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Growth and Ectomycorrhizal Development of Container-Grown *Quercus acutissima* Seedlings Inoculated with *Pisolithus tinctorius*[1]

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Pt菌의 人工接種에 依한 상수리나무 盆苗의 生長과 外生菌根發達[1]

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ABSTRACT

Containerized *Quercus acutissima* seedlings were inoculated with vegetative mycelial inoculum of *Pisolithus tinctorius* and were grown in a green-house. The ectomycorrhizal fungus, *P. tinctorius* (Pt) increased growth of the container-grown oak seedlings. Of three treatments, Pt inoculation stimulated remarkably primary lateral roots, shoot growth, and root collar diameter and leaf area development, compared with either non-inoculated or natural soil treatment. In addition, Pt-inoculated seedlings produced ectomycorrhizae. by 71.1 percent. However, noninoculated and naturally grown seedlings were free of natural ectomycorrhizal. Ectomycorrhizal roots developed more abundantly in the middle portion of the container than in the top or bottom portions.

Key words: ectomycorrhizae; Pisolithus tinctorius; *mycelial inoculum;* Quercus acutissima.

要 約

Pt 菌糸를 增殖培養後 人工接種한 상수리나무 盆苗는 溫室內에서 자랐으며, 外生菌根 Pt가 상수리나무 盆苗의 生長과 發達에 至大한 效果를 나타내었다. 本 實驗의 3가지 處理中 Pt 接種盆苗는 一次側根의 數와 길이, 樹高生長, 根部直徑, 葉面積의 發達에 顯著하게 나타나는 有意的인 因子였다. 그러나 菌根을 接種하지 않은 상수리나무 盆苗는 外生菌根의 形成을 볼 수 없었다. 또 Pt 接種盆苗에 있어서 外生菌根의 發達은 71.1%였으며, 대부분 外生菌根은 盆의 上部와 下部보다는 盆의 中央部位에서 增加하는 傾向을 보였다.

INTRODUCTION

Many scientists are enduring to increase wood products which must be produced on a fixed area of land. As a result of research on forest trees, attempts to use mycorrhizal fungi for the benefits of man are appearing.

Mycorrhizae, a symbiotic association between fungal hyphae and roots of higher plants, have been recognized since the last century, and mycotrophy has been intensively studied for more than 80 years.

Ectomycorrhizae in particular occur naturally in the world. It has been known for a long time that most of the common coniferous trees usually possess ectotrophic mycorrhizae. Even though sub-

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stantial amount of work has been done on them, our knowledge of how the fungi causes the ectotrophic mycorrhizae to form is very scanty.

Many coniferous trees cannot grow without ectomycorrhizae. Recent findings imply that any kind of tree seedlings with ectomycorrhizae are better than ones without ectomycorrhizae at all, especially in pines. This point has an important significance to large-scale afforestations with normally ectomycorrhizal trees in areas where their symbiotic fungi do not occur naturally (Marx, 1977).

The fungal partner of ectomycorrhizae is usually a basidiomycete. About 80 species of basidiomycetes have been experimentally demonstrated and are suspected to be mycorrhizal formers with forest trees (Trappe, 1962). Mycorrhizae require little attention in the management of natural forests. Although a lot of the basic mycorrhizal research has been accomplished with agronomic plants, a meaningful portion of plant growth and productivity can be attributed to the application of the mycorrhizal technology (Marks and Kozlowski, 1973). Practical application of mycorrhizae to plant productivity is more obvious in forestry than agriculture. Seedlings quality has been developed and increased by mycorrhizae use in the production of container and nursery-grown oak and pine seedlings. Futhermore, in man-made forests and nurseries, mycorrhizae need symbiotic elements essentially for the existence of higher plants. For instance, conditions in a forest nursery differ greatly from those in natural forests, and this may cause serious disturbances when seedlings are transplanted from the nursery to the field.

To solve such critical problems from their transplant, containerized oak seedlings inoculated with the ectomycorrhizal fungus, *Pisolithus tinctorius* (Pers.) Coker and Couch, may be a valuable alternative to noninoculated seedlings. Frequently, slow initial growth following outplanting of oak seedlings suffer from inadequate root system development and lack mycorrhizal associations that are common to naturally growing seedlings (Shemakhanova, 1962).

Recently, research on mass inoculation of pine seedlings with highly beneficial ectomycorrhizal fungus, *P. tinctorius*, has shown great potential benefits in tree nurseries, in artificial regeneration programs, and adverse sites (Marx *et al.*, 1976; Marx *et al.*, 1977; Marx *et al.*, 1982).

In addition, inoculation of oak seedlings with specific ectomycorrhizal fungi could potentially increase the root absorptive surface area as well as other physiological benefits (Garrett *et al.*, 1979; Dixon *et al.*, 1981). Futhermore, specific ectomycorrhizae, *P. tinctorius*, increased survival and growth of sawtooth oak seedlings (Anderson *et al.*, 1983).

The purpose of this study was to compare the potential value of inoculating container-grown seedlings with a particular species of ectomycorrhizal fungus, *Pisolithus tinctorius* (Pers.) Coker and Couch, compared with natural soil treated and noninoculated container-grown seedlings on growth and ectomycorrhizal development of Quercus acutissima seedlings.

MATERIALS AND METHODS

The study was conducted in a glass greenhouse at the Chonnam National University. Seedlings were grown for 23 weeks. Growth conditions consisted of approximately 65 percent full sunlight. Watering was done on alternate days, and ambient air and soil

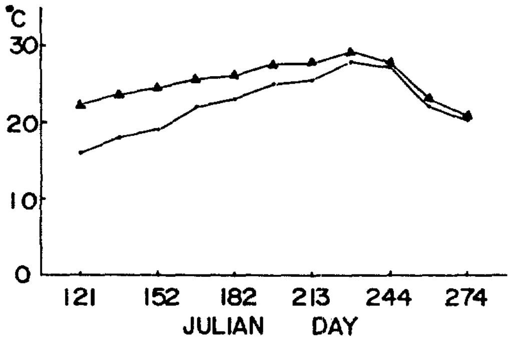

Fig. 1. Seasonal changes in mean soil and air temperatures
▲–▲ mean air temp.
●–● mean soil temp.

temperatures were shown in Figure 1. Inoculum of *Pisolithus tinctorius* (isolate 250) was provided by Koo *et al.* (1982) who introduced from Institute for Mycorrhizal Research and Development in Athens, Georgia, U. S. A. Inoculum cultured in sterile Petri dishes with Modified Melin-Norkrans' medium (Marx, 1969) at 25°C for about two weeks was transferred to preautoclaved one liter glass bottle filled with a 750 ml mixture of ground peat moss to passing a 20-mesh sieve and coarse vermiculite (1:24 v/v). Four hundred milliliters of MMN solution (pH 5.7) were added, stoppered with cotton, covered with aluminum foil, and autoclaved for 30 minutes at 15 psi. After inoculation all bottles were left in darkness at 25°C for about 4 months until fungus completely produced bright mustard yellow hyphae and prolific mycelial growth.

The vegetative mycelial inoculum grown in peat-vermiculite was removed from the bottles, bundled in cheesecloth, and rinsed under deionized water for two minutes. Excess water was removed by squeezing the inoculum in the cheesecloth by hand. After that the inoculum in polyethylene bag was stored for one day at 4°C. The 1,000 cc seedling cavities were inoculated by thoroughly mixing 35 ml of the squeezing inoculum into the growth medium. The sterile noninoculated containers and natural soil containers maintained not to be contaminated with Pt by covering them with aluminum foil. A homogeneous mixture (1:1 v/v) of loamy sand and finely chopped peat moss which had been steam-sterilized for 12 hours at 100°C was adopted as growth media. This growth medium was used to plant the acorns treated with either Pt-inoculated or noninoculated. The other was a natural soil treatment after surface soil was removed. The mixture of growth medium is an 1:1(v/v) ratio of peat moss and natural soil (loamy sand). Three treatments with three replicates were applied to randomized complete block design on the greenhouse bench. Acorns were collected at Bogil island, Chonnam province, during October of 1983. Collections were made from six different trees. Acorns which were visually infested by weevil or floated in water were discarded. All of the remaining acorns used were uniform in size, since acorn size is related to tap root growth. The acorns were soaked in tap water for 16 hours in polyethylene bags and stored at 4°C until used. On April 6, 1983, acorns which germinated with radicle of length of 0.5 mm were dipped into a 10 percent sodium hypochlorite solution for one minute and then rinsed in water. Pretreated acorns like this were planted at a depth of 2 cm in paper containers. One healthy acorn in each container was needed to remove sporadic germination.

The following data for individual seedlings were collected at the end of the growing period; sample of *Pisolithus* inoculated, noninoculated and natural soil inoculated seedlings from each treatment was randomly selected for analysis of stem diameter 1 cm above the root collar, length, dry weight, and number of lateral roots; leaf surface area and dry weight; percent of mycorrhizal short roots. Leaf surface areas were measured with a Lambda portable meter (model LI-3000).

Roots were carefully washed with tap water and examined for ectomycorrhizae. Percent of ectomycorrhizal short roots was calculated as the number of infected lateral root tips divided by the total number of root tips per root system. To confirm formation of ectomycorrhizae from ten seedlings picked randomly, selected lateral roots were cut from the tap root, and presence of the Hartig net of fungus mantle was examined under a microscope (150X).

RESULTS

Seedling growth was significantly increased in the container as a result of inoculation with *Pisolithus tinctorius* (Table 1).

Pisolithus – inoculated seedlings exhibited a significantly longer stem length than those from both noninoculation and natural soil in the container. Moreover, stem length of *Pisolithus*-inoculated seedlings was 40.6% and 20.7% greater,

Table 1. Means of growth variables of container-grown *Quercus acutissima* seedlings 160 days after treatment with Pt-inoculation, noninoculation and natural soil in the greenhouse.

Variables	Treatments		
	Pt-inoculation	Noninoculation	Natural soil
Shoot length (cm)	31.84 a[1]	22.64 b	26.37 b
Root collar diameter (mm)	2.74 a	2.28 b	2.33 b
No. of leaves	7.63 a	6.43 a	6.84 a
Leaf area (cm^2)	212.21 a	172.83 b	185.24 b
Stem dry weight (gm)	0.42 a	0.26 b	0.29 b
Leaf dry weight (gm)	0.67 a	0.53 b	0.55 b

[1] A common letter is not significantly different between treatments at the 0.05 level by Duncan's New Multiple Range Test.

respectively, than seedlings harvested from the two other treatments. Significant seedling growth differences in root collar diameter were also shown. Pt - inoculated seedlings resulted in larger diameter compared to noninoculated stock or natural soil stock. The inoculated ones had 20.0 percent and 17.6 percent longer roots, respectively, than the latter two treatments. In number of leaves, there were no significant differences among Pt - inoculation, noninoculation and natural soil treatments. Although differences were not significant, the seedlings inoculated with *Pisolithus* had a larger mean leaf number than the others at the end of the first growing season.

Leaf area was significantly greater in the Pt - inoculated seedlings than in the noninoculated seedlings. Furthermore, inoculation with *Pisolithus tinctorius* increased mean leaf area by 22.8 percent over the noninoculated seedlings. Pt - inoculated stock produced a stem weight increase of 61.5 and 44.8 percent, respectively, over the noninoculation or the natural soil grown seedlings. Pt - inoculated seedlings produced a greater leaf dry weight significantly than noninoculation and natural soil grown seedlings. Furthermore, the leaf dry weights of the inoculated seedlings were significantly greater by 0.53 g (26.4 percent) and 0.55 (21.8 percent), respectively, than the noninoculated seedlings and natural soil grown seedlings. Differences in primary lateral number due to Pt - inoculation were 49.6 percent and 36.7 percent greater, respectively, than the noninoculated and natural soil grown seedlings. Pt seedlings showed many short roots per centimeter of lateral root compared with non-mycorrhizal and natural soil seedlings (Table 2).

However, there was no difference in number of short roots per centimeter of lateral root between mycorrhizal and natural soil seedlings. In addition, when soil profile of container was divided into three zones such as top, middle, and bottom, respectively, 54% of the short roots egressed from top position (Fig. 2).

Pt - inoculated seedlings showed greater differ-

Table 2. Means of root variables of *Quercus acutissima* seedlings treated with and without *Pisolithus tinctorius.*

Variables	Treatments		
	Pt-inoculation	Noninoculation	Natural soil
No. of primary lateral roots	175 a[1]	117 b	128 b
No. of short roots of lateral roots	51.2 a	32.4 b	45.1 a
Total length of lateral roots (cm)	479.3 a	268.1 b	278.8 b
Primary lateral root weight (g)	1.15 a	0.45 c	0.81 b
Percentage of mycorrhizal short roots	71.1	0.0	0.0

[1] A common letter is not significantly different between treatments at the 0.05 level by Duncan's New Multiple Range Test.

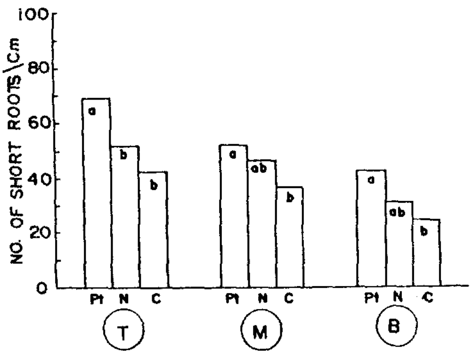

Fig. 2. No. of short roots per cm lateral roots of *Quercus acutissima* seedlings. Bars with the same letter are not significantly different between treatments (p=0.05).
Soil profile of container was divided into three zones;
T = top 7 cm, M = middle 7 cm,
B = bottom 7 cm

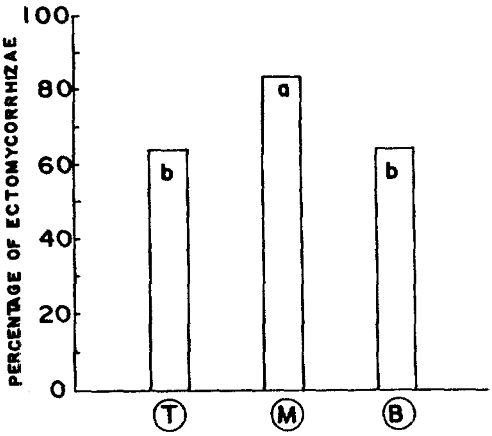

Fig. 3. Ectomycorrhizal development of *Quercus acutissima* seedlings with *Pisolithus tinctorius*. Bars with the same letter are not significantly different between treatments (p = 0.05).
Soil profile of container was divided into three zones;
T = top 7 cm, M = middle 7 cm,
B = bottom 7 cm.

ences in short root development from top, middle and bottom zones than the noninoculated and natural soil seedlings (Fig. 2).

Total length of lateral roots was significantly greater for the Pt– inoculated seedlings than for the noninoculated and natural soil seedlings. Pt– inoculated seedlings developed greater lateral roots in length than the other treatments. Pt– inoculated seedlings exhibited the total length of lateral roots 211.2cm, or 78.8%, and 200.5cm or 71.9%, longer, respectively, than the noninoculated and natural soil seedlings. Primary lateral root weight in the Pt– inoculated seedlings was 15.5 percent greater than in the noninoculated and natural soil grown seedlings. *Pisolithus tinctorius* formed ectomycorrhizae on approximately 71.1 percent of the short roots of seedlings in inoculated seedlings during one growing season (Fig. 3, Table 2).

Moreover, all inoculated seedlings produced abundant Pt– ectomycorrhizae. All noninoculated seedlings were free of ectomycorrhizae. When soil profile of container was divided into three zones, 83.9 percent of the ectomycorrhizae were observed in middle zone (Fig. 3). Thus, Pt seedlings in middle zones had significantly greater mycorrhizal short roots than those in both top and bottom zones.

DISCUSSION

In this study, *Quercus acutissima* seedlings inoculated with *Pisolithus* showed significantly greater growth than noninoculated and natural soil grown seedlings during the first growing season. In general, the result of this study demonstrated that Pt– inoculation seedlings had greater primary lateral roots and shoot growth, and root collar diameter and leaf area development. The result that inoculation of the containerized seedlings with *Pisolithus tinctorius* exhibited an increase in all growth variables and benefited to the growth of seedlings was similar to growth increment of reported by Marx (1979). Northern red oak exhibited superior stem length, stem weight, and leaf weight when compared to noninoculated seedlings.

It is generally recognized that mycorrhizae in trees normally cause a striking increase in growth and survival (Fisher and Cox, 1979). Many mycorrhizal researchers have demonstrated that mycorrhizal root symbionts play an important role in uptake of mineral nutrition. In *Pinus elliottii* and *Pinus radiata* experiments, tree roots with mycorrhizae were able to absorb more nutrients than those without mycorrhizae per unit dry weight or surface area (Lamb and Richards, 1971). In this study, stem length of *Pisolithus* inoculated seedlings was 40.6% greater than noninoculated seedlings (Table 1). The overall growth of the seedling inoculated with *Pisolithus* produced significantly greater numbers of primary lateral roots, mycorrhizal short roots and total length of lateral roots than noninoculated and natural soil seedlings (Fig. 3, Table 2). Primary lateral root number and short root number in Pt– inoculated seedlings were 49.6 percent and 58.0 percent greater, respectively, than in noninoculated seedlings. Furthermore, total length of lateral roots exhibited the remarkable growth differences among the Pt– inoculated, noninoculated and natural soil; Pt– inoculated seedlings increased 211.2cm (78.5%), and 200.5cm (71.9%) more, respectively, than noninoculated and natural soil seedlings. Seedlings maintained under high fertility and sterile soil hindered the ectomycorrhizal development on containerized oak seedlings. Moreover, soil fumigation removed the competing other microorganisms that inhibit development of Pt– ectomycorrhizae and spread of Pt hyphae in the container. Consequently, due to the fact that soil fumigation eliminated the indigenous symbiotic fungi, many lateral roots extended out from the container and short roots egressed, but they were noninfected (Ruehle, 1983). In this study, both experiment conditions the seedlings except the Pt– inoculated seedlings failed to form the abundant natural ectomycorrhizae in the primary lateral roots. However, the formation of short roots in the natural soil seedlings developed well compared to those of noninoculated seedlings which planted after natural soil was steriled. Thus, when few short roots develop, few mycorrhizae are able to develop. Furthermore, when excavated from the containers, short roots did not exhibit similar symptoms to ectomycorrhizal roots in the noninoculated and natural soil seedlings. As a result, fumigation markedly affected factors influencing mycorrhizal formation. In addition, the short roots infected with *Pisolithus* produced 71.1 percent ectomycorrhizae (Table 2). When soil profile of container was divided into three zones, most of the ectomycorrhizal roots developed in the middle portion of the container compared to the top and bottom portions of the containers. Inoculum of *P. tinctorius* must be incorporated deeper than 10 cm in soil for maximum ectomycorrhizal development on northern red oak in the nursery study (Marx, 1979). Thus, inoculum should be placed with the deeper portion of container.

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