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In vitro propagation of herbaceous peony (*Paeonia lactiflora* Pall.) by a longitudinal shoot-split method

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ABSTRACT

A procedure for the clonal propagation of Paeonia lactiflora Pall. cvs. Takinoyosooi and Sarah Bernhardt through shoot tip culture is described. Half strength Murashige and Shoog (1962) medium supplemented with 0.5 mg/ℓ 6-benzylaminopurine plus 1 mg/ℓ gibberellic acid promoted formation and growth of axillary buds. Continuous shoot multiplication was achieved by vertically splitting the shoot axis and subsequent division of elongated axillary shoots every 36 days. High frequency (57–100%) of rooting was obtained on paper-bridge liquid medium supplemented with 1 mg/ℓ indole-3-butyric acid. Half of the rooted plantlets were established on porous soil. Thus, 700 and 300 plants of cv. Takinoyosooi and Sarah Bernhardt could be theoretically obtained from a single bud in one year.

ABBREVIATIONS

BAP, 6-benzylaminopurine; GA, gibberellic acid; NAA, α-naphthaleneacetic acid; IBA, indole-3-butyric acid; MS, Murashige and Skoog (1962) basal medium.

INTRODUCTION

Herbaceous peony (Paeonia lactiflora Pall.) is one of the most important perennial ornamentals. Propagation of herbaceous peony is usually conducted by division of rhizome or crown by which 3 to 5 clones are obtainable per year (Miyazawa, 1967). However, this multiplication rate is too slow to meet the demand of production of popular cultivars or newly selected clones. Meyer (1976) induced callus from flower bud and root, but no differentiation of shoots and roots was obtained. Recently, Jin-Jing et al. (1987) obtained callus from embryos in seeds and succeeded in somatic embryo formation in a modified Linsmaier-Skoog (1965) medium supplemented with N-(3-methyl-2-butenyl)-1H-purine-6-amine (2iP) and NAA. However, the further development was arrested because the somatic embryos had no shoot meristems unlike normal seed embryos. Even if this problem was overcome, vegetative clonal propagation still remained unresolved. In the meantime, Kato and Hagiya (1984) attempted shoot tip culture and succeeded in the formation of axillary buds in half strength MS medium supplemented with GA and BAP. However, a successive multiplication method has not been established.

Previously, we (Hosoki et al. 1986, 1988) have described a rapid multiplication method for Japanese horseradish (Wasabi) (Eutrema japonica Maxim.) by shoot split method where the shoot axis was longitudinally split into halves to induce the formation and growth of axillary buds. By repeating the process and separating the axillary shoots, thousands of plants were obtained from one shoot tip in one year.

The present communication describes a rapid multiplication method for herbaceous peony by splitting shoot axis, the subsequent separation of axillary shoots and establishment of rooted plantlets in the nursery pan.

MATERIALS AND METHODS

In a preliminary experiment, shoot tips (2–3 mm long) of wild herbaceous peony (Paeonia lactiflora Pall.), which had been stored for 2 months at 0℃, were cultured in half strength MS medium (Murashige and Skoog, 1962) supplemented with 0.5 mg/ℓ BAP, 1 mg/ℓ GA, or 0.5 mg/ℓ BAP plus 1 mg/ℓ GA to reconfirm the formation of axillary buds according to Kato and Hagiya's method (1984). Other nutrient and culture conditions are described in the following: In the main experiment, rhizomes of commercial herbaceous peony cultivars Takinoyosooi and Sarah Bernhardt, were obtained in the early spring when buds on the rhizomes were still underground. A few scale leaves were removed and the buds were sterilized in a diluted solution of sodium hypochlorite (active chlorine 0.7%) and rinsed with a sterile water two times. Main shoot tips and

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axillary shoot tips (2-3 mm long) were excised after removal of small scale leaves under a dissecting microscope. The explants were placed on agar (0.8%) solidified medium (15 mℓ) in test tubes (2 cm ϕ, 15 cm long). The nutrient medium consisted of half strength Murashige and Skoog (MS)(1962) medium and Fe-EDTA, Ringe and Nitsch (1967) minor elements and vitamins and 3% sucrose at pH 5.6. BAP (0.5 mg/ℓ) plus GA (1 mg/ℓ) were supplemented as growth regulators for the initial culture. Supplement of only 0.5 mg/ℓ BAP was also tested for the subculture. All the test tubes were placed at 20℃ under 16 hr illumination from 52 $\mu E\ m^{-2}\ s^{-1}$ cool white fluorescent lamps.

RESULTS AND DISCUSSION

In a preliminary experiment using wild species, P. lactiflora, two to three axillary buds were formed in GA and BAP containing medium (Table 1). No axillary buds were formed in GA medium while a few buds were formed in BAP medium. Shoot length was the highest in the combination treatment while no difference was observed between the single treatments of GA and BAP. Leaf number was almost the same among the treatments. The promotion of axillary bud formation by combination of GA and BAP agreed with Kato and Hagiya's (1984) results. Therefore, the combination treatment of BAP and GA was adopted for multiplication of commercially important cultivars (main experiment). Ten shoot tips each of cv. Takinoyosooi and Sarah Bernhardt were cultured in 0.5 mg/ℓ BAP plus 1 mg/ℓ GA supplemented 1/2 strength MS medium for 3 and 2 months, respectively. Excised shoot tips elongated and formed axillary buds at most nodal positions. The elongated shoot axes with 3 to 5 axillary buds were then longitudinally split into halves as shown in Fig. 1, left. Some of the long shoots were further sectioned transversely into two so that four stem sections were obtained. Here, each section should contain at least one axillary bud. Otherwise, no more mutiplication occurred. The average numbers of split sections obtained in cv. Takinoyosooi was 3.8 shoots, and in cv. Sarah Bernhardt 3.3 shoots (Table 2). From the next division, 0.5 mg/ℓ BAP single treatment as well as GA and BAP combination treatment was used. Within about 35 days after culture, each section developed into shoots (2-3 cm long) and again produced secondary axillary buds at the nodes. Some of the axillary buds elongated and looked like branching shoots in BAP and GA combination treatment in both cultivars (Fig. 2, right). From this time on, multiplication was achieved only by division of axillary shoots (Fig. 1, right). About 33 days after culture, axillary

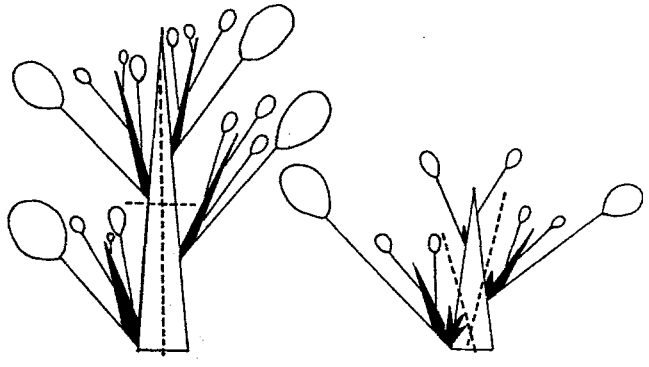

Fig. 1 Multiplication by shoot split and division of axillary shoots. Longitudinal split of main axis and transverse section if possible (left), and subsequent separation of axillary shoots (right).

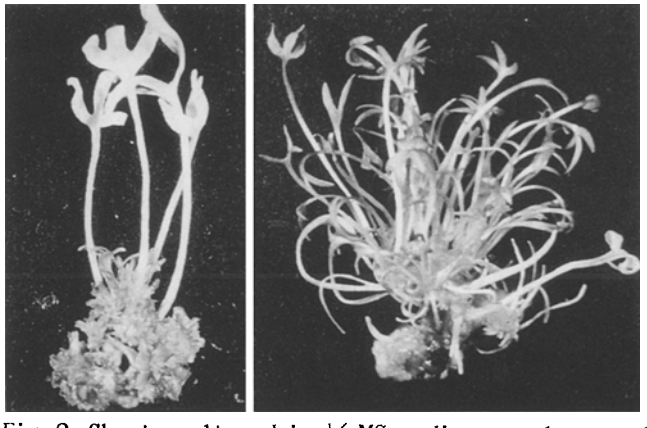

Fig. 2 Shoots cultured in ½ MS medium supplemented with 0.5 mg/ℓ BA (left) or 0.5 mg/ℓ BAP + 1 mg/ℓ GA (right)(cv. Takinoyosooi). Note minute axillary buds at the stem base in 0.5 mg/ℓ BAP medium (2 months after culture).

Table 1. Effect of GA, BAP and GA+BAP on formation of axillary buds, shoot length and leaf numbers on shoot in the wild species, Paeonia lactiflora.

Treatment (mg/ℓ)	No. of axillary buds (mean±SD)	Length of main shoot (cm) (mean±SD)	Leaf numbers on main shoot (mean±SD)
1 GA	0	1.4±0.2	4.3±0.4
0.5 BAP	0.2±0.4	1.5±0.3	4.8±0.7
1 GA + 0.5 BAP	2.4±0.4	2.4±0.3	5.4±1.4

Recorded 55 days after culture (5 explants per treatment).

Table 2. Multiplication by shoot split and division of axillary shoots in herbaceous peony cultivars.

Cultivar	The number of times of shoot split/division* (day-interval to next division)	No. of divided shoots (mean ± SD)		No. of elongated axillary shoots (mean ± SD)	
		0.5mg/ℓBAP	0.5mg/ℓBAP+1mg/ℓGA	0.5mg/ℓBAP	0.5mg/ℓBAP+1mg/ℓGA
'Takino-Yosooi'	1	——**	3.8 ± 1.1	——**	——***
	2 (35)	3.4 ± 0.6	3.0 ± 0.7	3.1 ± 0.6	2.5 ± 0.7
	3 (32)	2.4 ± 0.6	3.6 ± 0.6	0.8 ± 0.7	1.9 ± 1.4
	4 (32)	2.8 ± 0.5	3.8 ± 1.9	1.7 ± 1.3	3.1 ± 2.1
	5 (45)	2.6 ± 0.6	3.5 ± 0.3	0.9 ± 0.9	3.6 ± 1.9
'Sarah Bernhardt'	1	——**	3.3 ± 0.3	—— **	——***
	2 (36)	2.2 ± 0.8	2.0.± 0.7	2.1 ± 0.7	2.3 ± 0.8
	3 (34)	2.2 ± 0.5	4.0.± 1.6	1.6 ± 0.9	3.8 ± 1.8
	4 (33)	1.8 ± 0.8	2.4.± 0.7	1.6 ± 0.9	2.2 ± 1.4
	5 (45)	1.8 ± 0.5	2.8 ± 0.5	1.1 ± 0.9	1.9 ± 1.2

* Five to six explants used for each split/division, except initial culture for which 10 explants were used.
** Not treated.
*** Not recorded.

buds elongated to 2-4 cm long with new axillary buds in both cultivars. Again, shoot division was achieved. This continued division was possible at 36 day intervals at least upto 5 times without losing the capacity of axillary bud formation in both cultivars. The number of divided shoots and the number of elongated axillary shoots were somewhat greater in BAP and GA combination medium than in BAP single treatment. In BAP treated shoots, many minute axillary buds were observed around the nodal area at the lower stem position (Fig. 2, left). These bud aggregates were morphologically similar to those on underground rhizome or crown grown in the field. If this division was repeated in BAP plus GA medium at 36 day intervals for one year including the time for initial shoot elongation (2-3 months), more than 5000 shoots could be theoretically obtained from a single bud. This number is similar to that in multiplication of Japanese horseradish for which a similar shoot split method was developed (Hosoki et al. 1986, 1988). This multiplication method is much easier and more practical, compared with that of somatic embryogenesis from callus of seed embryo (Jin-Jing et al, 1987).

For rooting, shoot (2-3 cm long) was transferred to 0.1 mg/ℓ or 1 mg/ℓ of NAA and IBA supplemented agar solidified medium. The 0.1 mg/ℓ of both auxins produced roots only in 0-40% of the shoots in both cultivars whereas 1 mg/ℓ of them produced roots in 40-80% of the shoots but it took over 3 months (data not shown). Therefore, paper-bridge method on liquid culture was adopted to expose stem-base to more oxygen. One mg/ℓ of NAA or IBA was again used. Within 2 months, 100% IBA treated shoots produced roots in cv. Takinoyosooi and 57% in cv. Sarah Bernhardt (Table 3)(Fig. 3). NAA produced roots in 40% and 70% of the plants, respectively, but it also produced callus at the stem-base, which caused tissue decay by fungus attack after ex vitro culture. IBA treated plantlets with roots were transferred to a porous soil medium under plastic cover at 18-20℃ under 16 hr illumination from 52 $\mu E\ m^{-2}s^{-1}$ cool white fluorescent lamps. More than half of shoots were

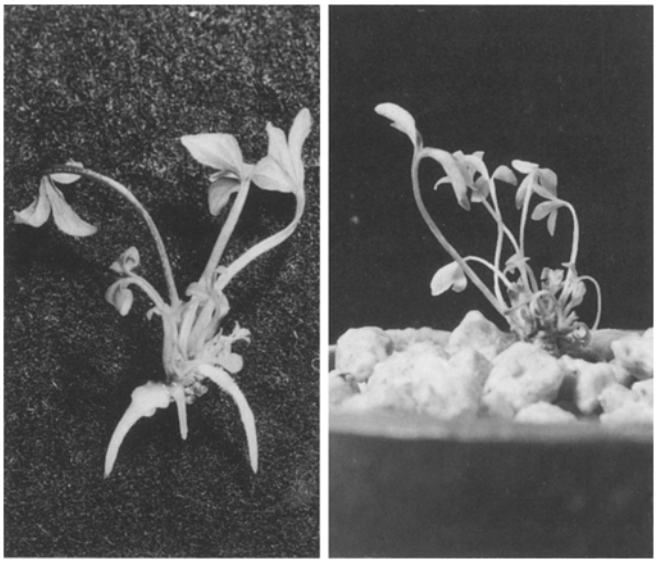

Fig. 3 A rooted plantlet of cv. Sarah Bernhardt (left) in ½ MS liquid medium supplemented with 1 mg/ℓ IBA on paper-bridge (2 months after culture) and the established plant in the soil (right).

Table 3. Effect of NAA and IBA on root and callus formation on shoots.*

Cultivar	Auxin (1 mg/ℓ)	Rooting frequency (%)	Root no. per explant (mean±SD)	Callus formation frequency (%)
'Takinoyosooi'	NAA	40	0.6±0.8	100
	IBA	100	2.3±1.6	0
'Sarah-Bernhardt'	NAA	70	2.6±3.0	100
	IBA	57	2.1±2.3	0

* Recorded on 2 months after culture (7 to 10 explants per treatment).

established. The remaining plants turned yellow. Such an incomplete establishment may be due to lift-up of the shoots above paper-bridge as the roots grew down during culture. Therefore, nutrient absorption became insufficient. Such a problem is being resolved by placing shreds of gauze about 4mm thick on the paper-bridge where roots penetrate into medium and good aeration is maintained. Although more efficient acclimatization after ex vitro culture must be established, we have demonstrated a clonal propagation system of commercial cultivars of Paeonia lactiflora by a shoot splitting method and subsequent division of axillary shoots.

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