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# Differentiation of Winter Buds of *Prunus yedoensis* Matsumura from Jeju Island Depending on the Collection Time and Media Conditions[1]

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# 濟州 自生 왕벚나무 (*Prunus yedoensis* Matsumura) 冬芽의 採取時期와 培地의 條件에 따른 器官誘導[1]

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## ABSTRACT

We tried to mass-propagate *Prunus yedoensis* from Jeju through tissue culture. We investigated the effect of bud collection time, the concentration of $NH_4NO_3$ in media and plant growth regulators(BAP, $GA_3$ and IBA) on the differentiation of winter buds. Buds, taken in February, flushed well regardless of various *in vitro* conditions. Bud flushing rate was significantly different depending on the collection time. BAP appeared to be effective on bud flushing. Sixty percent of buds taken in October flushed on the media containing 3.0 ㎎/ℓ BAP. No buds flushed on the medium supplemented with IBA. Buds, after flushing in BAP media, grew as foliated shoots and showed a rosette of leaves. When $GA_3$ supplemented to the BAP-containing media as a higher concentration than that of BAP, shoots elongated and developed into normal shoots. The combination of BAP 1.0 ㎎/ℓ and $GA_3$ 2.0 ㎎/ℓ is most recommendable for shoot elongation.

*Key words* : *BAP, flushing rate, $GA_3$, IBA,* Prunus yedoensis, *winter bud*

## 要 約

경제적으로 중요한 가치가 있는 제주 자생 왕벚나무를 조직배양을 통하여 대량 증식하고자 동아를 이용할 때 분화 및 생장에 미치는 기내 조건을 구명하고자 하였다. 동아의 채취시기, 배지의 $NH_4NO_3$의 농도, 생장조절물질의 종류 (BAP, $GA_3$, IBA) 및 농도에 따른 영향을 조사하였다. 동아의 채취시기는 2월이 적합하였으며 그 중 1,200 ㎎/ℓ $NH_4NO_3$의 농도에서 잎 전개율이 70%로 가장 높았다. 생장조절물질 처리구 중 BAP 3.0 ㎎/ℓ첨가 배지에서 채취시기에 관계없이 잎의 전개율이 높았다. 반면 IBA 처리구에서는 전혀 잎이 전개되지 않아 동아의 전개를 저해하는 것으로 나타났다. BAP 첨가 배지에서 전개된 동아는 줄기로 자라지 못하고 다발 모양을 이루었다. BAP와 함께 $GA_3$를 처리한 결과 $GA_3$의 농도가 BAP보다 높을 때 줄기 신장에 효과가 있는 것으로 나타났으며, BAP 1.0 ㎎/ℓ와 $GA_3$ 2.0 ㎎/ℓ 혼합 처리구에서 줄기의 생장이 가장 양호하였다.

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## INTRODUCTION

Nineteen species of *Prunus* grow in Korea and most of them have economic value in addition to ornamental importance for their beautiful flowers. *Prunus yedoensis* grow at the altitude of 450m to 850m in Mt. Halla. This species exhibits high variability in flower and tree form. The timber was valuable for furniture and ship in the past. *Prunus yedoensis* trees in Mt. Halla are distinguished genetically from those trees planted along the road in Korea and other countries (Kim, 1997) even though they own the same scientific name. In these days the demand for *P. yedoensis* is rapidly increasing due to beautiful spring flowers. Although *P. yedoensis* in Jeju is considered to be much valuable in many aspects, it is very difficult to propagate asexually such as cutting and grafting, possibly because of aging. In addition, seed germination rate is very low. Tissue culture technique can be applied to this species (Bonga, 1980; Ahuja and Muhs, 1985).

In Europe most *Prunus* species have been cultivated to produce cherries. Many researchers tried to produce virus-free plants through apical meristem culture of cultivars (Boxus and Quoirin, 1974, 1977) or transferred virus-resistant gene into *Prunus* species (Machado *et al.*, 1992; Tiziana *et al.*, 1995). However, there are few studies on *P. yedoensis* (Kim *et al.*, 1993; Koh *et al.*, 1997, 1998).

This study was performed to investigate the proper conditions to *in vitro* mass-propagate *P. yedoensis* using winter buds.

## MATERIALS AND METHODS

One tree (ca. 40 years old) was chosen at the altitude of 600m in Mt. Halla. The winter buds were taken from current-year branches of the crown every month from October in 1997 to February in 1998. Apical and/or lateral buds were used for culture except flower buds. Small scions were prepared as long as two centimeters and surface-sterilized in 70% ethanol for a minute and then in a 2% sodium hypochlorite solution for 20 minutes followed by three rinses with sterilized, distilled water. Before plated on media, scions were in flame in a short time.

In the first experiment, $NH_4NO_3$ concentrations of macro- and micro-nutrients were increased twice (800 ㎎/ℓ) and three times(1,200 ㎎/ℓ) as high as that of the basal one (400 ㎎/ℓ) based on Woody Plant Medium(WPM, Lloyd and McCown, 1984). Sucrose(30 g/ℓ) and agar(7 g/ℓ) were supplemented in all media. pH was adjusted to 5.7 before autoclaving in 121℃ at 1.5 ㎏/㎠ for 15 minutes. Ten milliliters medium was dispensed in a test-tube and capped with aluminium foil. Bud flushing rate only was investigated.

In the second experiment, buds collected in February were used. 1.0, 2.0 and 3.0 ㎎/ℓ 6-benzylamino purine (BAP), indole-3-butyric acid (IBA) and gibbellic acid ($GA_3$) were added to the basal WPM media, respectively. In the other experiment 1.0 ㎎/ℓ BAP was added in combination with 0.5, 1.0 or 2.0 ㎎/ℓ $GA_3$. Bud flushing rate, shoot formation rate, callus formation rate, shoot length and fresh weight were investigated. Each treatment consisted of two replications with 10 explants.

## RESULTS AND DISCUSSION

### 1. Flushing rate of buds depending on collection time and media containing various concentrations of $NH_4NO_3$

Within 5 days after plating, explants became brown in test-tubes and scales were separated from buds. Small green leaves appeared after a week. In the first culture stage, most buds were contaminated with fungi and had to be transferred to fresh media.

Bud flushing rate was various depending on the collection time. February buds, followed by January buds, showed the highest rate regardless of $NH_4NO_3$ concentration. However, there were no differences

among the others, i. e., October, November and December buds. Treatment of 1,200 ㎎/ℓ $NH_4NO_3$ seemed to be effective on flushing. Kim *et al.* (1993) reported that winter buds of *P. yedoensis* taken from Jeju in February flushed well in three different kinds of media and showed the highest flushing percentage in WPM. For successful flushing of buds, collection time appeared to be more important than the concentration of $NH_4NO_3$(Table 1).

**Table 1.** Differences of bud flushing rate of *P. yedoensis* depending on the concentrations of $NH_4NO_3$ and collection time[1].

| Concentration of $NH_4NO_3$[2] (㎎/ℓ) | Collection Time | | | | |
|---|---|---|---|---|---|
| | Oct. | Nov. | Dec. | Jan. | Feb. |
| 400 | 30 | 30 | 30 | 40 | 60 |
| 800 | 30 | 30 | 40 | 40 | 50 |
| 1,200 | 40 | 40 | 40 | 50 | 70 |

[1] Culture period was 3 weeks under 16-hour photoperiod.
[2] Other nutrients and vitamins were based on WPM medium.

All buds flushed grew into foliated shoots but leaves formed a rosette form. The leaves turned out to be yellow and fell off within a few days. It is supposed that other nutrients and/or growth regulators may be required. In the case of white birch, buds were dormant during the winter from October to February and were activated in March or April after winter(Rinne *et al.*, 1994). Hammerschlag (1982) suggested that a certain amount of chilling is needed to get the *in vitro* shoots from buds. *P. yedoensis* seemed to have the same habit on bud dormancy as other species.

### 2. BAP, $GA_3$ and IBA treatment in culture media

Bud flushing rate on the different culture media appeared to be similar among the growth regulators except IBA. No buds flushed in the media containing IBA(Fig. 1). In most treatments one bud established two normally-shaped leaves, no matter how many small leaves were formed on the medium containing BAP(Fig. 2).

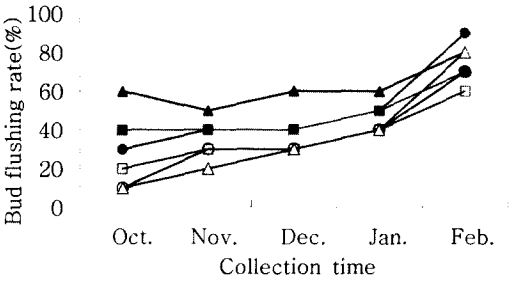


**Fig. 1.** Differences of bud flushing rate of *P. yedoensis* depending on the concentrations of BAP and $GA_3$, and collection time. Culture period was 2 weeks under 16 hours/day photoperiod.
● : BAP 1.0, ■ : BAP 2.0, ▲ : BAP 3.0,
○ : $GA_3$ 1.0, □ : $GA_3$ 2.0, △ : $GA_3$ 3.0

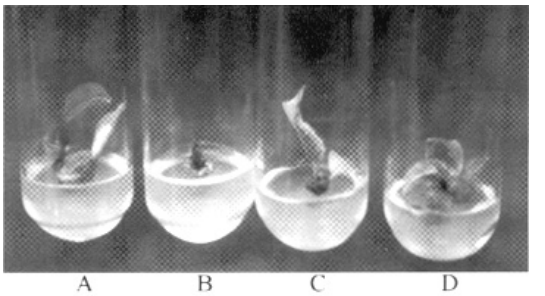


**Fig. 2.** The response of winter buds on the different plant growth regulators.
A : Control, B : IBA, C : $GA_3$, D : BAP

Even 60% of October buds flushed on the media containing 3.0 ㎎/ℓ BAP. It is suggested that BAP may break the dormancy of winter buds in this species. There are some reports that BAP stimulates bud flushing in *Prunus* spp(Snir, 1982; Sakatani, 1989; Kim *et al.*, 1993). Bud flushing rate was very similar between hormone-free media and $GA_3$-containing ones. However, buds in $GA_3$ media flushed and developed into shoots when they were collected in February when buds dormancy break in spring. Considering our results, February buds may have more cytokinins than others so that flushed well in all media and could developed shoots when the $GA_3$ was added exogenously. Bonga(1997) observed that chilling treated buds of *Larix* spp. had flushed *in vitro* but no shoot and showed embryo-like structure. On the other hands, buds

that had taken after winter developed into shoots. This report is very similar to our result. Most buds in IBA media did not flush and showed callus formation at the cut ends. It suggests that IBA works as inhibitor in bud flushing.

### 3. Shoot differentiation by treatment BAP+$GA_3$

Even though BAP was effective on bud flushing, a plantlet established still looked like a bundle of leaves and main shoot was too short to use further culture. It was required to elongate the internode of stems produced in BAP-containing media. On the other hand, $GA_3$ appeared to stimulate shoot elongation. For shoot development BAP-containing media were supplemented with $GA_3$. As the result of the treatment of $GA_3$, buds were elongated into shoots. $GA_3$, however, was affected by BAP. Three kinds of concentrations of $GA_3$(0.5, 1.0 and 2.0 ㎎/ℓ) were combined together with 1.0 ㎎/ℓ BAP (Fig. 3).

There was no difference in bud flushing rate following the ratio of BAP/$GA_3$. However, callus formation rate decreased depending on the ratio of BAP/$GA_3$ (Fig. 3). As BAP/$GA_3$ ratio became lower, shoot formation rate increased and shoot length become longer and longer(Table 2). This suggests that shoot development was affected by the ratio of BAP/$GA_3$ rather than absolute concentration of $GA_3$, and vice versa.

**Table 2.** Shoot length and fresh weight of bud differentiated on the media supplemented with BAP and $GA_3$.

| Concentration (㎎/ℓ) | Shoot Length (mm) | Fresh Weight (mg) |
|---|---|---|
| BAP 1.0+$GA_3$ 0.5 | 2.5±0.7 b* | 167.0±30.4 b |
| BAP 1.0+$GA_3$ 1.0 | 2.8±1.3 b | 204.8±67.9 a |
| BAP 1.0+$GA_3$ 2.0 | 5.0±2.2 a | 190.3±63.0 a |

* Means with the same letter are not significantly different at the $\alpha$=0.05 following Duncan's multiple range test.

It is known that gibberellin has a positive effect on shoot elongation. When the winter buds of *P. avium* were cultured, 0.1 ㎎/ℓ $GA_3$-$NH_4$ accelerated the differentiation rate(Jones and Hopgood, 1979). $GA_3$ was also reported to give an effect on the shoot elongation of the winter buds of *P. yedoensis*. As the protocol mentioned above, we could get many shoots with long stem internodes by adding $GA_3$ and BAP to the media at the first step.

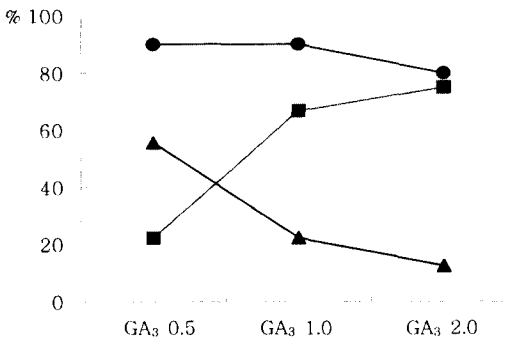


**Fig. 3.** Bud flushing rate, shoot formation rate and callus formation rate of winter buds of *P. yedoensis* on the media containing 1.0 ㎎/ℓ BAP, when supplemented with 0.5, 1.0 and 2.0 ㎎/ℓ $GA_3$, respectively. Basal media was WPM and culture period was 3 weeks.
● : Bud flushing rate, ■ : Shoot formation rate, ▲ : Callus formation rate

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