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Study on the Various Conditions of *In Vitro* Culture for Mass-propagation of *Prunus yedoensis* Matsumura[1]

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濟州 自生 왕벚나무(*Prunus yedoensis* Matsumura)의 器內 줄기 增殖을 위한 培養條件 究明[1]

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ABSTRACT

Multiple shoots were induced from *in vitro* shoot originated from winter bud of *P. yedoensis* from Jeju. Most explants grow in similar type among the five different media but affected by supplement of sucrose regardless of media. For mass-propagation various concentrations of BAP or GA_3 were treated in the medium respectively. BAP was very effective to produce multiple shoots and 3.5~9.5 shoots were formed on the explant. The shoots induced on the high levels of BAP have short internodes. No shoots were induced on the treatment of GA_3 but roots were induced on it. When GA_3 was supplemented with the medium containing BAP, multiple shoots were produced from the explants. The medium(WPM) containing with 0.5 ㎎/ℓ BAP and 4.0 ㎎/ℓ GA_3 was most effective to produce multiple shoots. When the explants were cultured for 8 weeks, 39.5 shoots were developed in average.

Key words : *BAP*, *GA_3*, *mass-propagation*, P. yedoensis

要 約

제주 자생 왕벚나무 동아에서 분화된 줄기를 이용하여 대량증식 하고자 기내배양조건을 구명하였다. 다섯 종류의 배지에서 모두 양호한 생장을 하여 차이가 나타나지 않았으나 sucrose가 첨가되지 않은 배지에서는 생장이 매우 저조하였다. BAP와 GA_3를 여러 농도로 처리한 결과 BAP 처리구에서 평균 3.5~9.5개의 줄기가 유도되었다. 그러나 BAP의 농도가 높아질수록 줄기의 길이생장은 저조하였다. GA_3 단독 처리구에서는 줄기가 발생하지 않았으나 BAP와 혼용처리 하였을 때 BAP 단독 처리구보다 더 많은 줄기가 발생하였으며, 길이 생장도 양호하였다. 그 중 BAP 0.5 ㎎/ℓ와 GA_3 4.0 ㎎/ℓ 조합에서 가장 많은 줄기가 발생하였다. 배양기간이 경과할수록 줄기형성은 많아졌으나 12주간 배양된 줄기는 정단부가 고사하였다. 배양 8주째 양호한 줄기를 다량으로 얻을 수 있었다.

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INTRODUCTION

Prunus species are distributed in the temperate or subtropical area in northern hemisphere. This is valuable genetic resource which provides cherries, timber and so on. *P. yedoensis*, which is indigenous to the Jeju(Mt. Halla) region, has very unique and various characters in many ways, and is widely planted in parks and roadsides for its beautiful flowers in spring in Korea as well as Japan. For their ornamental characteristics, the demands for this species has increased in recent years. However, most trees growing in Mt. Halla are too old to propagate by traditional clonal methods using cuttings. The seed germination rate is also very low. *Prunus* species has been cultured using embryos(Hurby, 1962), meristem tip(Boxus, 1971; Boxus and Quoirin, 1974, 1977) to produce virus-free plants. Recently, researchers transferred the genes resistant to poty pot virus mediated by *Agrobacterium* (Machado, 1992; James *et al.*, 1993; Tiziana *et al.*, 1995). As *P. yedoensis* is not a fruit baring plant, there were few studies(Kim *et al.*, 1993; Koh *et al.*, 1997; 1998).

To find an efficient method to propagate this species, we investigated mass propagation using *in vitro* culture techniques.

MATERIALS AND METHODS

1. Plant materials

Culture were established using winter buds from wild *P. yedoensis*(ca 40 years old) growing in Mt. Halla. They were taken from annual branches of the crown. Flower buds were removed from the scions. Apical and lateral buds were used for culture. Small scions were cut into two centimeters segments. These pieces were surface-disinfected in 70% ethanol for one minute and then in a 2% sodium hypochlorite solution for 20 minutes followed by three rinses with sterilized distilled water. Before being placed on media, scions were flamed very briefly. After inducing the bud flush *in vitro*, small shoots were used for mass-propagation experiment.

2. Media and Plant growth regulators

Five different growth media and two different concentrations of sucrose were tested; B_5 (Gamborg *et al.*, 1968), GD (Greshoff and Doy, 1972), MS (Murashige and Skoog, 1962), 1/2MS (half strength MS salts and full vitamins), and Woody Plant Medium (WPM, Lloyd and McCown, 1981). Media contained either 2%(w/v) of sucrose or no sucrose and solidified with 0.7%(w/v) agar, pH 5.7. Initial fresh weight measurements were made when explants had two apical leaves. Fresh weight of all explants were measured again 4 weeks later. Ten explants were cultured in each treatment and all treatments were replicated three times.

The effects of varying levels(0.5, 1.0, 3.0 and 5.0 mg/ℓ) of 6-benzylamino purine(BAP) and gibbellic acid (GA_3) on shoot multiplication were also tested. Plnats were grown in culture tubes with 10mℓ of media containing WPM salts and vitamins with 2% sucrose, pH 5.7, and solidified 0.7% agar. Various concentrations of GA_3(1.0, 2.0 and 4.0mg/ℓ) were supplemented with BAP(0.5, 1.0, and 3.0mg/ℓ). After 4 weeks, number and length of shoots induced from explants were measured. Five explants were cultured in each treatment and replicated three times. The measurements were made every 4 weeks(4, 8 and 12 weeks)

RESULTS AND DISCUSSION

1. Effects of culture media on shoot growth

We investigated the shoot growth in five different media. In plant tissue culture, 20~3 g/ℓ sucrose is usually supplemented in media(Pierik, 1987). We measured explants' fresh weight. Shoot growth is indicated by the increase in fresh weight during the culture period(Table 1).

The fresh weights of explants grown in media without sucrose significantly differ from those in sucrose media. Among the five media, MS medium was most effective in promoting shoot growth, both

with or without sucrose. Even though the control treatment cantaind no nutrients, shoots could survive and fresh weight was slightly increased. Explants on MS formed callus at the base of stem. Non significant statistical differences were observed among the media, the leaf characteristics such as color, size and shape were similar in most media. But all shoots seemed to need sucrose. It is well known sucrose affects the plant's growth in tissue culture. Tricoli *et al.*(1985) also reported that *Prunus serotina* need sucrose in *in vitro* to produce multiple shoots and the lower sucrose concentrations the less shoots formed.

Table 1. Shoot growth on the five media supplemented with or without sucrose[1].

Media	Sucrose	
	Free	2%
Control[2]	2.9±1.1 c[3]	8.2±8.5 c
B_5	7.5±3.2 ab	44.8±34.6 b
GD	6.9±3.3 ab	48.4±30.4 b
1/2MS	7.1±5.0 ab	51.3±30.0 b
MS	9.0±4.8 a	65.5±30.2 a
WPM	5.2±2.6 ab	42.8±25.8 b

[1] Shoot growth was indicated by measuring the fresh weight during the culture(FW after 4 weeks - initial FW, unit : mg)

[2] Control medium consisted of distilled water with 0.7 % agar.

[3] Means with the same letter are not significantly different at the $\alpha = 0.05$ according to Duncan's multiple range test.

2. Effect of BAP and GA_3 on shoot multiplication

First we investigated the effects of BAP and GA_3 independently. While BA was very effective in stimulating explants to form shoots in all concentrations, no shoots were formed in media with GA only(Table 2). In tissue culture of *Prunus* species, high level of BAP with low level of auxins such as IBA was usually used for shoot multiplication (Reeves *et al.*, 1983; Jona and Vigliocco, 1985; Almehde and Parfitt, 1986; Hammerschlag and Scorza, 1987).

The higher the BA concentration, the higher the number of shoots; however, shoots that developed in high BA levels were shorter than those on lower levels. Futhermore, some shoot tips died on high BA levels(Table 3). Reeves *et al.*(1985) also observed that high level of BAP inhibit shoot elongation. It is need to diminish BA concentration and contain GA_3.

This result is similar to reports of Tricoli (1982) and Tricoli *et al.*(1985). When he induced multiple shoots from winter buds of *Prunus serotina*, 1.0 ㎎/ℓ BAP was effective to produce many shoots but shoot elongation was inhibited. Druart(1988) reported that shoots could be vitrificated by high concentration of BAP. There was a result which apical part had became necrosis in high level of BAP(Almehdi and Parfitt, 1985). Considering the results, 0.5 ㎎/ℓ BAP is most adequate to apply to induce shoots. Morzadec(1997) reported that GA_3 affected rooting on *Cynara scdymus* shoot *in vitro* rather than NAA. Although the rooting effect of GA_3 in this species was not strong like in *Cynara scdymus*, there was an effect on root formation than shoot formation.

Table 2. Differentiation from the explants derived from winter bud of *P. yedoensis* on medium supplemented with various concentrations of BAP or GA_3. Culture period was 4 weeks.

PGRs[2](㎎/ℓ)	Shoot induction rate (%)	Root induction rate (%)
BAP 0.5	100	0
BAP 1.0	100	0
BAP 2.0	95	0
BAP 3.0	100	0
BAP 5.0	95	0
GA_3 0.5	0	25
GA_3 1.0	0	30
GA_3 2.0	0	25
GA_3 3.0	0	30
GA_3 5.0	0	30

[2] PGRs : Plant Growth Regulators

Table 3. Number and growth of shoots regenerated on the WPM medium supplemented with BAP for 4 weeks.

Concentration (㎎/ℓ)	No. of shoots induced	Length of induced shoots (㎜)	Fresh weight of explants (㎎)[1]
0.5	3.5±2.4 a[2]	12.0±6.0 a[2]	414.3±201.6 ab[2]
1.0	6.3±3.3 ab	7.2±6.1 b	640.0±294.4 a
2.0	7.3±1.9 ab	6.7±0.5 b	586.0±347.2 ab
3.0	9.0±0.8 b	< 5	325.0± 76.0 ab
5.0	9.5±3.3 b	< 5	223.3± 40.4 b

[1] Fresh weight(㎎) = FW after 4 weeks - Initial FW
[2] Mean±SD : Means with same letter are not significantly different at α=0.05.

3. Effect of the combination of BAP and GA_3 on shoot multiplication

In the first experiment, multiple shoots were induced in BAP. As Reeves *et al.*(1985) suggested that GA_3 was needed for elongation of rosettes shoots, various concentrations of GA_3 were supplemented with BAP(0.5, 1.0 and 3.0 ㎎/ℓ). The GA_3 concentrations were 1.0, 2.0 and 4.0 ㎎/ℓ. Compared to the treatment of BAP only, more shoots were formed in the combination of two plant growth regulators. Shoots were longer in the media with 4.0 ㎎/ℓ GA_3 than in the others(Table 4).

Table 4. The number and length of shoots induced on the media(WPM) supplemented with various concentrations of GA_3 and BAP after 4 weeks.

BAP (㎎/ℓ)	GA_3 (㎎/ℓ)	No. of shoots induced	Length of induced shoots (㎜)
0.5	1.0	8.8±0.9 bc[1]	13.7±2.2 b[1]
	2.0	8.2±1.7 c	15.2±3.6 ab
	4.0	11.7±2.1 a	17.1±1.9 a
1.0	1.0	8.0±2.0 c	13.6±2.5 b
	2.0	7.8±1.3 c	13.6±2.1 b
	4.0	12.4±3.7 a	15.1±2.5 ab
3.0	1.0	7.9±1.7 c	8.9±0.9 c
	2.0	9.9±3.3 ab	8.7±0.9 c
	4.0	11.6±3.6 a	9.2±1.7 c

[1] Mean±SD, Means with same letter are not significantly different at α=0.05.

There were two types in shoot formation; formed at basal callus and developed at every axillary buds. The apical parts of the most explants which produced many shoots had turned into brown and died. GA_3 may affected shoot elongation as well as shoot formation when it contained with BAP in this species. The role of GA_3 in shoots were very different from when it treated in alone. The number and length of produced shoots were different depending on the culture period(Table 5).

Table 5. The number and the length of shoots induced from the shoots depending on the culture periods[1].

Culture Period (weeks)	Number of shoots induced	Shoot length (㎜)
4	15.4±8.4	12.7±5.5
8	39.5±22.5	18.3±8.9
12	23.8±9.0[2]	16.9±9.1

[1] Culture medium is WPM supplemented with 0.5 ㎎/ℓ BAP and 4.0 ㎎/ℓ GA_3 in the culture flask.
[2] Dead shoots were excluded from the counting.

As culture period past, more shoots were developed. In 12 weeks culture, however, shoots that developed in early had become necrosis. Considering this, the lifetime of *in vitro* shoot of *P. yedoensis* seemed about less than 12 weeks. It is longer than *Prunus persica*. According to Reeves (1983), dead rate of *P. persica in vitro* was 8.1% within 4 weeks and 62.5% in 8 weeks. It appeared that 4~8 week culture was suitable to get healthy shoots. Shoot formation continue till 12 weeks, however, apical meristem of shoots were died and very thin because of high density of shoots in the culture vessel.

P. yedoensis from Mt. Halla was mass-propagated using tissue culture. Shoot growth *in vitro* was affected by sucrose rather than nutrients of media. BAP was effective on shoot multiplication. Even though GA_3 did not act to produce shoots like BAP in alone, it showed synergic effect to produce multiple shoots when it supplemented with BAP. It seemed that 4~8 weeks was suitable for culture periods.

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