EFFECT OF ECTOMYCORRHIZAL INFECTION ON THE GROWTH AND PHOTOSYNTHETIC CHARACTERISTICS OF THREE SPECIES OF PINE SEEDLINGS GROWN UNDER ELEVATED CO_2 CONCENTRATIONS

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ABSTRACT

After 18 weeks, elevated CO_2 (720 μmol·mol^{-1}) increased significantly the ectomycorrhizal development. The phosphate concentration (P) in needles of *Pinus densiflora* and *Larix kaempferi* inoculated with *Pt* or EC was significantly higher than that without inoculation at both CO_2 concentrations. *Pt* or EC inoculation had led to significantly increase the physiological activities, such as the PAR saturated net photosynthetic rates (*Psat*), maximum net photosynthetic rate at saturated CO_2 concentration (*Pmax*), carboxylation efficiency (CE), RuBP regeneration rate of *A/Ci* curve and water use efficiency (WUE) of *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings grown at both CO_2 concentrations (360 and 720 μmol·mol^{-1}) relative to non-inoculated seedlings. Moreover, dry mass and stem diameter of inoculated *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings significantly higher than those non-inoculated seedlings.

INTRODUCTION

Pine species (*Pinus koraiensis*, *P. densiflora* and *Larix kaempferi*) can survive and grow on the nutrient-poor soil and after disturbances with symbiosis of ectomycirrhizae, such as *Pisolithus tinctorius* (*Pt*) and others [*Smith and Read*, 1997]. With increasing atmospheric CO_2 concentrations the pattern and amount of precipitation are now predicted to undergo great changes [*IPCC*., 1996]. Symbiosis with ectocymorrhizae usually act as an efficient root system for absorbing water and essential nutrients (nitrogen and phosphate) [*Smith and Read*, 1997; *Quoreshi et al*., 2003]. The photosynthetic adjustment in down-wards is frequently observed in plants grown under high CO_2 concentration, because of dilution effects of nutrient reduction of enzyme activities, enhanced accumulation of photosynthates in photosynthetic organs and sink strength [*Farrar and Williams*, 1991]. However, the activity of the ectomycorrhizal plants should be enhanced at high CO_2 concentration because symbiotic ectomycorrhiza provide water and essential elements, and also act as a large carbon sink [*Ceulemans and Mousseau*, 1994]. Therefore, we hypothesis that the inoculation of ectomycorrhiza increase physiological activity and growth of host plants without down-regulation under high CO_2 concentration. To tackle this hypothesis, the three pine species were inoculated with ectomycorrhiza and cultivated at high CO_2 concentration.

MATERIALS AND METHODS

The seedlings of *Pinus koraiensis*, *P. densiflora* and *Larix kaempferi* were grown in a phytotron at the Hokkaido Research Center, Forestry and Forest Products Research Institute (FFPRI), Sapporo (Japan) with a natural sunlight, day/night temperature range of 26/16 ^{o}C and humidity range of 55 –75 % during the study period lasting 18 weeks. The seedlings inoculated with each ectomycorrhiza, e.g. *Pisolithus tinctorius* (Pers.) Coker et Couch (*Pt*) and Diehard Ecto drench (EC) – (*Pt* + *Rhizopogon* spp. + *Laccaria* spp. + *Scleroderma* spp.), were naturalized in a rhizo-box. The rhizo-boxes were allocated at random such that half of the seedlings experienced ambient CO_2 (360 μmol·mol^{-1}) and the other half experienced elevated CO_2 (720 μmol·mol^{-1}) [*Koike*, 1995]. After 18 weeks, the net photosynthetic rates were examined using an open gas exchange system (LI-6400, Li-Cor, Lincoln, NE) at PAR saturation (1000 – 1200 μmol·m^{-2}s^{-1}), 25 ^{o}C of the leaf temperature and 50 – 70 % of the relative humidity. And then, the inoculation rate of ectomyucorrhiza (IRE) was determined according to the following formula:

$$IRE\ (\%) = ER/(ER+NR)100$$

where EF and NR respectively denote the number of ectomycorrhizal and non-ectomycorrhizal roots. Shoot and root dry mass of each seedling was measured after dried at 60 ^{o}C for 1 week using an electronic balance (HR-202, A&D, Japan). The dried samples were then ground to a fine powder in a vibrating sample mill (Wonder Blender, Osaka Chemical Co., Osaka, Japan). To determine the concentration of phosphorus (P), the samples were digested by a microwave digestion system (O·I analytical, College Station, TX) and then used for ICP analysis (IRIS, Jarrel Ash, Franklin, MA, USA).

RESULTS AND DISCUSSION

After 18 weeks, *Pt* or EC inoculation had led to significantly increase in dry mass and stem diameter of *P. densiflora* and *L. kaempferi* at both CO_2 concentrations, relative to non-inoculated seedlings. In *P. koraiensis*, *Pt* or EC inoculation increased significantly the dry mass and stem diameter relative to non-inoculated seedlings grown at elevated CO_2 concentration. Moreover, elevated CO_2 increased significantly the ectomycorrhizal development. The phosphate concentration (P) in needles of *P. densiflora* and *L. kaempferi* inoculated with *Pt* or EC was significantly higher than that without inoculation at both CO_2 concentrations. However, we did not find any difference in P concentration in needles between inoculated and non-inoculated *P. koraiensis* seedlings grown at ambient CO_2 concentration. The PAR saturated net photosynthetic rates (P_{sat}) of *P. koraiensis*, *P. densiflora* and *L. kaempferi* inoculated with *Pt* or EC were clearly higher than that of non-inoculated seedlings at both CO_2 concentrations, and the maximum net photosynthetic rate at saturated CO_2 concentration (P_{max}) was higher than that of non-inoculated seedlings (Fig. 1).

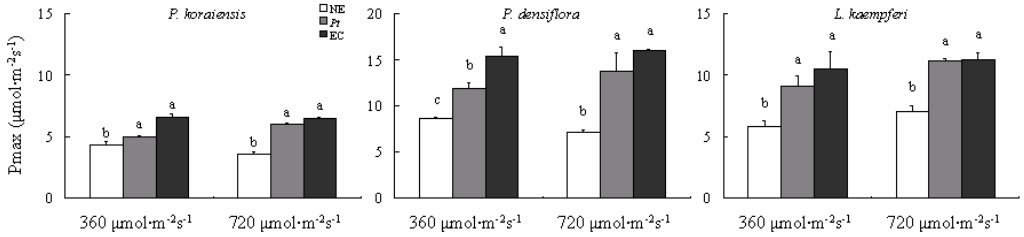

Fig. 1. Maximum net photosynthetic rate at saturated CO_2 concentration (Pmax) in the needles of *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings. NE represented non-inoculated seedlings. *Pt* and EC represented seedlings by *Pisolithus tinctorius* or Ectodrench, respectively.

Moreover, the carboxylation efficiency (CE) and RuBP regeneration rate of the A/C_i curve for *P. densiflora* and *L. kaempferi* inoculated with *Pt* or EC were significantly higher than those of non-inoculated seedlings at both CO_2 concentrations and *P. koraiensis* at elevated CO_2 concentration, especially inoculated with EC. The water use efficiency (WUE) of seedlings inoculated with *Pt* or EC grown at both CO_2 concentrations was significantly raised. Moreover, net photosynthetic rate of non-inoculated seedlings grown for 18 weeks at elevated CO_2 concentration tended to be down regulated; in contrast, *Pt* or EC inoculated seedlings showed no down-regulation at elevated CO_2 concentration. The activity of ectomycorrhiza may therefore enhance physiological function related to water and phosphate absorption in *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings at elevaed CO_2 concentration. Consequently the dry mass and stem diameter of inoculated *P. koraiensis*, *P. densiflora* and *L. kaempferi* seedlings significantly increased than those of non-inoculated seedlings.

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