Micropropagation of *Physocarpus amurensis*©

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***Physocarpus amurensis* (Maxim.) Maxim. is an endangered deciduous shrub in the family of Rosaceae only native in China. Micropropagation of *P. amurensis* was conducted by taking axillary buds from field plants and apical buds from greenhouse seedlings as explants. Protocols were developed for axillary bud and apical bud sprouting, axillary-bud- and apical-bud-derived shoot proliferation, enlongating and strengthening of proliferated apical-bud-derived shoots, in vitro rooting of axillary-bud-derived shoots and ex vitro rooting of enlongated and stronged apical-bud-derived shoots.**

INTRODUCTION

Physocarpus amurensis is a deciduous shrub in the family of Rosaceae. The genus *Physocarpus* includes approximately 20 species in the world. The world geographic distribution of this species is centered in North America, and only one species found in China (Zhou, 1986). *Physocarpus amurensis* is the only species distributed in China, it is a relic species (Lu, 1999). It is not only an excellent ornamental shrub (Chen, 2003), but also a medicinal tree species (Yang, 2004). There is a small isolated population on the peak area of Maoershan in our University Forest at Maoershan Town, ShangZhi City, Heilongjiang Province, China. Because of extremely narrow distribution, population spread was restricted. There was almost no seed regeneration. Clonal propagation is crucial to evolution and survival of the population. Currently, the areas of distribution and the population size of this species in China are much less than in the past. So it was believed an endangered plant species and must be protected (Qin et al., 1993).

Tissue culture techniques have been extensively used for mass propagation of forest and cultivated tree species (Khurana et al, 2003). The micropropagation of *P. amurensis* offers important conservation possibilities, which have the potential to support in situ protection strategies (Bensone et al., 2000). Ex situ conservation using in vitro methods provides a "safe" repository for populations of *P. amurensis* derived from locations severely at risk. In vitro propagated plants may be transferred to ex vitro environments. The rapid production of large numbers of in vitro plantlets, (without seasonal dependency) for ex situ commercial cultivation purposes reduces the risks of *P. amurensis* plants being sampled from wild habitats and thus safeguards existing natural populations. The aim of this study was to develop an effective micropropagation method for *P. amurensis* for commercial and conservative purposes.

MATERIALS AND METHODS

Initiation. We collected the axillary buds from the mature mother dormant trees (1 or 2 years old) and apical buds from 3-month-old greenhouse seedlings of *P. amurensis* as explants of micropropagation.

Axillary bud explants were surface sterilized with 70% (v/v) ethanol for 10 sec (two times, 5 sec for each), and then sterilized with 10 min immersion in 0.1% mercuric chloride containing two drops of Tween-20, followed by being rinsed 3–5 times in sterile water and aseptically transferred to tissue culture tubes containing woody plant medium (WPM) basal medium supplemented with 0.01 $mg \cdot L^{-1}$ TDZ for axillary bud sprouting in vitro.

Apical buds explants were washed under running tap water for 30 min to remove superficial contamination and were surface sterilized with 75% (v/v) ethanol for 10–20 sec, then sterilized with 3 min immersion in 3% H_2O_2 containing two drops of Tween-20, followed by being rinsed 3–5 times in sterile water and aseptically transferred to tissue culture tubes containing Murashige and Skoog (MS) basal medium.

For all the experiments, MS (Murashige and Skoog, 1962) basal culture medium was used, supplemented with 2.5% (w/v) sucrose and 0.65% (w/v) agar. The pH was adjusted to 5.8 before adding agar. The media was subsequently autoclaved under 105 KPa at a temperature of 121 °C for 20 min. Explants were placed in a culture tube (50 ml) with 25 ml of MS media and kept under controlled conditions at 25±2 °C, with a 16-h photoperiod (irradiance of 30~40 $\mu mol \cdot m^{-2} \cdot s^{-1}$) under cool white light, relative humidity 60%–70%. The development state was observed after 30 days culture.

Multiplication. Multiplication media for shoots derived from axillary and apical bud explants were MS supplemented with different concentration NAA and BA. Because of the mean length of multiple shoots from apical buds of greenhouse seedlings were very short (< 1 cm), and not suitable to root, the shoots were elongated and strenthened. The MS medium supplemented with different concentration IBA and GA_3 were used as the shoot elongation and strengthening medium (strengthening refers ot the fact that the shoots obtained during proliferation are too weak for rooting and need to be subcultured one more cycle before rooting).

Rooting. Rooting of shoots derived from axillary buds was in vitro on MS medium supplemented with 0.1 $mg \cdot L^{-1}$ IBA. In vitro rooting of the multiplicated shoots from apical buds was not good, therefore, ex vitro rooting was tried. The basal end of multiplied microshoots from apical buds (approx. 2–4 cm long) were immersed in liquid with different concentration of NAA for 30 min or dipped 1000 $mg \cdot L^{-1}$ NAA for 10 sec, after thoroughly washed under running water to remove the adhered agar. The treated microshoots were transferred to plastic plots (33 × 22 × 11 cm) containing steam-sterilized peat and vermiculite mixture (2 : 1, v/v). The plots were transferred into a culture room maintained at 25±2 °C with a relative humidity of 80%–90%. The plantlets were harvested after 30 days and washed carefully to expose the roots. Shoots survival rate, rooting rate, total primary roots, and root length were measured.

RESULTS AND DISCUSSION

Shoot Regeneration from Axillary Buds of Natural Field Plants. A MS medium supplemented with 1.0 $mg \cdot L^{-1}$ NAA and 1.0 $mg \cdot L^{-1}$ BA was the best for proliferation. Mean number of multiple shoots per bud was 18 shoots (Fig. 1) and the shoot growth was normal. A MS medium supplemented with 2.0 $mg \cdot L^{-1}$ NAA and 0.6 $mg \cdot L^{-1}$ BA was the best for strong growth and mean length of multiple shoots was >2 cm. A MS medium fortified with 0.1 $mg \cdot L^{-1}$ IBA was suitable for in vitro root development. In vitro rooted healthy shoots transferred directly to plastic plot containing steam-sterilized peat and vermiculite mixture (2 : 1, v/v) revived growth after 30 days of transplantation and 65% plantlets survived in field conditions.

Figure 1. Proliferation of axillary bud derived shoots of *Physocarpus amurensis*.

Figure 2. Ex vitro rooting of enlongated and strengthened apical bud derived shoots of *Physocarpus amurensis* (1 month).

Table 1. Effect of benzyladenine and naphthaleneacetic acid on proliferation of apical bud sprouted shoots of *Physocarpus amurensis*

Treatment media	BA mg·L^{-1}	NAA mg·L^{-1}	Preliferation coefficient*	Regeneration rate (%)
1	0.8	0.4	> 18.5	100
2	0.8	0.6	5.3	100
3	0.8	0.8	3	66.7
4	1.0	0.4	7	100
5	1.0	0.6	7.5	100
6	1.0	0.8	9.3	100
7	1.2	0.4	9	66.7
8	1.2	0.6	> 10.7	100
9	1.2	0.8	7.7	100

Note: *mean number of multiple shoots per bud.

Micropropagation from Apical Buds of Greenhouse Germinated Seedling. The MS medium supplemented with 1.0 mg·L^{-1} NAA and 1.0 mg·L^{-1} BA was the best for apical bud sprouting, and bud sprouting rate could reach 100%. NAA at 0.4 mg·L^{-1} in combination with BA at 0.8 mg·L^{-1} was the best hormone combination for shoot proliferation. Mean number of multiple shoots per bud was >18 (Table 1), but the mean length of multiplied shoots were very short (< 1 cm), and not suitable to root. The short shoots were elongated and strengthened**.** MS medium supplemented with 0.5 mg·L^{-1} IBA and 0.3 mg·L^{-1} GA_3 was the best for shoot elongation and strengthening (Table 2). The best treatment for ex vitro rooting of the elongated and stronged shoots was soaking the microshoots in 100 mg·L^{-1} NAA for 30 min (Fig. 2). The rooted plantlets were transferred to field conditions with 93.1% survival rate (Table 3). In view of Debergh and Maene (1981) ex vitro rooting accounts 35%–75 % reduction of the total cost of plants propagated through tissue culture, current result implies prospective for commercial micropropagation of *P. amurensis*.

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Table 2. Effect of gibberellic acid and indolebutyric acid on elongation of apical bud derived shoots of *Physocarpus amurensis*.

Treatment media	IBA mg·L^{-1}	GA_3 mg·L^{-1}	No. of shoots	Proportion of shoots of different length (%)			
				≥ 3cm	2~3cm	1~2cm	≤ 1cm
1	0	0	10	0	0	0	100
2	0	0.5	11	0	0	90.9	9.1
3	0	1.0	30	3.3	30.0	66.7	0
4	0	2.0	28	25.0	35.7	35.7	3.6
5	0	3.0	31	45.2	25.8	29.0	0
6	0.5	0.3	33	63.6	36.4	0	0

Table 3. Effect of NAA on ex vitro rooting of enlongated and strengthened apical bud derived shoots *Physocarpus amurensis* (±SE: standard error).

NAA mg·L^{-1}	Shoots tested	Survival shoots	Survival rate (%)	No. of roots (strip)	Length of roots (mm)
0	28	4	14.29	10.5±2.10a	11.37±1.96a
50	29	5	17.24	11±1.53a	19.4±4.39ab
100	29	27	93.1	18.67±3.33bc	29.98±0.79c
200	25	22	88	20.5±1.55c	21.39±3.27bc
1000	30	14	46.67	13.67±1.33ab	17.35±2.74ab

Note: Means follow by different letter indicate significant difference at 5% level.