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Original article

Cutting propagation of *Quercus acutissima* clones after rejuvenation through serial grafting

HK Moon [1], JS Yi [2]

[1] *Institute of Forest Genetics, Forestry Administration, Suwon, 441-350;*
[2] *Department of Forestry, College of Forestry, Kangweon National University, Chooncheon, 200-701, Republic of Korea*

Summary — Vegetative propagation of 34- to 67-year-old *Quercus acutissima* trees was successfully achieved from rooted cuttings. Semi-hardwood ramets which were obtained from the clones established through grafting twice onto the 2-year-old rootstocks showed 60% rooting (ranging from 20% to 100%). After grafting only once, average rooting frequency was 11% (ranging from 0 to 34%) using cuttings dipped in 500 ppm indole-butyric acid (IBA) solution. The rooting medium consisted of equal volumes of peatmoss and perlite. Cuttings were watered by intermittent mist and grown in the greenhouse at 25 ± 3 °C for more than 5 weeks. For rooting, there was no significant difference between ortet age but marked differences were observed among the clones. Although most of the rooted cuttings did not sprout new buds in the current year, they usually produced slowly-growing buds and/or revealed plagiotropic growth at the following year.

***Quercus acutissima* / rejuvenation / serial grafting / semi-hardwood cutting**

Résumé — Bouturage de *Quercus acutissima* après greffage en cascade. *La multiplication végétative de* Quercus acutissima *âgés de 34 et 67 ans a été réalisée avec succès. Deux générations successives de greffage sur des porte-greffes de 2 ans ont permis d'obtenir des boutures semi-ligneuses manifestant un taux d'enracinement de 60% (variation totale de 20% à 100%). Après la première génération de greffes le taux d'enracinement n'était que de 11% (0% à 34%); les boutures étaient trempées dans de l'acide indolbutyrique 500 ppm, le substrat était constitué d'un mélange en quantités égales de tourbe et de perlite. Les boutures étaient élevées sous mist intermittent dans une serre à 25°C (plus ou moins 3°C) pendant 5 semaines. L'âge de l'ortet n'avait pas d'effet sur les taux d'enracinement; par contre des variations importantes entre clones ont été observées. Bien que la plupart des boutures n'aient pas débourré durant l'année de l'enracinement, elles produisirent des petites pousses l'année suivante, qui étaient dans certains cas plagiotropiques.*

Quercus acutissima */rejuvénilisation / greffage en cascade / bouture semi-ligneuse*

INTRODUCTION

In research and usage, little attention has been paid to hardwoods partly due to the forestry policy which focused on conifers and our habitual ways of thinking. Among hardwoods, oaks were considered just for usage such as fuel, tool handles, small furniture, acorns and timber for black forest mushroom (*Lentinus edodes* (Berk) Sing). However, the consumption of oak timber has gradually increased along with the development of wood-processing techniques and the diversity of wood demands (Lee *et al*, 1989).

At the Institute of Forest Genetics in Suwon, Korea, an Oak Improvement Project which aimed at clonal conservation of selected trees and the development of an efficient propagation method were started in 1982. However, grafting incompatibility caused considerable loss of clones in the clone bank which was established in 1984. Two methods are now highly recommended for oak propagation from rooted cutting and/or *in vitro* culture to overcome the obstacles. Although some successes have been reported in vegetative propagation whether by rooted cuttings or by *in vitro* culture (Spethmann, 1985; Manzanera and Pardos, 1990), oaks are still difficult to root compared with other forest trees (Skinner, 1953; Flemer, 1962). Previous studies have revealed the possibility of asexual multiplication of juvenile oak trees by semi-hardwood cuttings and tissue culture (Moon *et al,* 1987, 1988). However, the same propagation method was not successful with adult oak trees. The method described for effective rejuvenation may open the way for mass-propagation of oak species and was obtained through serial grafting of *Q acutissima* clones. This is the first report on the rejuvenation of this species using repeated grafting.

MATERIALS AND METHODS

Experiment 1

Eight clones of *Q acutissima* plus trees, 34- to 62-years-old, provided the first ramets for serial grafting followed by rooted cuttings. Scions, collected in February 1988, were wrapped in plastic bags containing moist cotton and stored in a refrigerator at 4 °C. Scions having 2 or 3 buds were grafted onto the 2-year-old rootstocks of the same plus trees in March 1988 and maintained in the greenhouse. In July 1988, semi-hardwood cuttings were taken from the shoots of the growing scions. The second grafting (using ramets from the first grafts in 1988) and semi-hardwood cuttings (using ramets from the second grafts in 1989) followed by the procedures of 1988 were carried out in March 1989 and July of the same year, respectively. Cuttings 10–12 cm in length, with 2 or 3 leaves were used. The proximal ends of cuttings were dipped into 500 ppm indolebutyric acid (IBA) solution for 3 s prior to being applied with a Captan and talc mixture. The rooting medium was formulated by mixing equal volumes of peatmoss and perlite then sterilized by autoclaving at 121 °C. Cuttings were watered with intermittent mist and maintained in a greenhouse (where solar screens were installed to give 30% shade) at 25 ± 3 °C for up to 5 weeks. Each clone provided 4–44 cuttings. After transplantation of the rooted cuttings into vinyl pots (height x width = 18 x 7 cm) containing an artificial soil mix, observations were made periodically during the winter to investigate survival and growth.

Experiment 2

Because the rootability of the cuttings taken from second grafts was significantly increased, further experiments were conducted using just the second grafts. For experiment 2, the first and subsequent graftings were done in March 1989 and March 1990, respectively. A total of 22 clones grafted twice were used for semi-hardwood cuttings in July 1990 (table I). Cutting procedures were followed by the methods described for experiment 1.

RESULTS AND DISCUSSION

The rooting percentage varied among the clones and according to grafting times (fig 1). After the first graft in experiment 1, Kyonggi (KG) clone 5 showed 34% of rooting but the average rooting percentage of all 8 clones was 11%; which implies that the physiological age of ortets (34–62 yr) was not changed by a single grafting. After the second graft, however, Chunbuk (CB) clones 17 and 30 showed rootabilities of 64 and 93%, respectively. This suggested that the scions may have been rejuvenated by the juvenile rootstocks (Doorenbos, 1954; Franclet, 1983; Siniscalco and Pavolettoni, 1988).

The age of the ortet has been reported as being one of the important factors for successful vegetative propagation (Isebrands and Crow, 1985). The results from both experiments, however, showed no recognizable differences in rootability by the tested ages after grafting. Serial grafting increased the rooting frequency of the cuttings derived from KG clone 5 which was 62-years-old, whereas CB clone 41 and Chungnam (CN) clones 1, both were 36-year-old, did not show such marked improvement. These results suggest that clonal differences are critical for efficient vegetative propagation of this species. When the cuttings of 60-year-old *Quercus robur* and *Quercus petraea* were incorporated, varied rootability (0%–40%) was observed (Spethmann, 1985).

Rejuvenation which could be obtained by serial grafting to young root stocks seems to be an essential step for efficient asexual propagation from adult trees (Hackett, 1985). Doorenbos (1954) and Paton *et al* (1970) also reported similar results using ivy and eucalypts, respectively. Moon *et al* (1988) reported successful rooting from almost all the ramets of *Q acutissima* obtained from the second grafts of the 2-year-old rootstocks. Although high concentrations of rooting substances were applied to ramets, direct cuttings from adult branches of the same species did not root at all. These investigations strongly

Table I. Rooting of cuttings of *Q acutissima* plus tree clones which were rejuvenated by repeated grafting onto the 2-year-old rootstocks.

Clones	*Ortet age* [a] *(yr)*	*No of cuttings*	*No of rooted cuttings*	*Rooting percentages*
Chunbuk (CB)				
2	36	8	3	37.5
3	35	21	11	52.4
5	36	17	16	94.1
8	34	3	1	33.3
9	36	15	10	66.7
11	35	15	11	73.3
18	34	18	15	83.3
20	36	11	6	54.5
23	35	10	8	80.0
25	35	17	11	64.7
26	34	22	18	81.8
29	34	3	3	100
36	35	19	16	84.2
Chungnam (CN)				
2	36	14	7	50.0
3	38	20	14	70.0
9	39	19	11	57.9
11	38	11	5	35.9
14	34	14	5	35.9
15	34	5	1	20.0
Kyonggi (KG)				
3	67	45	10	22.2
8	56	7	7	100
Kyongbuk (KB)				
3	50	23	14	60.9
Total of 22 clones		337	203	60.2

[a] Represents the age of mother trees at the time of cutting propagation.

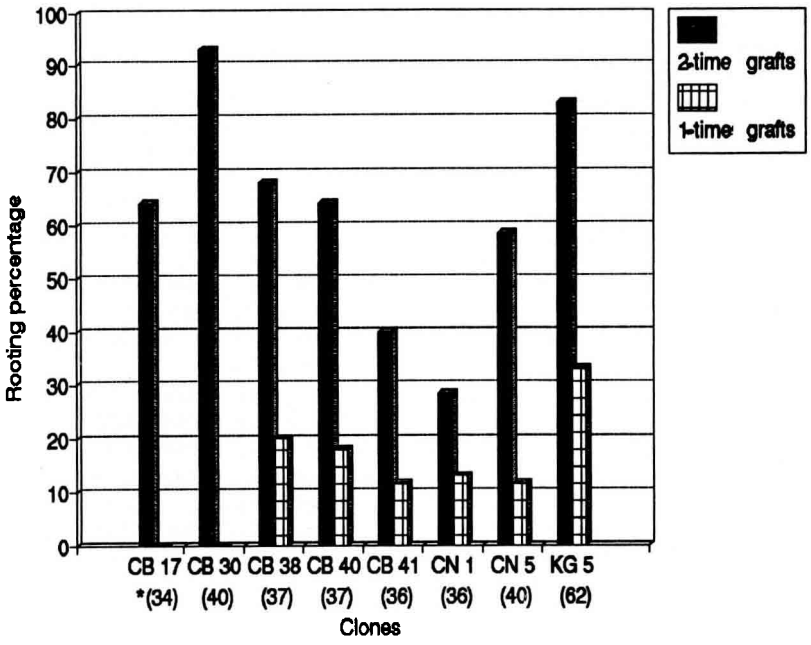

Fig 1. The effect of serial grafting on rooting of 8 clones of *Q acutissima*. * Represents the age of mother trees at the time of cutting propagation.

suggest that the rooting enhancement shown in figure 1 and table I resulted from rejuvenation by serial grafting onto juvenile understocks. The results obtained from the rooting frequencies of the plus tree clones, allowed the clones tested to be classified into 4 groups: 1) very easy to root (CB 5, CB 29 and KG 8); 2) easy to root (CB 9, CB 11, CB 18, CB23, CB26, CB36, CN 3 and KB 3); 3) difficult to root (CB 3, CB 20, CN 2, CN 9 and CN 11) and 4) very difficult to root (CB 2, CB 8, CN 14, CN 15 and KG 3). Siniscalco and Pavolettoni (1988) reported that rootability of eucalypt cuttings was significantly increased by repeated grafting on to juvenile rootstocks, more than 6 times, and also inferred that rejuvenation could be gradually improved. In this study, however, we did not graft more than twice because the rootability reached higher than 60%.

Rooted cuttings usually developed 1 or 2 primary roots. Relatively high number of ramets produced a callus or callus with roots at the basal end of the shoot. This type of plant eventually died after transplanting into the artificial soil mix. Normal rooted cuttings were kept in the greenhouse during the first winter. Most of the rooted cuttings did not sprout new buds during the current year; they usually produced slowly-growing buds and/or revealed plagiotropic growth at the following year.

Recent advances in *in vitro* culture systems provide another possible approach for rejuvenation of woody plants. Serial subculture onto the media containing cy-

tokinins revealed rejuvenation of the mature explants (Franclet, 1983; Hackett, 1985; Fouret *et al*, 1986; Pierik, 1990). We also observed that 60-year-old *Q acutissima* could be propagated effectively when the explants were cultured *in vitro* on a medium for multiple branching (data are not shown). In order to develop a reliable rejuvenation system and/or certify the status of rejuvenation, more extensive studies on morphological, physiological, biochemical and molecular biological aspects are being undertaken.

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