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Growth Stimulation of *Alnus firma* and *Robinia pseudoacacia* by Dual Inoculation with VA Mycorrhizal Fungi and Nitrogen-Fixing Bacteria and Their Synergistic Effect[1*]

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VA 内生菌根菌과 窒素固定菌으로 二重接種한 사방오리나무와 아까시나무의 生長促進과 接種의 相乘效果[1*]

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ABSTRACT

To evaluate potential of VA mycorrhizal fungi for promoting growth of nitrogen-fixing trees and efficiency of fungal inoculation in relation to soil fertility and dual inoculation with bacteria, *Alnus firma* was grown for six months in pots with steam-sterilized soil after inoculation with *Glomus mosseae*, and *Robinia pseudoacacia* was grown in the fumigated field after inoculation with native *Glomus* sp. and *Gigaspora* sp.

In unfertilized *Alnus* plants, 27% increase in dry weight(or 18% in height) was observed by *Glomus* inoculation, while plants inoculated with both VA mycorrhiza and actinomycete(crushed nodule inoculum) showed synergistic effect of 83% dry weight increase over uninoculated plants. In fertilized *Alnus* plants, mycorrhizal inoculation alone or dual inoculation with actinomycete resulted in depression of height and dry weight of plants. In case of *Robinia*, dual inoculation stimulated height growth by 23% (or dry weight by 25%) over the control in unfertilized field, while 13% more height growth(or 21% more dry weight) was observed in fertilized field.

It is concluded that VA mycorrhizae, especially *Glomus mosseae*, have a potential for growth enhancement in *Alnus*, that synergistic effect of dual inoculation(mycorrhiza + actinomycete) exists in both *Alnus* and *Robinia*, and that responses of these plants to VA mycorrhiza are more pronounced in unfertilized soil.

Key words : *VA mycorrhiza* ; *dual inoculation* ; Glomus mosseae ; Gigaspora ; Alnus firma ; Robinia pseudoacacia ; *Nitrogen-fixing bacteria*.

要 約

内生菌根이 窒素를 固定하는 樹木의 生長에 미치는 影響과 施肥에 따른 菌根菌의 效率을 評價하기 위하여, 사방오리나무 幼苗를 花盆에서 *Glomus mosseae*로 接種하고 아까시나무를 苗圃場에서 *Glomus* sp. 와 *Gigaspora* sp.로 接種하여 6個月後에 接種效果를 調査하였다.

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施肥하지 않은 사방오리나무의 경우에 土壤燻蒸後 G. mosseae로 接種하여 乾重量이 27%, 苗高生長이 18% 增加하였고, G. mosseae와 窒素固定菌으로 二重接種하여 乾重量이 83% 增加하는 相乘效果를 가져왔다. 施肥한 사방오리나무의 경우에는 菌根接種이나 二重接種으로 因하여 生長이 減少하였다. 아까시나무의 경우에 施肥하지 않은 苗木에서 二重接種으로 因하여 乾重量이 25%, 苗高生長이 23% 增加하였으며 施肥한 경우 二重接種으로 苗高生長이 13% 增加하였다. 위와같은 實驗으로 Glomus mosseae는 사방오리나무의 生長을 促進하고, 菌根菌과 根瘤菌의 二重接種으로 相乘效果가 나타나며, 菌根菌의 效果는 土壤肥沃度가 낮을때 더 크게 나타난다고 結論짓는다.

INTRODUCTION

Mycorrhizae are a common feature in most of the trees growing in natural forest ecosystem. Particularly vesicular-arbuscular(VA) mycorrhizae are associated with many tree species including nitrogen fixer in forests, such as legumes and alders(Trappe, 1979). Nodulated plants require large amounts of phosphorus during the process of biological nitrogen fixation(Bergersen, 1971), and are benefited from VA mycorrhiza for phosphorus uptake(Barea and Azcon-Aguilar, 1983). Phosphorus is a limiting factor for tree growth due to low content of available phosphate in forest soil(Pritchett, 1979).

Robinia and *Alnus* species are commonly planted in Korea for reclamation and improvement of poor forest soil. These two groups of plants are known to form endomycorrhiza, but in the *Alnus* VA mycorrhizae are not readily observed in natural environment(Lee and Koo, 1983). Recently Joo (1986) reported successful formation of VA mycorrhiza in *Alnus hirsuta*, using *Glomus* as an inoculum. *Glomus* was also used successfully in growth stimulation of sweetgum trees(Kormanik *et al*., 1981) and in mulberry trees in Korea to improve the growth and nutrition(Kim and Moon, 1986).

The objectives of present study were to evaluate the potential of *Glomus mosseae*, one of the widely used VA mycorrhizal fungi, as an effective inoculum for *Robinia* and *Alnus*, and efficiency of the fungus in relation to soil fertility.

MATERIALS AND METHODS

Experiments with *Alnus firma*

1) Culture of Mycorrhizal Inoculum and Inoculation.

Glomus mosseae was introduced from Institute for Mycorrhizal Research and Development in Athens, Georgia, U.S.A. as a soil inoculum containing mixture of soil, spores, and chopped sorghum roots. The fungus was pot-cultured twice using *Sorghum bicolor* var. Pioneer 931(U.S.A.) as a host. Pot culture was initiated by adding 100cc of the *Glomus* inoculum to 4*l* of steam-sterilized sandy loam supplemented with 25cc of hydrated lime to each pot. *Sorghum* seeds were sown and grown in greenhouse for six months before used as an inoculum. final inoculum consisted of 250ml mixture of soil and chopped roots. Spore counts indicated presence of 700-800 spores per 100cc of soil inoculum. Inoculum was added and mixed with the pot soil before transplanting *Alnus* seedlings.

2) Preparation for *Frankia* Inoculum.

Fresh root nodules were harvested in the fall from 1-year-old seedlings in a nursery of *Alnus firma*. The nodules were kept in a refrigerator for about a month before used as an inoculum. About five grams of root nodules were gently crushed in a mortar and pestle and nodule slurry was added to the pots for inoculation before sowing of *Alnus* seeds.

3) Cultivation of *Alnus firma*

Seeds of *Alnus firma* were collected in fall from a single mother tree at 20 years of age in Yongjon-ri, Seungju-gun, Chonnam Province, and germinated in vermiculite for about a month. After cotyledons were fully expanded, two seedlings were transferred to each pot containing 4*l* of steam-sterilized soil(1 : 1 mixture of nursery sandy loam and sand). Seedlings were grown for six months in heated greenhouse under the natural light from December to June of following year.

4) Experimental Design

A 4 x 2 factorial design was employed in this study to compare four kinds of fungal treatments at two levels of soil fertility. Each pot containing 4*l* of steam-sterilized soil received one of the following four treatments : 1) no inoculation to serve as control, 2) inoculation with *Frankia*, 3) inoculation with *Glomus mosseae*, 4) dual inoculation with *Frankia and Glomus mosseae*. For fertilizer treatment 5.0g of organic fertilizer(10% N, 10% P, 10% K plus other components) was added to each pot. Twenty pots with two seedlings each were assigned for each treatment.

Experiment with *Robinia pseudoacacia*

Polyethylene pots(30cm in diameter, 40cm deep) buried in nursery bed were used in this experiment. Nine pots were placed in a group to receive a single treatment. Pot soils plus entire nursery bed were fumigated for 4 days with methyl bromide as described by Koo *et al*.(1982). *Rhizobium* inoculum was prepared by crushing fresh root nodules of 1-year-old *Robinia pseudoacacia* seedlings. About one gram of crushed root nodules was added to each pot for inoculation. For mycorrhizal inoculation, 50 ml chopped roots of 2-year-old infected *Cryptomeria japonica* were used which contained mixed spores of *Glomus* sp. and *Gigaspora* sp.

After inoculation with *Rhizobium* and mycorrhizal fungi, seeds were sown and seedlings were fertilized once with 13g of organic fertilizer, and grown for five months until harvest in October for dry weight (above ground portion) measurement. Experimental design was same as *Alnus firma*, except each treatment consisted of nine pots in a group and replicated six times. A total of 392 pots were employed in this experiment.

RESULTS

Table 1 shows height growth and dry weight increase of *Alnus firma* seedlings inoculated with *Glomus mosseae*. Plants grew slow during the first four months due to slow germination and relatively low temperature in greenhoure (about 20℃ during the day and 15℃ at night). In unfertilized pots, plants inoculated with *Glomus mosseae* grew 27% faster in dry weight (6.36g) than uninoculated control plants(4.99g), or 18% faster in height. The effect of mycorrhizal inoculation started to show about four months after germination of seedlings. *Frankia* alone showed similar effect, and dry weight of *Frankia*-inoculated seedlings was 38% heavier than uninoculated control plants. Synergistic effect of mycorrhizal and actinorhizal association was shown in dual inoculation, in which inoculated plants(9.13g) were 83% heavier than uninoculated plants(4.99g).

In fertilized plants, the effect of mycorrhizal inoculation was not shown at all or instead reduced the growth of host plants. As shown in Table 1, mycorrhizal inoculation in fertilized plants slightly depressed the dry weight increase of the host plants, while *Frankia* inoculation alone stimulated the dry weight increase by 19% compared with uninoculated plants. In fertilized plants, dual inoculation with

Table 1. Growth of potted *Alnus firma* seedlings inoculated with *Glomus mosseae* and *Frankia* in either fertilized or unfertilized condition in greenhouse. Pot soil was steam-sterilized before inoculation. Seeds were sown on November 20 and plants were grown for six months.

Inoculation Treatment	Unfertilized				Fertilized			
	Height(cm)		dry weight (g)	Number of nodules	Height(cm)		dry weight (g)	Number of nodules
	Apr. 28	June 8			Apr. 28	June 8		
No Inoculation	11.0	43.5	4.99	8.2	18.1	58.9	8.83	6.1
Frankia	13.4	53.7	6.89	15.6	21.4	66.4	10.5	10.2
Glomus	12.3	51.3	6.36	10.2	17.4	63.0	8.46	11.2
Glomus + *Frankia*	15.6*	68.0*	9.13*	7.0	15.2	61.3	8.12	17.6

* ; Significantly different from others at 5% level.

mycorrhiza and actinomycete (8.12g) reduced dry weight more than mycorrhizal inoculation alone(8.46g). When height growth data are compared, mycorrhizal plants were shortest on Apr. 28(5 months after germination), but showed some recovery at the end of the experiment, and slightly taller than non-mycorrhizal plants.

Root nodules were counted when plants were harvested, and number of nodule clusters is shown in Table 1. In unfertilized pots, nodule formation was more abundant in *Frankia*-inoculated plants than in dual inoculation. In case of fertilized pots, dual inoculation resulted in most abundant nodule formation of about 18 nodule clusters per plant, even though dry weight of these plants were smaller than plants with other treatments.

Table 2 shows growth of *Robinia pseudoacacia* seedlings inoculated with mycorrhizal fungi and *Rhizobium*. In unfertilized treatment, plants inoculated with both mycorrhiza and *Rhizobium* resulted in 23% more height growth or 25% more dry weight increase than uninoculated control plants. Mycorrhizal or *Rhizobium* inoculation alone was not much effective in growth stimulation of the host plants. In fertilized treatment, mycorrhizal inoculation alone stimulated host growth by 11% in height or 18% in dry weight, while *Rhizobium* inoculation alone failed to stimulate the host growth. Synergistic effect of dual inoculation was also shown in fertilized plants, and it corresponds to 13% more height growth or 21% more dry weight than uninoculated control plants.

Table 2. Growth of *Robinia pseudoacacia* seedlings inoculated with mycorrhizal fungi and *Rhizobium* in either fertilized or unfertilized condition. Nursery bed was fumigated and seeds were sown in early May and plants were grown for five months.

Inoculation	Unfertilized		Fertilized	
Treatment	Height (cm)	Dry Weight(g)	Height (cm)	Dry Weight(g)
No Inoculation	74	32	132	68
Rhizobium	67	24	127	69
Mycorrhiza	79	33	147	80
Mycorrhiza + *Rhizobium*	90	40*	150	82

* Significantly different from others at 5% level.

DISCUSSION

In Tables 1 and 2 it is clearly shown that nitrogen-fixing plants inoculated with endomycorrhizal fungi grow much faster and heavier than non-mycorrhizal plants. However, soil fertility appeared to be very important in determining the efficiency of mycorrhiza. In case of *Alnus*, mycorrhizal stimulation of host growth was shown only under unfertilized condition, indicating that plants are benefited most from mycorrhizal symbiosis when soil nutrient availability is a limiting factor for growth. The enhanced growth of plants infected by vesicular-arbuscular mycorrhizal fungi results primarily from improved uptake of soil phosphate(Hayman, 1983 ; Rose and Youngberg, 1981). Particularly nitrogen-fixing plants are known to require large amounts of phosphate for active growth and nodulation(Gibson, 1976 ; Kucey and Diab, 1984). The possibility of mycorrhizae for increasing the effectiveness of *Rhizobium* was suggested by Ross and Harper (1970), and further substantiated by Schenck and Hinson(1973), and Kucey and Paul(1982). Daft *et al*.(1975) found natural population of VA mycorrhiza in *Robinia* plants growing in coal spoils and emphasized the importance of mycorrhiza in colonizing harsh environme . Double inoculation with VAM and *Rhizobium* resulted in 19% increase in soybean yield(Bagyaraj *et al*., 1979). 38% increase in cowpea yield(Islam and Ayanaba, 1981), and dry weight increase in *Leucaena*(Manjunath *et al*., 1984).

Actinorhizal plants are known to form tripartite symbiosis between plants, actinomycetes, and mycorrhizal fungi(Harley and Smith, 1983). Rose (1980) examined many actinorhizal plants and found 25 species in 7 families either ectomycorrhizal or endomycorrhizal. *Alnus* species commonly form abundant ectomycorrhizae in natural forest ecosystem(Lee and Koo. 1983), but are known very specific in selecting ectomycorrhizal symbiont (Molina, 1981). On the other hand, *Alnus* forms VA endomycorrhiza in natural environment(Green *et al*., 1979), and does not appear to be specific in selecting endomycorrhizal symbiont(Molina, 1981). For example, Joo(1986) successfully inoculated *Alnus hirsuta* seedlings with *Glomus mosseae* and *Gigaspora*. Present study with *Alnus firma* also confirmed the VA mycorrhizal formation as a predominant form of mycorrhiza.

Many studies with *Alnus* indicate the importance of endomycorrhize in nitrogen fixation (Hall *et al*., 1979). Wheeler *et al*. (1986) reported the improved nitrogen fixation of *Alnus* in Scotland by VA mycorrhiza. Photosynthetic activity was stimulated by mycorrhizal formation in *Alnus hirsuta* which resulted in five fold increase in dry weight of the inoculated seedlings grown in sand (Joo, 1986). In the present study, inoculated *Alnus* seedlings grew 27% more in dry weight than control plants, and this small effect compared to Joo's result(1986) appeared to be due to the relatively fertile soil used in the present study.

In Table 1, initial growth depression of the dual inoculation treatment(measured on Apr. 28) was observed in fertilized pots. It suggested the possibility that early stage of mycorrhizal establishment might have caused excess carbohydrate drain to the fungus before the fungus took an active role in nutrient absorption from the soil. This kind of initial depression of host growth is commonly observed in mycorrhizal research (Powell and Bagyaraj, 1984). For example, mycorrhizal fungi competed with host plants for carbohydrate in ryegrass (Buwalda and Goh, 1982), and in sunflower (Koide, 1985). Kucey and Paul(1982) found that about 4% of photosynthate were utilized by mycorrhizal fungus in faba beans.

Present study showed that dual inoculation of nodulating plants with nitrogen-fixing bacteria and mycorrhizal fungi resulted in synergistic enhancement of host growth. Particularly, the synergistic effect was pronounced under unfertilized condition. *Alnus* and *Robinia* are primarily planted for reclamation and improvement of poor soil in temperate zone. Therefore, successful mycorrhizal formation in these tree species should be considered as a prerequisite step for production of good quality planting stocks, especially when these trees are to be planted in poor site. Further study may be needed to select more species and strains of VA mycorrhizal fungi to be used as inocula for nitrogen-fixing woody plants.

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