韓國林學會誌 86(3) : 391 - 398. 1997
Jour. Korean For. Soc. 86(3) : 391 - 398. 1997

Micropropagation of Juvenile and Mature Trees of Sawtooth Oak(*Quercus acutissima* C.)[1]

Heung Kyu Moon[2], Yang Youn[2] and Jae Seon Yi[3]

상수리나무 幼木과 成熟木의 器內繁殖[1]

文興奎[2] · 尹 陽[2] · 李在善[3]

ABSTRACT

Present study describes a method on the application of efficient tissue culture systems for the micropropagation of juvenile and mature sawtooth oak(*Quercus acutissima*). Nodal segments with axillary buds were used as initial explant sources. WPM(Woody Plant Medium) was the best in growth and proliferation of shoot among the media tested. Although the single effect of zeatin revealed on two dorminant shoot elongation with normal growth until the elevation of levels up to 3.0mg/l, BAP(N^6-benzyl amino purine) usually showed better response than zeatin on shoot multiplication and/ or elongation. In addition, the incorporation of BAP and zeatin onto the culture media represents more effectiveness in shoot proliferation and its growth. Optimum concentrations of BAP and zeatin were 0.5 and 0.05~1.0mg/l, respectively. Ninety percent of the proliferated shoots was rooted on half-strength GD (Gresshoff and Doy, 1972) medium containing 0.5mg/l IBA(indole butyric acid) in 4 weeks after culture. More than 70% of the rooted plantlets survived after 5 months of transplanting into artificial soil mix containing equal amount of peatmoss and perlite.

Among 27 plus tree clones which were grafted twice onto the juvenile rootstocks, only 4 clones revealed the possibility for shoot multiplication through tissue culture system. The capacity for the micropropagation using mature explant sources was highly depended on clonal differences compared with those of ortet age. More than 90% of rooting ratio was obtained from the best responding clone. Among the 7 rooting media tested, GD medium was the best for rooting. The most effective rooting was obtained on half-strength GD medium containing 0.2 to 2.0mg/l IBA. More than 60% of rooted plantlets survived after 5 months of transplanting into the artificial soil mix.

Key words : shoot multiplication and rooting, media and PGRs effect, juvenile and selected mature oak

요 약

참나무류의 효율적인 기내 번식법 개발을 위하여 상수리나무 유묘와 수형목을 재료로 액아를 배양하였다. 5가지 배지 가운데 WPM 배지에서 줄기 분화 및 생장이 가장 양호하였다. 싸이토키닌중 zeatin 3.0mg/L의 농도에서 줄기 분화 및 생장에 효과를 보였으나 대체로 BAP의 처리가 다경 줄기 분화 및 생장에 주효하였다. BAP와 zeatin의 공조처리는 다경줄기 유도 및 생장에 더욱 효과가 있었으며, 적정 농도는 BAP 0.5, zeatin은 0.05-1.0mg/L이었다. 기내발근은 1/2 GD 배지에 0.5mg/L IBA 처리로 90% 이상 발근되었고, 인공 배양토에서 70% 이상 활착되었다.

[1] 接受 1997年 7月 10日 Received on July 10, 1997.
[2] 임목육종연구소 Forest Genetics Research Institute, Forestry Administration, Suwon 441-350, Korea
[3] 강원대학교 산림과학대학 임학과 Department of Forestry, College of Forestry Sciences, Kangwon Nat'l Univ., Chooncheon 200-701, Korea

유령 대목에 2회 접목된 27개 수형목 클론을 재료로 시험한 결과는 4개 클론만이 기내 증식의 가능성을 보였다. 재유령화된 수형목의 기내 번식은 모수령의 영향보다는 클론간의 차이에 크게 좌우되었다. 기내 반응이 우수한 수형목 클론은 중식후 90% 이상 발근되었다. 발근배지는 염류를 반감시킨 GD배지에 IBA 0.2-2.0mg/L 처리시 주효하였다. 발근묘는 인공 배양토에 이식하여 5개월 후 60% 이상 활착되었다.

INTRODUCTION

Oaks are one of the most important broadleaved trees which occupied 27% of the total forest stocks in Korea. Among this genera, *Quercus acutissima* has been considered as more valuable species due to its straight shape, high timber quality as well as productivity for mushroom. However, natural or conventional reforestration of this species are still difficult for their low survival rate after transplanting, severe variation on acorn production, the problems related to acorn storage and insects. Moreover most of the selected plus trees for tree improvement programs appeared as low seed production and severe variation of acorn fecundity (Moon et al., 1989). Although vegetative propagation via *in vitro* or rooted cuttings has been suggested to overcome the obstacles, these kind of approaches are still considered as difficult partly due to the lack of extensive studies(Moon et al., 1987; 1988; 1989; 1991). The object of present study was to develop reliable micropropagation systems of *Quercus acutissima* from juvenile and mature tissue by modifying culture conditions using a broad spectrum dosage of plant growth regulators and media.

MATERIALS AND METHODS

1. Juvenile materials

Seedlings of *Q. acutissima* were obtained by germinating 8 acorns in wet artificial soil mix. They were placed in a growth chamber maintained 25±2℃ of temperature, 16 hr of photoperiod, and periodically watering conditions. Nodal segments with apical and axillary buds from 3-month-old seedlings were used as initial explants. To avoid exudation of water phenolic compounds or tannins, initially cultured juvenile explants were re-cultured onto same fresh media after 3 to 5 days of inoculation and for mature explants, tissues were manipulated onto the water. Shoots were proliferated continuously from the WPM supplemented with 0.5mg/l BAP and 0.01mg/l NAA(α-naphthalene acetic acid) at 3 to 4 weeks of interval for 18 months. To obtain a sufficient number of *in vitro* stock sources, each proliferated shoot was subcultured on WPM containing 1% activated charcoal for 4 weeks. To test the ability for shoot proliferation, *in vitro* grown stem node(ca. 1.5cm in height) containing 1 to 2 axillary buds without shoot apex were cultured on various media such as : SH(Schenk and Hildebrandt, 1972), GD(Gresshoff and Doy, 1972), MS(Murashige and Skoog, 1962), B_5(Gamborg et al., 1968) and WPM(Lloyd and McCown, 1980). Different levels of BAP, zeatin, and IBA were employed onto the media just mentioned above.

The pH of the media was adjusted to 5.5-5.7. The media were sterilized in an autoclave at 1.5 atmospheres(121℃) for 15min. The cultures were incubated at 25±2℃ under a 16 hr photoperiod with 3,000Lux. Each shoot was subcultured on half-strength GD medium containing 0.5mg/l IBA for rooting. Rooted plantlets were transferred into greenhouse conditions for acclimatization.

2. Mature materials

Among the plus trees of *Q. acutissima* which were selected at the Forest Genetics Research Institute in Korea, total 27 clones were used for this experiment. These clones had been established by grafting onto the 2-year-old seedlings twice. The current year shoots 15 to 30cm long were used as explants. For initial establishment of shoot culture, excised stem pieces(2cm in length with 1 or 2 axillary buds) were individually transferred to test tubes(2.4×15cm) containing 8ml of WPM with 0.5mg/l BA and 0.01mg/l NAA. Subcultures were followed by 2 or 3 weeks of interval. Data were collected after 5, 11, and 17

weeks from the final subculture. To obtain more reliable result, rooting experiment was carried out using Chonbuk(CB) 17 clone which represented high shoot proliferation rate as well as reproducibility. Media used for this experiment are as follow : GD, WPM, BTM(Chalupa, 1981), B_5, MS, LP(Quoirin et al., 1977), and SH. In all experiment for root induction, various levels of IBA were associated. Cultures were maintained under the same conditions as specified for juvenile materials culture.

RESULTS AND DISCUSSION

1. Juvenile materials

1) Shoot proliferation

Basal parts of explants usually began to swell or formed calli first, then bud burst and growth were started. Most of the explants cultured on cytokinin contained media showed bud burst within a week, whereas those on hormone free media failed to bud burst. All the multiflied shoots formed calli(0.3-1.0cm in diameter) at the basal part. The callus usually represent white or green color and the size of the callus increased along with the concentration of growth regulators. These kinds of calli have frequently been reported in oak tissue culture(Chalupa, 1981; Vieitez et al., 1989). Vieitez et al.(1989) suspected that the basal callus might act as a sink or a storage place for cytokinins or nutrients.

BAP gave better results in shoot proliferation than zeatin(Table 1 and 2). Effective concentration of BAP on WPM was 0.2 to 0.5mg/l. Among the media tested, WPM showed the best response in shoot elongation and shoot induction. Some explants growing on GD or MS developed normal shoots, but those shoots frequently showed retar-

Table 1. Effect of various media and BAP levels on shoot proliferation of microshoots derived from juvenile *Q. acutissima*.

BAP (mg/l)	Media[a]	No. of shoots cultured	No. of shoots induced (x±SD)[b]	Length of shoots (x±SD)[b] (cm)
0	1	28	0.5 ± 0.2	0.9 ± 0.5
	2	30	0.5 ± 0.4	0.9 ± 0.7
	3	30	1.0 ± 0.2	0.5 ± 0.4
	4	30	0.7 ± 0.5	0.8 ± 0.6
	5	29	1.1 ± 0.3	1.4 ± 0.9
0.2	1	29	1.5 ± 0.7	1.9 ± 1.4
	2	30	2.6 ± 1.7	1.6 ± 0.8
	3	30	2.5 ± 1.3	2.1 ± 1.5
	4	30	2.5 ± 1.3	2.1 ± 1.4
	5	29	4.8 ± 1.7	2.3 ± 1.5
0.5	1	30	1.6 ± 0.7	1.7 ± 1.2
	2	30	3.0 ± 1.6	1.5 ± 0.7
	3	30	2.3 ± 1.1	2.0 ± 1.2
	4	30	1.9 ± 1.2	1.7 ± 0.9
	5	30	4.5 ± 1.7	1.8 ± 1.2
1.0	1	28	1.3 ± 0.4	1.2 ± 1.1
	2	30	2.8 ± 1.2	1.7 ± 0.9
	3	30	2.4 ± 1.9	1.6 ± 1.1
	4	30	1.6 ± 0.7	1.6 ± 0.9
	5	.36	4.3 ± 1.7	2.1 ± 1.5
3.0	1	30	1.5 ± 0.8	0.9 ± 0.6
	2	30	2.9 ± 1.5	1.3 ± 1.0
	3	30	2.0 ± 1.3	1.5 ± 0.9
	4	30	1.3 ± 0.7	1.6 ± 0.9
	5	26	2.7 ± 1.2	1.6 ± 0.9

[a] Media 1 : SH, 2 : GD, 3 : MS, 4 : B_5, 5 : WPM

[b] Mean ± standard deviation

Table 2. Effect of various media and zeatin levels on shoot proliferation of microshoots derived from juvenile *Q. acutissima*.

Zeatin (mg/l)	Media[a]	No. of shoots cultured	No. of shoots induced (x±SD)[b]	Length of shoots (x±SD)[b] (cm)
0	1	28	0.5 ± 0.2	0.9 ± 0.5
	2	30	0.2 ± 0.4	0.9 ± 0.7
	3	30	0.5 ± 0.2	0.5 ± 0.4
	4	30	0.7 ± 0.5	0.8 ± 0.6
	5	29	0.7 ± 0.3	0.9 ± 0.7
0.2	1	30	0.7 ± 0.5	0.8 ± 0.7
	2	30	0.6 ± 0.5	0.4 ± 0.3
	3	30	0.3 ± 0.2	0.2 ± 0.1
	4	30	0.4 ± 0.3	1.2 ± 0.9
	5	30	0.9 ± 0.3	2.3 ± 1.5
0.5	1	30	0.8 ± 0.6	0.5 ± 0.4
	2	30	1.3 ± 0.6	0.9 ± 0.6
	3	30	0.5 ± 0.4	0.2 ± 0.1
	4	30	0.7 ± 0.5	1.0 ± 0.9
	5	30	1.0 ± 0.2	2.3 ± 1.5
1.0	1	30	0.8 ± 0.6	0.7 ± 0.6
	2	30	1.5 ± 0.5	0.6 ± 0.4
	3	30	0.4 ± 0.3	1.2 ± 0.5
	4	30	0.7 ± 0.5	1.4 ± 1.2
	5	30	1.0 ± 0.3	1.8 ± 1.2
3.0	1	30	0.8 ± 0.6	0.6 ± 0.5
	2	30	1.5 ± 0.7	0.6 ± 0.5
	3	30	1.2 ± 0.4	0.8 ± 0.7
	4	30	0.6 ± 0.5	1.4 ± 1.1
	5	30	1.1 ± 0.6	1.1 ± 0.8

[a] Media 1 : SH, 2 : GD, 3 : MS, 4 : B_5, 5 : WPM
[b] Mean ± standard deviation

dation or hyperhydricity. Generally, cultures maintained on the media containing BAP showed higher multiplication rate and rapid elongation than zeatin treated one(Table 2).

Table 3 summarizes the results of the combination effect of BAP and ZN on WPM. Although there were no significant differences among the treatments, great results were obtained from media containing 0.5mg/l BAP and 0.05 to 1.0 mg/l zeatin. From the results, we could suggest that the interaction of BAP and zeatin might be more effective than that of BAP alone. Lee et al.(1985) and Shoyama et al.(1992) reported similar results using BAP.

Even though a lot of reports had described clonal differences or position effect in oak tissue culture(Favre and Juncker, 1987; Moon et al., 1987; San-Jose et al., 1988; San-Jose et al., 1990), we did not observed those effects. These results seem partly due to the juvenility and uniformity of explants used. One of the problems in shoot cultures of this species was shoot-tip browning or necrosis. Shoot-tip necrosis occurred after 3 weeks in culture. Frequency of necrosis ranged from 5 to 20%, and necrotic state became intensive with the time lapse in cultures. When dominant shoot-tip was dead by the necrosis, subdominant shoot buds were developed to replace the dead one. It has been suggested that apical necrosis of *in vitro* cultures is attributed to calcium deficiency, lack of cytokinins, and the presense of auxin in the culture medium(Vieitez et al., 1989). In previous study, Moon et al.(1987) suggested that shoot-tip necrosis could possibly be prevented by shortening the subculture period.

2) Root induction

More than 90% of the explants were rooted on the half-strength GD medium containing 0.5mg/l

Table 3. Interaction effect of BAP and zeatin on shoot proliferation of microshoots derived from juvenile *Q. acutissima*.

Cytokinins (mg/l)	No. of shoots cultured [a]	No. of shoots induced (x±SD)[b]	Length of shoots (x±SD)[b](cm)
BAP 0.2 + ZN 0.05	30	3.5 ± 1.1	2.4 ± 1.7
ZN 0.1	30	4.7 ± 1.2	2.2 ± 1.5
ZN 0.5	30	3.6 ± 0.8	2.3 ± 1.3
ZN 1.0	30	4.4 ± 1.6	1.7 ± 1.3
BAP 0.5 + ZN 0.05	30	4.5 ± 1.6	2.2 ± 1.4
ZN 0.1	30	4.6 ± 1.6	1.9 ± 1.5
ZN 0.5	30	4.9 ± 1.2	1.8 ± 1.6
ZN 1.0	30	4.9 ± 1.9	1.8 ± 1.4

[a] Explants were cultured on WPM for 4 weeks.
[b] Mean ± standard deviation

IBA(data are not shown). Roots were initiated from swollen part. In many cases, roots with some callus were formed, but direct rooting without callusing was also occasionally observed.

The number of adventive primary roots were 2.5cm in average number. The number and/ or growth of roots induced from excised single shoot cultures were highly effected by the quality of shoots than culture media. When the rooted micropropagules were transplanted into the greenhouse conditions, mean survival rate of 70% was observed. After 5 months, the height of these shoots reached 10cm. We suspect that the low survival rate of plantlets may be due to the decay of callus formed at the basal part of the plantlets. Recently, Tomita and Kondo(1991) reported that the survival rate of plantlets was decisively depended on the callusing at the time of root induction. These results represent the strong relationships between direct rooting and their survival after transplantation. After 4 years, more than 90% plantlets were still surviving at field.

2. Mature materials

1) Shoot proliferation

Table 4 summarizes the results of 27 clones cultured on WPM containing 0.5mg/l BA and 0.01mg/l NAA for 17 weeks. Axillary bud burst and elongation varied with clones. General features observed from the stem node cultures were categorized as follows : 1) failure of the bud expanding, 2) callogenesis on the upper axillary zones and, 3) abnomal shoot growth. Although explants with apical buds showed rapid shoot elongation, these sources revealed press multiplication effect compared with those the subapical buds containing explants. Lignified explants at the time of primary culture reduced the percentage of bud break and their growth, while greenish explants showed good response. Favre and Juneker(1987) observed similiar results in *Q. robur*.

Clonal variation in shoot growth was apparent after 11 weeks in culture. After 4 weeks of primary culture, only single shoot was elongated, and the length varied with 1 to 6cm in all clones tested(Table 4). After 17 weeks in culture, some clones produced multiple shoots, whereas the others died gradually. *In vitro* response(bud burst or shoot growth) was not influenced by ortet age. This results suggested that the bud burst or shoot elongation at the initial culture seem to be more influenced by the developmental stage and physiological state of the explants. From the 4 clones of similiar ortet age, clone Chungnam 12 and Chonbuk 44 were dead but Chonbuk 17 and Chonbuk 1 showed shoot development after 17 weeks in culture.

Clonal variation in shoot cultures of oak trees has also been observed frequently in juvenile tissue culture(Moon et al., 1987; San-Jose et al., 1988, 1990; Shoyama et al., 1992). San-Jose et al.(1988) reported that *in vitro* response of five clones of *Q. robur* was influenced greatly by both clones and types of explant, and the interclonal differences in shoot productivity were unrelated to the juvenility of the clone. In our results, we

Table 4. Clonal reactivity for *in vitro* shoot proliferation of *Q. acutissima* plus tree clones rejuvenated after twice grafting onto the 2-year-old understock.

Clones[a]	Age[b]	No. of explants cultured[c]	No. of shoots obtained from each culture period[d]			Range of shoot length (cm)
			5 wks	11 wks	17 wks	
CB 1	35	16	16	19	26	1.0-6.0
3	37	24	24	20	20	1.0-3.0
5	38	4	4	5	7	1.5-3.0
8	36	9	8	3	2	1.5-2.5
14	37	6	6	3	3	1.0-2.0
15	38	15	13	16	26	1.0-2.0
17	36	15	15	30	70	1.0-3.5
20	38	9	9	7	5	1.0-3.0
23	37	9	9	6	4	1.0-3.5
32	39	20	18	10	6	1.0-2.5
33	38	1	1	0	0	1.0-1.5
36	37	13	7	1	1	1.0-1.5
40	39	12	12	3	1	1.0-1.5
41	38	10	10	10	17	1.5-3.0
42	36	10	10	5	3	1.0-2.0
44	36	5	5	2	0	1.0-2.0
CN 3	40	18	18	18	17	1.5-2.5
5	42	16	16	5	4	1.0-2.5
6	42	8	6	2	1	1.0-2.5
8	38	19	18	17	17	1.0-3.0
9	39	5	5	1	2	1.0-2.5
11	39	8	5	2	2	1.0-3.0
12	35	9	6	0	0	1.0-1.5
14	36	6	4	3	2	0.5-1.5
KG 3	69	16	16	14	7	1.0-2.5
5	54	30	30	30	98	1.0-5.0
PS 3	48	10	5	1	0	1.0-1.5

[a] CB : Chonbuk, CN : Chungnam, KG : Kyonggi, PS : Pusan
[b] Represent the actual year of mother trees grafted onto understock at the time of initial culture
[c] Explants were cultured on WPM containing 0.5mg/l BAP and 0.01mg/l NAA.
[d] Shoots size longer than 1.5cm were counted.

could classify the clone Chonbuk 1, Chonbuk 15, Chonbuk 17, and Kyonggi 5 as higher shoot forming clones.

Previous studies on *Eucalyptus*(Siniscalco and Pavolettonai, 1988) tissue culture has been reported that 6 times of successive grafting could improve the rootability. Douglas fir(Franclet, 1981) could also be rejuvenated after 5 times of successive grafting onto the juvenile rootstock. The above results suggested that the rejuvenation is attained progressively. Boulay(1979) dealt with adult *Sequoia sempervirens*. reported that juvenile characteristics and rootability could be attained by successive subculturing onto cytokinin media. On the other hand, our results revealed that shoot proliferation was more influenced by the clonal differences, and the successive subculture to fresh media could not overcome the obstacles. This observation indicates that attention should be paid to clonal selection to propagate from adult trees using tissue culture. Our results imply that mature trees of *Q. acutissima* can be micropropagated using two times successive graft, but further research is needed to rejuvenate the recalcitrant clones.

2) Root induction

Table 5 and 6 show the rooting of clone Chonbuk 17. Root induction was observed between 7 to 20 days from initial culture. After 4 weeks in culture, 93.1 % of the explants produced root on GD medium, whereas 30 % of the explants were rooted on SH medium(Table 5). Half-strength of

Table 5. Effect of several media on the rooting of mature clone Chonbuk 17 microshoots.*

Type of media	IBA free		IBA 0.5mg/l	
	% of rooting	No. of mean root	% of rooting	No. of mean root
GD	76.7	1.5	93.1	1.9
WPM	30.0	1.1	90.0	1.7
BTM	46.7	1.4	83.3	2.0
mB5	43.3	1.5	70.0	1.8
MS	36.7	1.4	46.7	2.0
LP	10.0	2.0	46.7	1.5
SH	3.3	1.0	30.0	1.4

* Data were taken from 35-120 microshoots per treatment.

Table 6. Effect of various IBA levels and GD media on the rooting of mature clone Chonbuk 17 microshoots.*

IBA (mg/l)	GD		1/2 GD	
	% of rooting	No. of mean root	% of rooting	No. of mean root
0.0	74.0	1.3	84.2	1.4
0.2	72.4	2.1	90.5	3.3
0.5	90.3	1.5	89.7	1.6
1.0	70.4	1.3	85.7	1.4
2.0	80.0	1.6	85.5	1.3

* Data were taken from 27-58 microshoots per treatment.

GD media showed a slightly better rootability than full-strength(Table 6), and best rooting ratio (90.5%) was obtained on the former media supplemented with 0.2mg/l IBA. The higher rootability implies that this clone may be rejuvenated by double grafting and successive *in vitro* subculture (Boulay, 1979; Franclet, 1981; Moon et al., 1988; Siniscalco and Pavolettonai, 1988). The rooted plantlets were eventually transferred to polyethylene pots containing a 1 : 1 mixture of peatmoss and perlite, and acclimatized in the greenhouse. Sixty percentage of the plantlets survived after 5 months, and showed normal growth.

CONCLUSION

Our results imply that mature trees of *Quercus acutissima*(plus tree clone) can be micropropagated after rejuvenation of explants via grafting, or by repeated subculturing onto BAP contained media.

In vitro proliferation from the explants derived from juvenile trees of this species could be successfully achieved on WPM supplemented with 0.5mg/l BAP and 0.05-1.0mg/l zeatin.

IBA showed effective rootability for both of juvenile and mature trees. Optimum concentrations for rooting were 0.5 or 0.2-2.0mg/l IBA in both origins, respectively.

Exudation of phenolic substances or tannins could be prevented by some technical skills, i. e., shortening the subculture interval to fresh media and manipulating tissues in water.

The big obstacle for the development of micropropagation systems from this species was the clonal differences on *in vitro* response. This problem also occurred on the tissue culture using juvenile materials.

Further studies on rejuvenation of source plants are still required to develop reliable masspropagation systems from hard-to-regeneration clones of this species.

LITERATURE CITED

1. Boulay, M. 1979. Multiplication et clonge rapidu du *Sequoia sempervirens* Parla culture *in vitro*. In : micropropagation d'arbres forestiers. Assoc Fote-Cellulose, domaine de l'Etancon, 77370 Nangis, France, pp 49-56.
2. Chalupa, V. 1981. Clonal propagation of broad-leaved forest trees. Commun. Inst. Forest. Cechosl. 12 : 255-271.
3. Favre, J.M. and B. Juncker. 1987. *In vitro* growth of buds taken from seedlings and adult plant material in *Quercus robur* L. Plant Cell, Tissue and Organ Culture 8 : 49-60.
4. Franclet, A. 1981. Rajeunissement et propagation vegtetative de lignieux. Annales AFOCEL 1980, pp 12-39
5. Gamborg, O.L., R.A. Miller, and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Res. 50 : 151-158.
6. Gresshoff, P.M. and C.H. Doy. 1972. Development and differentiation of haploid *Lycopersicon esculentum*(tomato). Planta 107

: 161-170.

7. Lee, B,C,. J.H. Kim and J.I. Park. 1985. Induction of plantlets by bud culture in *Quercus acutissima*. Res. Rep. Inst. For. Gen. Korea 21 : 104-108.
8. Lloyd, G. and B. McCown. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Proc. Intl. Plant. Soc. 30 : 421-427.
9. Moon, H.K., Y.H. Park, K.Y. Lee, and W.W. Kim. 1987. Rooted cuttings of *Quercus acutissima* by rooting substances and cutting media. Res. Rep. Inst. For. Gen. Korea 23 : 38-46.
10. Moon, H.K., J.H. Kim, and J.I. Park. 1987. Position effect of axillary buds on shoot multiplication and rooting in bud culture of *Quercus acutissima*. J. Korean For. Soc. 76 : 370-375.
11. Moon, H.K., M.H. Park, K.Y. Lee, and Y.H. Park. 1988. Rooted cuttings using juvenile semihardwood of some useful oaks and rooted cuttings of grafted seedlings of *Quercus acutissima* plus tree. Res. Rep. Inst. For Gen. Korea 24 : 42-46.
12. Moon, H.K., and M.H. Park. 1989. The induction of sprouts and the subsequent rooting of cuttings by BAP spray with the top-pruned *Quercus acutissima* seedlings. Res. Rep. Inst. For. Gen. Korea 25 : 188-192.
13. Moon, H.K., Y.H. Park, K.Y. Lee, and S.I. Hwang. 1989. Acorn variation of 27 *Quercus* spp. plus tree half-sib families. Res. Rep. Inst. For. Gen. Korea 25 : 193-197.
14. Moon, H.K., Y.H. Park, K.Y. Lee, and B.S. Lee. 1991. Effect of cutting length, nodal position, leaf trimming type, and blanching on rooting from cuttings of *Quercus acutissima* seedlings. Res. Rep. Inst. For. Gen. Korea 27 : 80-84.
15. Moon, H.K., Y. Youn., Y.I. Hyun, and S.K. Lee. 1991. *In vitro* shoot proliferation from seedlings of half-sib families of sawtooth oak(*Q. acutissima* C.). Res. Rep. Inst. For. Gen. Korea 27 : 75-79.
16. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15 : 473-479.
17. Quoirin, M., P. Lepoivre, and P. Boxus. 1977. Un premier bilan de 10 annees de recherches sur les cultures de meristemes et la multiplication "*in vitro*" de fruitiers ligeneux. C.R. Rech. 1976-1977. et Rapports de Synthese, Stat. Cult. Fruit. et Maraich. Gembloux, pp 93-117.
18. San-Jose, M.C., A. Ballester, and A.M. Vieitez. 1988. Factors affecting in vitro propagation of *Quercus robur* L. Tree Physiol. 4 : 281-290.
19. San-Jose, M.C., A.M. Vieitez. and A. Ballester. 1990. Clonal propagation of juvenile and adult trees of sessile oak by tissue culture techniques. Silvae Gen. 39 : 50-55.
20. Schenk, R.U. and A.C. Hildebrandt. 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can. J. Bot. 50 : 119-204.
21. Shoyama, Y., Y. Sasaki, I. Nishioka, and T. Suzaki. 1992. Clonal propagation of oak(*Quercus acutissima*). In : Biotechnology in Agriculture and Forestry. Vol 18. High-Tech and Micropropagation II(ed. by Y.P.S. Bajaj) Springer-Verlag Press. pp 179-192.
22. Siniscalco, C., and L. Pavolettonai. 1988. Rejuvenation of *Eucalyptus× Trabutii* by successive grafting. Acta Hort. 227 : 98-100.
23. Tomita, M. and T. Kondo. 1991. Factors affecting *in vitro* plantlet regeneration from axillary buds of *Q. acutissima* derived from stump sprouts. Res. Rep. For. Tree Breed. Inst. Jap., pp 33-41.
24. Vieitez, A.M., C. Sanchez., and C. San-Jose. 1989. Prevention of shoot-tip necrosis in shoot cultures of chestnut and oak. Sci. Hort. 41 : 151-159.