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SHORT COMMUNICATION

Effect of *Frankia* inoculation on the growth of *Alnus sieboldiana* on unsterilized soil

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Abstract The effect of inoculation with *Frankia*, a N-fixing actinomycete, on the growth of *Alnus sieboldiana* seedlings was studied on unsterilized soil from a nursery and an alder stand (forest of *Alnus firma*). The seedlings of *A. sieboldiana* were inoculated with *Frankia* before or after a 2-month culture on sterilized vermiculite, during which they nodulated, and transplanted to unsterilized soil from the nursery and the alder stand. The control seedlings were also cultured on sterilized vermiculite for about 2 months and transplanted to unsterilized soil without *Frankia* inoculation. The seedling growth, nodulation and N-fixing activity were measured 3, 10 and 16 weeks after the transplantation. Growth and nodule biomass of the seedlings inoculated with *Frankia* and those grown on the alder soil were better than those without inoculation with *Frankia* and grown on the nursery soil, respectively. The seedlings inoculated before spontaneous nodulation grew better than those inoculated at the transplantation. Nitrogen-fixing activity measured by acetylene reduction assay at 16 weeks after the transplantation was higher in the seedlings grown on the soil from the nursery than on the soil from the alder stand.

Keywords *Alnus* · *Frankia* · Nodulation · Unsterilized soil

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Introduction

Actinorhizal plants, such as *Alnus*, *Casuarina*, and *Myrica*, fix atmospheric nitrogen in root nodules symbiotically formed by an actinomycete *Frankia* (Baker and Schwintzer 1990). Because of their capacity for nitrogen fixation, nodulated plants can grow better than unnodulated plants. This seems to be the case in the plants nodulated after inoculation with an isolate of *Frankia* in sterilized soil (Yamanaka and Okabe 1995; Yamanaka et al. 2005). In the nursery and field, uninoculated actinorhizal plants are spontaneously nodulated by indigenous strains of *Frankia*, which suggests that *Frankia* inoculation is not necessary for growth improvement of introduced plants. In the present study, we evaluated the effect of *Frankia* inoculation on seedling growth and nitrogen fixation in *Alnus sieboldiana* cultured in pots containing nursery soil and forest soil (alder stand) without sterilization. The growth and N-fixing activity of *A. sieboldiana* inoculated with *Frankia* before and after a 2-month aseptic culture (before and after nodulation) were also compared on the unsterilized soils.

Materials and methods

Plant materials

Seeds of *A. sieboldiana* soaked in running water for several days were surface-sterilized in 95% ethanol for 1 min and then in 1% sodium hypochlorite solution for 5 min. They were then rinsed 5 times in sterilized distilled water and put aseptically in petri dishes, which contained 0.9% agar. The petri dishes were sealed with Para-films and placed in a growth chamber maintained at 28°C under continuous light of 10,000 lux. After germination, the seedlings were