韓國林學會誌 79(3) : 302-308. 1990.
Jour. Korean For. Soc. 79(3) : 302-308. 1990.

Effects of Inoculation with Mycorrhizal Fungi, *Pisolithus tinctorius* and *Glomus* sp. on the Rooting of *Quercus acutissima* Carr. Cuttings at Various Ortet Ages[1]*

Jong Ju Kim[2] and Kyung Joon Lee[2]

모래밭 버섯菌과 *Glomus* 菌根菌의 人工接種이 年齡이 다른 상수리나무에서 採取한 揷穗의 揷木發根에 미치는 影響[1]*

金鍾周[2] · 李景俊[2]

SUMMARY

This study was conducted to determine the effects of mycorrhizal inoculation and ortet ages on the rooting of *Quercus acutissima* cuttings. The cuttings taken from 12-week-, 2-year-, 4-year-, and 20-year-old plants were rooted in mid summer in the rooting medium (vermiculite 2 : peatmoss 1 by volume) with or without *Pisolithus tinctorius* (Pt) and *Glomus* sp. inocula and IBA under intermittent misting system in an open shed.

The average percentages of rooting were about 82%, 49%, 29%, and 13% for cuttings taken from 12-week-, 2-year-, 4-year-, and 20-year-old seedlings, respectively. Pt inoculation enhanced rooting of cuttings at all age classes, except 12-week-old seedlings, with the highest enhancement (22%) observed in cuttings taken from 20-year-old trees. The highest percentage of rooting in each age group was 88.9% in 12-week-old seedlings treated with Pt plus 3,000ppm IBA, 75% in 2-year-old plants with 1,000ppm IBA, 58.3% in 4-year-old plants with 3,000ppm IBA and 22% in 20-year-old plants.

The addition of *Glomus* sp. fungus inoculum failed to enhance rooting. Pt mycorrhizal inoculation enhanced root dry weight, length, and diamter of adventitious roots at cuttings taken from 12-week- and 20-year-old trees, except the cuttings taken from 4-year-old seedlings. Rooted cuttings had more total nitrogen content in the leaves than unrooted cuttings, and the greater rooting response was associated with the higher phosphorus content in the leaves.

Key words : *Rooting of cuttings, mycorrhizal inoculation, age effect,* Pisolithus tinctorius, Glomus sp., Quercus acutissima.

要 約

本 研究의 目的은 12週, 2年, 4年, 20年生 상수리나무 母樹에서 採取한 揷穗에 모래밭버섯菌(*Pisolithus tinctorius*)과 *Glomus* sp. 內生菌根菌을 人工接種하여 揷穗의 發根, 不定根 發達, 發根特性 등을 調査하여, 菌根菌의 人工接種이 상수리나무의 揷木發根에 미치는 影響을 알아보는데 있다.

菌根菌을 人工接種한 것과 接種하지 않은 培地(버미쿨라이트 : 피트모스=2 : 1, v/v)에 1989年 8月初에 揷木을 實始하였다. 揷穗는 午前 7時에서 午後8時까지 20秒間 作動하고 2分間 멈추는 簡歇灌水시스템 하

[1] 接受 1990年 5月 15日 Received on May 15, 1990.
[2] 서울大學校 林學科 Dept. of Forestry, Seoul Nat'l Univ., Suwon 441-744, Korea.
* This research was financed by Korea Science and Engineering Foundation. Their support and encouragement are greatly appreciated.

에, 약 25/18℃(낮/밤)의 溫度, 78%의 遮光條件 下에서 約 10週間 培養하였다.

12週, 2年生, 4年生, 20年生 모수에서 採取한 揷穗의 平均發根率은 각각 82.4%, 48.1%, 29.2%, 12.5%로 나타났다. 모래밭버섯균의 人工接種은 12주된 실생묘를 제외하고 모든 年齡의 揷穗發根을 促進하였고, 특히 20년생의 경우에는 대조구에 比較하였을 때, 그 效果가 두두러지게 나타났다. 揷穗의 發根率이 가장 높은 處理는 12週된 實生苗의 경우, 모래밭 버섯과 3,000ppm IBA 處理(88.9%), 2年生 幼苗는 1,000ppm IBA(75%), 4年生 萌芽는 3,000ppm IBA(58.3%), 20年生은 모래밭 버섯과 3,000ppm IBA 處理(22%)에서 나타났다. *Glomus* sp. 내생균근균의 接種은 삽수의 初期發根을 促進시키지 않았다. 뿌리 乾重量, 發根된 揷穗當 一次根의 길이와 直徑등에 있어서 모래밭 버섯과 3,000ppm IBA를 함께 處理한 것이 가장 높았다.

葉內 全窒素 含量은 處理間에는 差以가 없었으나, 發根된 揷穗가 發根되지 않은 揷穗보다 더 많은 것으로 나타났다. 그리고 發根率이 높은 處理가 葉內 全燐酸 含量이 높게 나타났다.

INTRODUCTION

Oaks are dominant species in Korean forest ecosystem, but have been recognized as minor broad-leaved species. Today, much interest in the development and utilization of oak resources has stimulated research on better management and improvements of their potential use in Korea (Lee *et al*., 1989). However, works on the vegetative propagation of oaks by cutting have been rather limited because they are difficult-to-root species (Skinner, 1952 ; Flemer, 1962), although successful adventitious root formation from the cuttings of young *Quercus acutissima* seedlings has been reported Moon *et al*., 1987 ; 1988).

Recently, potential use of mycorrhizal fungi has received much attention in agriculture (Menge, 1983). The idea of mycorrhizal stimulation of plant growth was based on the observation that mycorrhizal fungi could enhance the plant growth by releasing plant growth substances, auxins, into the culture medium and some times in the absence of actual root infection (Levisohn, 1956), and protect the roots from the soil-borne pathogens (Dehn, 1982). Survival and early leaf development of the rooted cuttings might be also improved by mycorrhizal infection on roots (Lee and Koo, 1985).

The objectives of this study were to determine the effects of mycorrhizal fungus inoculations and ortet ages on the rooting of *Q. acutissima* Carr. cuttings.

MATERIALS AND METHODS

Mycorrhizal Inocula

Pisolithus tinctorius #1392 (Pt) was introduced from Institute for Mycorrhizal Research and Development in Athens, Georgia, U.S.A. and was used in this experiment. Mycelium of the Pt was mass cultured for 6 months in the mixture of vermiculite and peat moss (77 : 3 by volume) moistened with MMN (Modified Melin and Norkrans) liquid medium as described by Koo *et al*. (1982) and Marx and Bryan (1975).

Large resting spores of VAM fungus were collected from the soils in the rhizosphere of *Fraxinus americana* L. growing in Seoul Nat'l Univ. Arboretum located in Suwon, Kyonggi-do, Korea. The wet-sieving and decanting method described by Gerdemann and Nicolson (1963) was used for collecting VA fungal spores. The fungal spores used was putatively identified as *Glomus* sp. by the keys described by Trappe (1982) and Schenck and Perez (1987). They were surface-sterilized with 2% Chloramin T solution for 10 minutes and cultured in open pots in greenhouse for 6 months as Gilmore (1968) described.

Plant Materials and Rooting Medium

Information on ortets used in this study is shown in Table 1. *Q. acutissima* shoots were prepared as follows : they were cut into about 7㎝ in length and two leaves on the upper part of cuttings were also cut in a half size, and other leaves were removed. The rooting media was a mixture of vermiculite and peatmoss in the ratio of 2 : 1 (v/v).

Table 1. Ages and origins of *Q. acutissima* ortets used in this study.

Ages	Origins
12-week-old seedlings	Univ. Forest[1]
2-year-old seedlings	Univ. Nursery[2]
4-year-old seedlings	Univ. Nursery[2]
20-year-old tree	Arboretum[3]

[1]: Seeds were collected at Interior station, Univ. Forests located in Kwangju-gun, Kyonggi-do.
[2]: University nursery in Suwon.
[3]: University Arboretum located in Suwon.

IBA Application and Inoculation

The lower cut end of cuttings were dipped in various concentrations of IBA solutions for 5 sec. and planted into rooting medium.

The Pt inoculum was washed thoroughly with tab water, layered on the rooting medium, and covered with additional 3cm thick of the rooting medium. VAM inocula were also placed at 3cm depth of the rooting substrates.

Culture Condition of Cuttings & Experimental Designs

Cuttings were cultured for 10 weeks during mid summer in a misting bed roofed with semitransparent plastic slates intercepting 78 percent of full sun light. An intermittent misting system was operated during the day, from 07 : 00 to 20 : 00, and the water misted for 20 seconds every two minutes. Air temperature was about 25℃ during the day and around 18℃ at night.

A completely randomized design with three replications per treatment was used. Two sets of experiments were designed in this study. One set of experiment consisted of following treatments of 3,000ppm IBA, Pt, *Glomus*, 3,000ppm IBA plus Pt, 3,000ppm IBA plus *Glomus*, and control for cuttings taken from 12-week-, 4-year-, 20-year-old plants. Another set of experiment used cuttings taken from 2-year-old seedlings with the treatments of 500ppm IBA, 1,000 ppm IBA, 3,000ppm IBA, 5,000ppm IBA, Pt, and control. A treatment had 36 cuttings and total cuttings used in the experiment were 864.

Measurements

All of the cuttings harvested in early October 1989, and the number and the length of all the adventitious roots longer than 2mm in length were recorded. Cuttings with one or more roots were classified as the rooted. Number of cuttings survied, diameter of adventitious roots, and root dry weight were measured.

Total nitrogen and phosphorus contents in the leaves were determined with micro-Kjeldahl method (Bremner and Mulvaney, 1984) and molybdovanado-phosphoric acid method (Kitson and Mellon, 1944), respectively. The results were compared by Duncan's new multiple range test and the Chi-square test.

RESULTS AND DISCUSSION

Rooting of Cuttings

Figure 1 shows the rooting of IBA-treated or nontreated cuttings of *Q. acutissima* inoculated with Pt or *Glomus* sp. in the rooting medium. The overall percentages of rooting were 82.4%, 29.2%, and 12.5% for cuttings taken from 12-week-old, 4-year-old, and 20-year-old trees, respectively.

In cuttings taken from 12-week-old seedlings, there was no significant effect of mycorrhizal inoculation on the rooting. However, the highest rooting (88.9%) was observed in the treatment of Pt with 3,000ppm

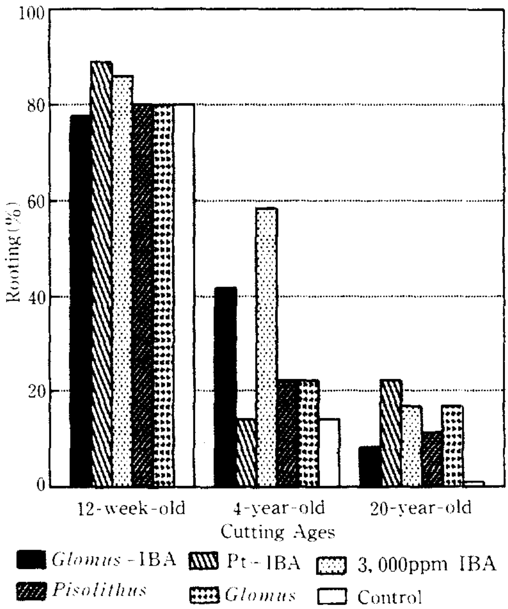

Fig. 1. Rooting of *Q. acutissima* cuttings treated with mycorrhizal inoculum and (or) 3,000 ppm IBA.

IBA. In cuttings taken from 4-year-old seedlings, 58.3% of the cuttings treated with 3,000ppm IBA were rooted, and 41.7% rooted in *Glomus* sp. inoculum plus 3,000ppm IBA, compared to 16.7% of cuttings rooted in the control. The Chi-square and Duncan's new multiple range test indicated that the differences among or between the treatments at rooting of cuttings taken from 4-year-old seedlings were significant at the 5% level (Table 2).

Table 2. Effects of mycorrhizal inoculations and auxin on rooting of cuttings taken from 12-week-, 4-year-, and 20-year-old trees.

	Treatments	12-week	4-year	20-year
Rooting (%)	*Gl.*-IBA	77.8a*	41.7ab	8.3b
	Gl.	80.1a	22.2bc	11.1b
	Pt-IBA	88.9a	13.9c	22.2a
	Pt	80.1a	22.2bc	16.7ab
	IBA	86.1a	58.3a	16.7ab
	Control	80.1a	13.9c	0.0c
Root Length (cm)	*Gl.*-IBA	7.7bc	7.8ab	5.7a
	Gl.	8.8b	5.1b	6.2a
	Pt-IBA	10.6a	10.3a	7.2a
	Pt	7.5bc	7.6ab	7.8a
	IBA	7.0bc	5.9b	6.7a
	Control	7.1c	9.1ab	0.0b
Root Diameter (mm)	*Gl.*-IBA	0.91bc	1.87a	1.53ab
	Gl.	0.93b	1.51a	1.42ab
	Pt-IBA	1.06a	1.70a	1.61a
	Pt	0.82c	1.87a	1.53ab
	IBA	0.83bc	1.56a	1.35b
	Control	0.83bc	1.53a	0.0c
Number of roots	*Gl.*-IBA	2.8ab	1.7a	1.8a
	Gl.	1.9b	1.2a	1.7a
	Pt-IBA	2.0ab	0.8a	2.1a
	Pt	2.0ab	1.7a	1.2ab
	IBA	2.9a	1.8a	2.4a
	Control	1.9b	1.4a	0.0b
Root dry weight (mg)	*Gl.*-IBA	226ab	201a	18bc
	Gl.	231ab	83b	35bc
	Pt-IBA	348a	101b	90a
	Pt	207b	173a	65ab
	IBA	227ab	228a	43abc
	Control	186b	94b	0.0c

IBA : 3,000ppm Indolebutyric acid solution.
Gl. : *Glomus* sp. fungus inoculation.
Pt : *Pisolithus tinctorius* fungus inoculation.
* : The same letters in a column are not significantly different at 5% level by Duncan's new multiple range test.

In cuttings taken from 20-year-old trees, cuttings treated with Pt inoculum plus 3,000ppm IBA showed 22.2% of rooting response. However, controlled cuttings produced no roots at all. Significant differences in rooting of cuttings were noted among the treatments at 5% level by Duncan's new multiple range test (Table 2).

On another set of experiment, with the cuttings taken from 2-year-old seedlings, the addition of Pt inoculum alone to the rooting medium also stimulated rooting of cuttings (55.6%), when compared to the control (36.1%) as shown in Figure 2 and Table 3. The cuttings treated with 1,000ppm IBA had the highest percent of rooting (75%), while 3,000ppm IBA showed lower percent than control, suggesting that optimum concentration of IBA for rooting of *Q. acutissima* cuttings was around 1,000ppm (Moon *et al.*, 1987).

Lee and Koo (1985) working with hybrid poplar in Korea demonstrated that inoculation of cuttings of hybrid poplar with *Pisolithus tinctorius* markedly enhanced rooting and survival in a nursery bed. Navratil and Rochon (1981) also reported enhanced root and shoot development of poplar cuttings induced by Pt inoculation, even though cultivar variation was

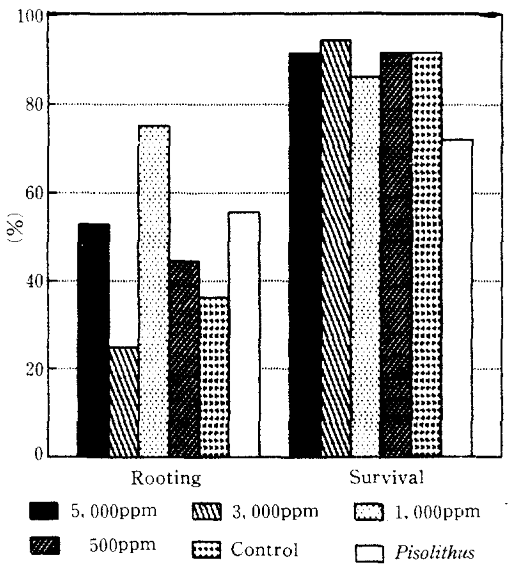

Figure. 2. Rooting and survival of cuttings taken from 2-year-old seedlings treated with *Pisolithus tinctorius* inoculum and(or) various concentration of IBA.

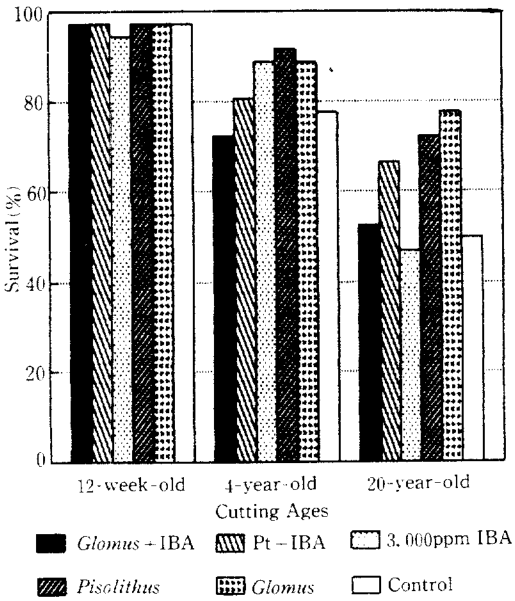

Figure. 3. Survival of *Q. acutissima* cuttings treated with mycorrhizal inoculm and (or) 3,000 ppm IBA.

noticed in response to the Pt inoculation.

In our study, the occurrence of mycorrhizal infection in the newly formed roots and occurrence of hyphae at the cut end of cuttings were not confirmed, though a moist propagation bed could encourage the spore germination in case of *Glomus* inoculation as observed by Hepper (1984).

Survival of Cuttings

Average survival rates of cuttings taken from 12-week- and 4-year-old seedlings were 96.8% and 84.9%, respectively (Figure 3). In cuttings taken from 20-year-old trees, differences in survival rates were markedly evident between the cuttings inoculated with Pt (77.8%) and the controlled cuttings (49.4%).

Length, Diameter, and Number of Adventitious Roots

In cuttings taken from 12-week-old and 4-year-old seedlings, length of adventitious roots was significantly longer in the Pt inoculation plus 3,000ppm IBA treated cuttings than in the controlled (Table 2). Effects of *Glomus* inoculation on rooting was not as apparent as those of Pt.

In cuttings taken from 12-week-old seedlings and 20-year-old trees, diameter of adventitious roots was thicker in Pt plus IBA treatment than in control, as shown in Table 2. And also the 3,000ppm IBA induced more new roots than control in cuttings from 12-week- and 20-year-old trees.

Root Dry Weight

In cuttings taken from 12-week-old seedlings, the addition of Pt inoculum plus 3,000ppm IBA produced 87% more root dry weight per cuttings than the control (Table 2). And in cuttings taken from 4-year-old seedlings, the 3,000ppm IBA, *Glomus* sp. plus 3,000 ppm IBA, and Pt inoculation increased 143%, 114%, and 84% more root dry weight, respectively, than those of the control. In 2-year-old seedlings, the 1,000ppm of IBA produced more root dry weight than other treatments (Table 3).

Total Nitrogen and Phosphorus Contents

Total nitrogen content in the leaves was greater in rooted cuttings than in unrooted ones (Tables 4 and 5). This indicates that stored nitrogen in leaves de-

Table 3. Effects of mycorrhizal inoculation and auxin on rooting, length, diameter, number, and dry weight of adventitious roots formed from cuttings taken from 2-year-old seedlings.

Treatments	Rooting (%)	Root length (cm)	Root diameter (mm)	Number of roots	Root dry weight (mg)
5,000ppm IBA	52.8b*	5.3a	1.97ab	1.8a	164a
3,000ppm IBA	25.0c	8.0a	2.02ab	2.0a	125a
1,000ppm IBA	75.0a	3.5a	2.55a	1.8a	205a
500ppm IBA	44.4bc	4.3a	1.97ab	1.5a	167a
Pt	55.6ab	2.0a	1.99ab	1.6a	147a
Control	36.1c	4.3a	1.72b	1.6a	131a

IBA : Indolebutyric acid solution.
Pt : *Pisolithus tinctorius* fungus inoculation.
* : The same letters in a column are not significantly different at 5% level by Duncan's new multiple range test.

Table 4. Total nitrogen(%) and phosphorus contents (mg/g) in leaves of rooted and unrooted cuttings from 12-week-old and 4-year-old plants.

Treatments	Nitrogen		Phosphorus	
	12-week	4-year	12-week	4-year
Rooted cuttings				
Glomus	2.1	1.8	1.75	1.77
Glomus+IBA	2.0	2.5	1.12	1.13
Pt+IBA	1.9	1.9	3.16	1.76
Pt	1.9	2.2	0.90	1.12
IBA	1.9	1.9	0.65	3.13
Control	1.9	2.1	0.26	0.78
Unrooted cuttings	1.7	1.6	1.12	1.10

IBA : 3,000ppm IBA solution
Pt : *Pisolithus tinctorius* fungus inoculation

Table 5. Total nitrogen(%) and phosphorus contents(mg/g) in leaves of rooted and unrooted cuttings taken from 2-year-old seedlings.

Treatments	Nitrogen	Phosphorus
Rooted cuttings		
5,000ppm IBA	2.4	2.81
3,000 〃	1.9	0.65
1,000 〃	1.7	3.92
500 〃	2.2	2.36
Pt	1.9	3.05
Control	2.1	0.91
Unrooted cuttings	1.7	0.90

Pt : *Pisolithus tinctorius* fungus inoculation

creased during the rooting process and recovered by absorption of adventitious roots after root emergence. The higher phosphorus content might be related to high percentages in rooting response (Tables 4 and 5). Haun and Cornell(1951) and Hartmann and Kester(1983) mentioned that the nitrogen nutrition of the stock plants had a greater effect on rooting response of the cuttings than did phosphorus.

CONCLUSIONS

The main point of interest of this study was to determine the effect of mycorrhizal inoculation on rooting of *Q. acutissima* cuttings. The enhancement of root development by the addition of mycorrhizal inoculum was observed, but there were some variations among the different ortet ages. Especially, the addition of ectomycorrhizal inoculum of *Pisolithus tinctorius* to rooting medium enhanced root initiation, the rate of cuttings survived, and root development of *Q. acutissima* cuttings taken from 20-year-old trees. Those characteristics such as dry weight, length, and diameter of adventitious roots might be augmented by the Pt fungus inoculation. Therefore, mycorrhizal application to cutting is recommendable for the production of well rooted cuttings, particularly when ortets are relatively old.

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