

In Vitro Propagation of Japanese Pear Rootstocks

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(Received September 21, 1987)

(Accepted February 12, 1988)

Seedlings of *Pyrus serotina* Rehd. and *Pyrus beturaefolia* Bunge. are commonly used as rootstocks for Japanese pear (*Pyrus serotina* Rehd.). But these seedlings show a wide range of variation because of their self-incompatible and heterozygous characters. These have hindered the progress in line selection or breeding of excellent rootstocks which are tolerant to winter injury, fruit hard-end disorder, and pests and diseases, including soil adaptability.⁵⁾ Therefore, the efficient method for clonal propagation of these rootstocks is necessary for future breeding. On the other hand, rootings in softwood and hardwood cutting of adult pear rootstocks are generally thought to be difficult.¹⁾

Recently, in vitro propagation has become practical means for rapid and large proliferation of some fruit species,⁴⁾ but this method was rarely applied to pear rootstocks. In this study, we explored the methods for in vitro propagation of Japanese pear rootstocks.

Plant materials were taken from old *Pyrus beturaefolia* Bunge., *P. serotina* Rehd., *P. calleryana* Decne., *P. dimorphophylla* Makino, *P. aromatica* Kikuchi et Nakai and *P. hondoensis* Nakai et Kikuchi trees (>30 years old) grown in the orchard of Tottori Univ. These shoot tips and axillary buds were collected in May, and sterilized in 70% ethanol for 3 min and sodium hypochlorite solution (containing 0.5% of available chlorite plus 0.05% of Tween 20) for 15 min and rinsed 4 times with sterile water. The meristems of shoot tips and axillary buds were dissected less than 0.5 mm length under a binocular microscope. These explants were placed in test tubes (3×12 cm) containing 10 ml of half-strength Murashige and Skoog (MS) salt mixture³⁾ supplemented with 0.1 mg/l IBA and 1 mg/l BA. The cultures were grown at 25°C under 16-hr photoperiod with a light intensity of 35 μE/m²/s in cool white fluorescent tubes.

At the established stage in the culture, the shoot growth and proliferation of *P. beturaefolia* was most vigorous (**Fig. 1**). Shoot growth and proliferation of other rootstocks except for *P. calleryana* and *P. dimorphophylla* were poor at the first three subcultures, but gradually became better thereafter. The establishment of the first culture step in *P. calleryana* and *P. dimorphophylla* were difficult and these failed to proliferate. The types of basal media were tested for subculture of *P. beturaefolia*. The following 4 media were examined: MS, half-strength MS (1/2 MS), one fourth-strength MS (1/4 MS) and woody plant medium (WPM).²⁾ Among these media WPM generally showed the best results in the shoot proliferation (**Fig. 2**).

A rooting response of the shoot of *P. beturaefolia* was tested. An auxin was essential to induce rooting in culture. The best rooting was obtained with half-strength MS supplemented with 1 mg/l IBA (**Table 1**). But in this method the plantlets often died on transplantation due to the considerable callusing in the basal end of shoots. To examine the effect of pretreatment with high concentrations of auxin on the root formation, the basal ends of shoots were inserted into the agar media containing IBA and incubated for 24 hr and then transplanted on the auxin free media supplemented with 0.1 mg/l kinetin. As a result, the treatment with 50 mg/l IBA for 24 hr reduced the callusing of shoot ends (**Table 2**) and increased subsequent survival of shoots to 73.7%.

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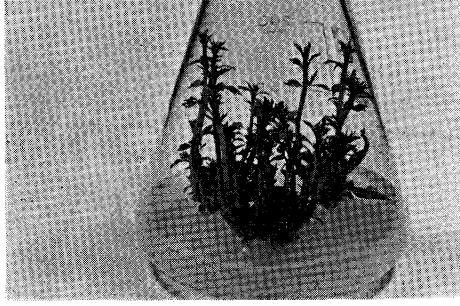


Fig. 1. Shoot proliferation of *Pyrus beturaefolia*.

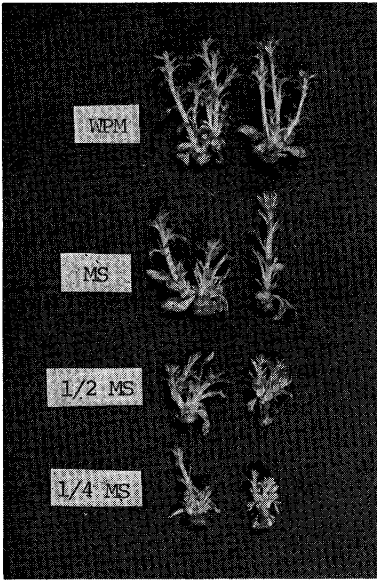


Fig. 2. Effect of several media on the shoot proliferation of *Pyrus beturaefolia*.

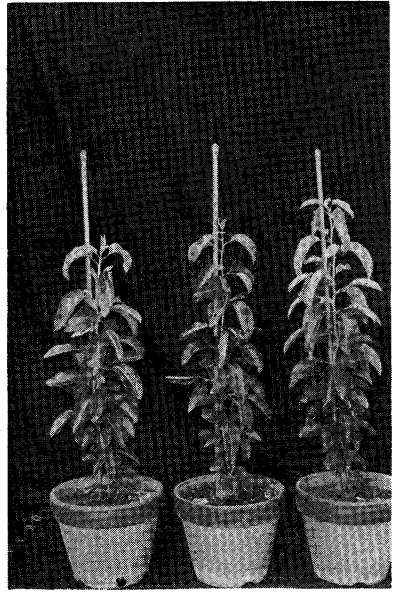


Fig. 3. Plantlets of *Pyrus beturaefolia* 2 months after transferring to a pot.

Table 1. Effects of IBA and NAA at different concentrations in MS and 1/2 MS media on the percentage of rooting of *Pyrus beturaefolia*.

Media	Auxin concentration (mg/l)				
	0	0.01	0.1	1.0	10.0
MS + IBA	0 (-) ^a	20.0 (-)	20.0 (±)	60.0 (++)	60.0 (++)
1/2MS + IBA	0 (-)	0 (-)	40.0 (±)	80.0 (++)	40.0 (++)
MS + NAA	0 (-)	0 (-)	0 (-)	0 (+)	0 (+)
1/2MS + NAA	0 (-)	0 (±)	40.0 (+)	60.0 (++)	0 (++)

^a Degree of callusing from none (-) to severe (++)

Table 2. Effect of pretreatment with IBA at different concentrations in MS and 1/2 MS media on the percentage of rooting of *Pyrus beturaefolia*.

Media	IBA concentration (mg/l)		
	25	50	100
MS ^a	0 (-) ^b	20.0 (+)	20.0 (±)
1/2MS	60.0 (+)	80.0 (+)	40.0 (+)

^a All media contain 0.1 mg/l kinetin.

^b Degree of callusing from none (-) to severe (±).

Rooted plantlets were transferred to peatmoss and gradually acclimatized for potting. After the transplant to the soil, most of the plantlets began to grow well. All leaves of the plantlets were large and round, and showed a complete adult phase (Fig. 3).

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《和文要約》

茎頂培養によるニホンナシ台木の大量増殖について

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種々のニホンナシ台木を用いて茎頂培養による増殖法を検討した。葉条増殖には、0.1 mg/l の BA と 0.1 mg/l の IBA を含む WPM 培地が良好であった。また、葉条基部を 50 mg/l の IBA で 24 時間処理した後、2分の1の濃度の MS 培地に植え替えると、葉条基部のカルス形成が抑制され、発根率、馴化育成率がともに向上した。