Korean J. Medicinal Crop Sci. 10(4) : 249-252(2002)

Micropropagation of *Kalopanax pictus*(Thunb.) Nakai by bud cultrue

Bong Kyu Kim*, Yong Sub Yi**† and Joong Hoon Ahn*

* *Department of Forest Resources, Konkuk University, Seoul, 143-701, Korea*
** *Biotechnology Division, Korea Forest Research Institute, Suwon 441-350, Korea*

ABSTRACT : **Several plant growth regulators were examined for on their effect on the *in vitro* propagation of *Kalopanax pictus*(Thunb.) Nakai on WPM medium. Among the cytokinins tested, BA at 13.32 μM appeared to be the most effective for multiple axillary shoot formation. Although the addition of 2.89 μM GA_3 promoted stem elongation, it produced morphologically abnormal leaves and stems. For rooting of the shoots, 4.9 μM IBA seemed to be more effective than 2.69 μM NAA. When the regenerated plants were transferred on artificial mixture containing vermiculite and peat-moss (1 : 1, v/v), 81% of them survived and grew normally.**

Key words : *Kalopanax pictus, in vitro*, micropropagation
Abbreviation : NAA-1-naphtalene acetic acid; GA_3-gibberellic acid; BA-6-benzyladenine ; IBA-indole-3-butyric acid

INTRODUCTION

Kalopanax pictus, belongs to the family *Araliaceae* and is distributed in China, East Siberia, Korea, and Japan (Gerd Krussmann, 1977). It is a deciduous tree or shrub reaching up to 30 m in height in its habitat, and has palmately lobed leaves. The color of the flowers is white in 20-30 cm long panicles composed of racemose branchlets. Fruits are globose and blue-black. Conventional propagation is by rooted cuttings and seeds. However, rooted cuttings die from damping-off diseases and seeds have been difficult to germinate due to dormancy and immaturity problems (Yeoung et al.,2001).

The trees have been used widely as a traditional medicine. It was reported that the trees had saponins, syringin and liriodendrin as lignans, coniferin, alkaloid, and anti-bacterial compounds (Prakash and Jeffrey, 1997; Park, 2001). Recently, fresh buds and leaves of the trees in spring have been used as edible vegetables due to their special flavor and nutrition for health. Therefore, the continuous collection from wild trees is becoming one of the reasons that the wild population is being destroyed and depleted.

The demand for vegetables cause intensive cultivation. Therefore, it is necessary to supply more propagules for cultivation. A method for large scale propagation of *Kalopanax pictus* through bud culture is described in this manuscript. Using this method, hundreds of rooted plants from a few buds were successfully acclimatized within 6 months.

MATERIALS AND METHODS

Preparation of explants and surface disinfection

Before the collection of explants from 7-10 years old *Kalopanax pictus*, it was transplanted to a greenhouse for 2 months. The explants were taken from the elongated stems and were first washed using two drops of tween 20. The buds were then sterilized with 2% (w/v) sodium hypochlorite for 10 min and then rinsed three times with sterile distilled water. After cutting into

† Corresponding author (Phone) : Yong Sub Yi, 031-290-1118, E-mail : yongyi67@yahoo.co.kr
Received 4 July, 2002 / Accepted 28 August, 2002

1.5 cm pieces, the explants were initiated on Woody Plant Medium (WPM) basal medium (Lloyd and McCown, 1981) for a week to screen contamination. The screened explants were subcultureed on the same medium for three weeks intervals.

Culture medium and culture condition

The basal medium used for culture was WPM containing 2% sucrose (Sigma, USA) and 0.25% phytagel (Sigma P-81) and autoclaved at 120℃ for 20 min. All media were adjusted to pH 5.6 with KOH or HCl prior to autoclaving. The cultures were incubated at 25±2℃ under a 16-h photoperiod of 60 $\mu E\ m^{-2}\ s^{-1}$ photosynthetic photon flux provided by Cool White fluorescent tubes. The basal medium was supplemented with various concentration of growth regulators, 2.22-22.20 μM benzyladenine purine(BA), 2.32-23.25 μM kinetin, and 2.28-22.80 μM zeatin for shoot induction. The developed multiple shoots were separated and transferred to subculture medium of the same composition for further multiplication. The shoots were subcultured at 4 weekly intervals.

Rooting of shoots and transfer of plantlets to potting mix

The shoots (23 cm long) were transferred to a rooting medium (WPM containing 2% sucrose and 0.7% phytagel) containing 1.07-16.11 μM naphthalene-acetic-acid (NAA), and 0.98-14.70 μM indole-3-butyric acid (IBA) for rooting. Shoots with well developed roots were transferred to pots after washing in distilled water to remove the phytagel. The pots contained peat moss and vermiculite (1 : 1, v/v). Plantlet acclimatization was achieved in a chamber with 70-80% relative humidity and a temperature of 28±2℃. After twelve weeks, acclimatized plants were transferred to pots containing garden soil and sand in equal proportions and kept in the shade for another 45 weeks prior to being planted out in the nursery.

RESULTS AND DISCUSSION

Newly induced shoots appeared after three weeks of initiation, and multiple shoots were arose from the base of the initially induced shoots (Figure 1). At the

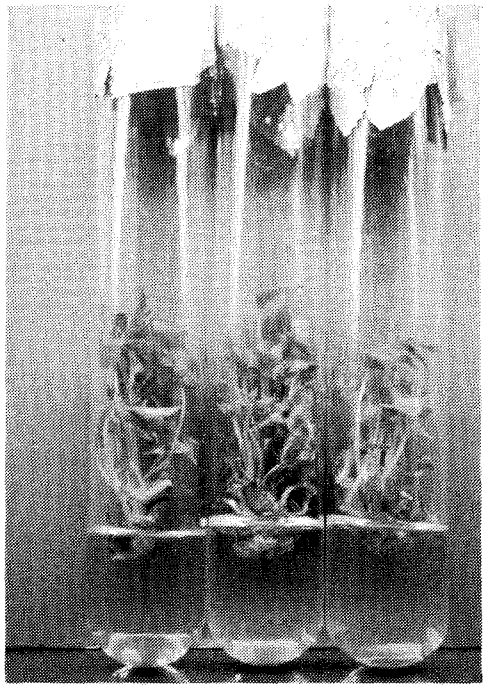

Fig. 1. Multiple shoot induction from explants at 4 weeks after culture on WPM basal medium supplemented with 13.32 μM BA.

Table 1. Effects of growth regulators on induction of multiple shoots from buds of *Kalopanax pictus*.

Growth regulator Concentration(μM)		Shoot length (㎝)[a]	Mean number of shoots induced per shoot
Control		1.46±0.39c[b]	1.6d[b]
BA	2.22	1.56±0.39c	1.9d
	4.44	1.83±0.45bc	2.8bc
	13.32	2.32±0.38a	4.2a
	22.2	2.01±0.54ab	2.9bc
Kinetin	2.32	1.77±0.53c	2.3c
	4.65	1.70±0.61bc	2.6bc
	13.95	1.47±0.37c	2.0cd
	23.25	1.46±0.43c	1.9cd
Zeatin	2.28	1.41±0.59c	1.4d
	4.56	1.93±0.38c	1.7bc
	13.68	2.28±0.47ab	3.7ab
	22.80	1.95±0.40c	3.0bc

a : Mean ±standard deviation.
b : Means followed by the same letter were not significantly different as indicated by Duncan's multiple range test at the 0.05 % or less probability.

initiation of shoot induction, decontamination step was important step as most explants, which were contaminated, browned and finally were dead. The longest average shoot length and mean number of shoots per explant was with 13.32 μM BA, although kinetin and zeatin were found to induce multiple shoots (Table 1). The treatment of kinetin and zeatin produced leaves that appeared not fully elongated. Also, kinetin induced necrosis at the base of the shoots which then advanced to the top of shoots. The results indicated that a kind of kinetins was more important than concentration.

When BA and GA_3 were used in combination, the number of induced shoots appeared higher in 1.45 μM GA_3, but the shoot length was higher in 2.89 μM GA_3 (Table 2). The shoots induced by treatment of GA_3 were abnormal elongated (Figure 2). Especially, after four weeks of inoculation, callus at the base of the shoots became brown (data not shown). The results indicated that GA_3 induced the elongation of cell and tissue (Daykin et al., 1997).

For rooting of shoots, IBA and NAA were treated (Table 3). After a small callus was formed at the base of the shoot, four through two roots of 1 to 2 cm in length grew (Figure 3). Root length was high at 4.9 μM IBA and the number of roots induction was high at 0.98 μM IBA. High concentration of auxins did not lead good results. The plantlets were transferred to pots containing peat moss and vermiculite (1 : 1, v/v). For hardening, the pots were covered by vinyl film under culture condition for a month. After a hardening period, the potted regenerants rooted, grew in height, and developed new leaves (Figure 4).

Table 2. Effects of GA_3 on the shoot elongation of *Kalopanax pictus*.

Growth regulators(μM)		Shoot length (㎝)[a]	Mean nunber of shoot induced per shoot
BA	GA		
Control (PGR's free)		1.46±0.39c[b]	1.6d[b]
2.22	1.45	1.95±0.82b	1.9a
	2.89	3.68±0.46a	1.6a
	8.67	3.37±0.82a	1.1a

a : Mean ±standard deviation.

b : Means followed by the same letter were not significantly different as indicated by Duncan's multiple range test at the 0.05 % or less probability.

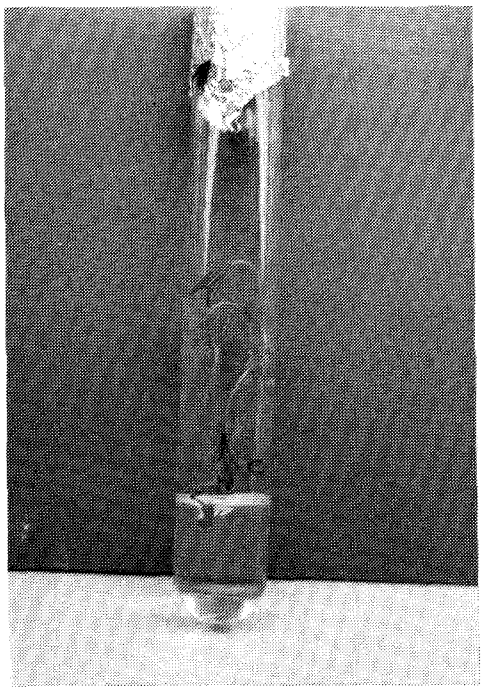

Fig. 2. Shoot elongation at 4 weeks after culture on WPM basal medium supplemented with 2.89 μM GA_3.

Table 3. Effects of auxins on rooting of shoots of *Kalopanax pictus.*

Growth regulators concentration(μM)		% of root formation	Root length (cm)[a]	No. of roots induced per shoot[a]
Control		10	0.35±0.21	0.75±0.21
IBA	0.98	40	0.64±0.23	4.25±1.75
	2.45	60	1.50±0.62	3.58±1.73
	4.90	60	1.98±0.77	3.25±1.86
	14.70	20	0.33±0.13	1.50±0.58
NAA	1.07	25	0.56±0.25	1.40±0.55
	2.69	55	1.71±0.60	1.90±0.83
	5.37	40	1.30±0.34	1.50±0.53
	16.11	30	1.17±0.45	1.50±0.55

a : Mean ±standard deviation.

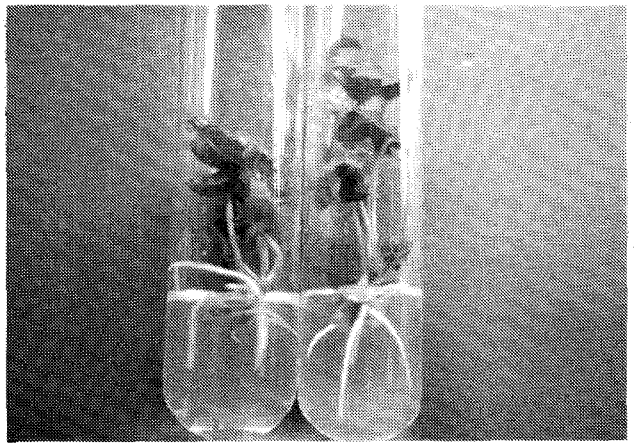

Fig. 3. In vitro rooting from shoots at 4 weeks after culture on WPM basal medium supplemented with 4.9 μM IBA.

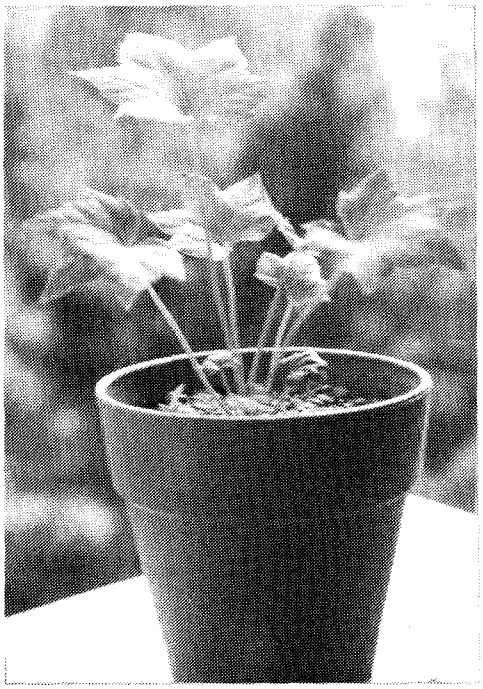

Fig. 4. Plant growing for transfer to the field.

LITERATURE CITED

Gerd K (1977) Manual of cultivated broad-leaved tree & shrubs. Timber press pp 198-199.

Kang HS, Lee DK (1998) Site and growth characteristics of *Kalopanax septemlobus* growing at Mt. joongwang in Pyungchang-gun, Kangwon-do. Journal of Korean Forestry Society. 87 : 483-492.

Daykin A, Scott IM, Francis D, Causton DR (1997) Effect of gibberellin on the cellular dynamics of dwarf pea internode development. Planta 203 : 526-535.

Kaur K (1998) Plants obtained from Acacia catechu WILLD using mature nodal segments. Plant Cell Reports 17 : 427-429.

Laloue M, Pethe C (1982) Dynamics of cytokinin metabolism in tobacco cells. In : Plant growth substances 1982. Proc. 11th Int. Plant Growth Sunst. (ed. PF Wareing pp185-195). New York, Academic Press p 683.

Lloyd G, McCown B (1980) Commercially feasible micropropagation mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Proc. Int. Plant Prop Soc 30 : 421-427.

Park, HJ (2001) *Kalopanax saponin* A is a basic saponin structure for the anti-tumor activity of hederagenin monodesmosides. Planta medica. 67 : 118-121.

Prakash MD, Jeffrey BH (1997) Plant Biochemistry. Academin Press Inc., California. P 554.

Shim KK, Ha YM, Lee SJ, Shim KB (1997) Mass propagation of Betula pendula 'Purpurea' through axillary bud culture. J. Kor. Soc. Hort. Sci. 38 : 776-782.

Sun SH, Hall RB (1995) Multiple shoot induction from ex vitro and in vitro derived stem node culture of Populus alba L. × P. grandidentata michx. Korean J. Plant Tissue Culture 22 : 131-135.

Tomita M, Kondo K (1991) Factors affecting in vitro plantlet regeneration from axillary buds of Quercus acutissima derived from stump sprouts. Res. Rep. For. Tree Breed. Inst. Japan, pp. 33-41.

Yeoung YR, Lee MH, Kim BS, Kim HK, Kim JH (2001) Seed germination and softwood cutting technique of *Kalopanax pictus* Nakai. Korean Journal of Plant Resources 14 : 53-59.