical, College Station, TX) and analyzed by a ICP (IRIS, Jarrel Ash, Franklin, MA). The mean values of the inoculation rate, the P concentration in needles, P_{max}, CE, WUE, the shoot and root growth and stem diameter were all studied using the *t*-test implemented with the Stat View 5.0 software (SAS Institute, Cary, NC, USA).

Results and Discussion

Ectomycorrhizal development, i.e. the infection rate of ectomycorrhiza, increased significantly under elevated CO_2 concentrations ($P<0.05$) in each species. Moreover, for each species at each [CO_2], the P concentrations in needles inoculated with *Pt* increased significantly relative to non-inoculated (control) seedlings (Table 1). Ectomycorrhizal plants enhance the rate of photosynthesis over non-mycorrhizal plants as a result of improved plant nutritional status, including N and P (SMITH & READ 1997). In general, phosphorus in needles affects photosynthesis through RuBP regeneration (SHARKEY 1985), and through the peak carboxylation velocity or peak capacity of electron transport (HARLEY & SHARKEY 1991). As expected, the P_{max} and CE of *Pt* inoculated seedlings of each species at each [CO_2] were significantly higher than those of control seedlings. Moreover, the WUE of *Pt* inoculated seedlings of each species at different [CO_2] showed significant increases compared with control seedlings (Table 2). We believe that ectomycorrhiza development in inoculated seedlings and the associated increasing P levels in needles enhance the P_N, i.e. P_{max} and CE, and WUE of each species (Table 1, 2). Moreover, increased physiological activities in inoculated seedlings increased the growth rate over control seedlings (dry mass and stem diameter) (Fig. 1).

Table 1. The infection rate of ectomycorrhiza (IRE) and P in needles of *P. densiflora*, *P. koraiensis* and *L. kaempferi* seedlings inoculated with *Pt* and non-inoculated seedlings grown at ambient (36 Pa) and elevated (72 Pa) [CO_2]. The statistical differences in infection rate and P in needles were compared for each [CO_2] concentration and between *Pt* and controls. (**P*<0.05, ***P*<0.01, ****P*<0.001).

	CO_2 concentration	IRE (%)	P in needles ($mg \cdot g^{-1}$)	
			control	*Pt*
P. densiflora	Ambient	55.13 *	0.74 *	1.23
	Elevated	66.51	0.77 *	1.23
P. koraiensis	Ambient	62.53 *	1.49 ***	1.75
	Elevated	84.88	1.36 **	1.78
L. kaempferi	Ambient	56.13 *	0.83 ***	1.20
	Elevated	76.15	0.79 **	1.18

Table 2. Maximum net photosynthetic rate at saturated CO_2 concentration (P_{max}), carboxylation efficiency (CE) and water use efficiency (WUE) of photosynthesis in the needles of three conifer seedlings grown at ambient (36 Pa) and elevated (72 Pa) [CO_2]. Statistical difference of P_{max}, CE and WUE was compared between control plants and *Pt*. (**P*<0.05, ***P*<0.01, ****P*<0.001).

	CO_2 concentration	P_{max}		CE		WUE	
		control	*Pt*	control	*Pt*	control	*Pt*
P. densiflora	Ambient	7.11 *	9.74	0.12 *	0.27	10.52 ***	14.27
	Elevated	6.09 ***	13.67	0.12 **	0.35	11.45 **	19.50
P. koraiensis	Ambient	4.89	5.00	0.11	0.13	12.2 **	22.6
	Elevated	4.52 *	6.06	0.08	0.17	24.3 **	30.7
L. kaempferi	Ambient	5.58 ***	11.21	0.17	0.26	18.4 ***	29.4
	Elevated	8.51 *	12.52	0.20	0.29	22.6 *	33.6

The P_N of control seedlings of *P. densiflora* and *P. koraiensis* grown at elevated [CO_2] for 180 days exhibited down-regulation compared with ambient [CO_2], so that the seedlings had reduced P_{max} and CE; in contrast, *Pt* inoculated *P. densiflora* and *P. koraiensis* seedlings show no down-regulation at elevated [CO_2] (Table 2). This phenomenon has been observed in other studies, in which plants were grown in fertile soils (VOGEN & CURTIS 1995) or with mycorrhizae (STADDON & al. 1999). Phosphorus limitation (CONROY & al. 1986) and reduced sink strength (STITT 1991, ROGERS & al. 1998) have been proposed as mechanisms inducing down-regulation, via a reduction of RuBP regeneration capacity and reduced Rubisco activity (SHARKEY & al. 1994). We therefore conclude that *Pt* inoculated seedlings display increased nutrient and water uptake, leading to improved plant nutritional status and a more vigorous physiological response, in particular photosynthetic activity; and that these responses moderate the down-regulation at elevated [CO_2].

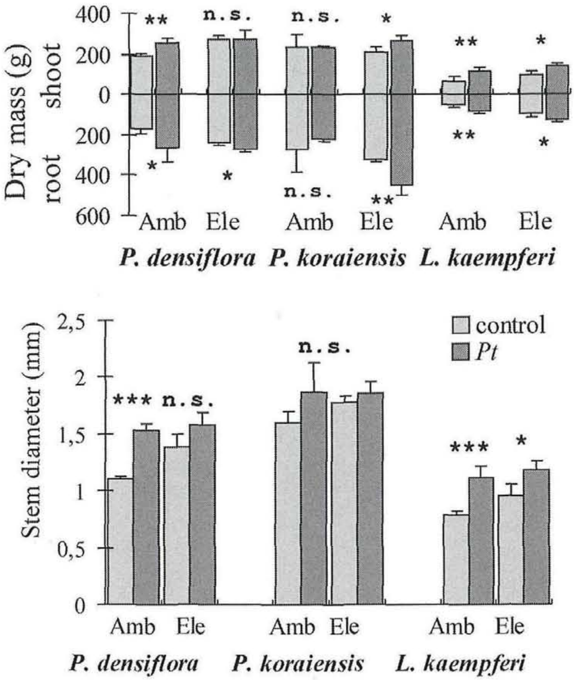

Fig. 1. Effect of ectomycorrhizal inoculation by *Pt* on the shoot and root dry mass and stem diameter of *P. densiflora*, *P. koraiensis* and *L. kaempferi* seedlings grown at 36 Pa (Amb) and 72 Pa (Ele) [CO_2]. (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

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