Regeneration of *Platycodon grandiflorum* Through Adventitious Shoots Formation From Cotyledon and Hypocotyl Explants©

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An efficient protocol for mass propagation of *Platycodon grandiflorum* 'Samidare-murasaki' and 'Sentimental Blue' was developed using explants divided into two halves of expanded cotyledon and hypocotyl segments. The direct formation of shoots from these explants was most active on the Murashige-Skoog (MS) medium supplemented with BA (1 mg·L^{-1}) and NAA (0.1 mg·L^{-1}). In this research, 20–23 shoots were initiated from cotyledon explant and 8–9 shoots were initiated from hypocotyl segment 6 weeks after the beginning of culture. Most of adventitious shoots were directly formed at the proximal cut end of proximal half of a cotyledon, and successively at the proximal cut end of distal half. The distal cut end of the hypocotyl was more active in the formation of adventitious shoots than the proximal cut end. Rooting of the adventitious shoots was promoted in the MS medium supplemented with IBA (1 mg·L^{-1}), while NAA (0.1 mg·L^{-1}) had little effect on the rooting. After acclimatization, the regenerated plants grew normally and flowered in a greenhouse.

INTRODUCTION

Balloon flower, *Platycodon grandiflorum* (Jacq.) A. DC., a perennial plant of Campanulaceae, is native to Japan, Korea, and the northern region of China. The plant, so-called balloon flower, is popular as a cut flower and pot plant. In addition, the plant is useful for the production of medicines, since the root of the plant contains several kinds of saponin and inullin.

The traditional propagation methods for this plant are from seeds or by division. The former often results in non-uniform plants by genetic segregation and the latter is not very high in the rate of propagation. Therefore, the method of micropropagation of the plant was developed not only for obtaining a number of elite clones, but as culture system for future genetic transformation research. As for micropropagation of *P. grandiflorum*, several studies have been reported using different types of explants, for example, node (Hosoki et al., 1995; Yamamoto 2004); shoot tip and axillary bud (Yonemitsu et al., 1998), cotyledon, hypocotyl, and leaf (Kasumi et al., 1999); and leaf segment (Iijima et al., 2003). The present paper describes micropropagation through direct adventitious shoot formation using divided halves of expanded cotyledon and hypocotyl segment, and the difference in potential of adventitious shoot formation among the positions within these explants.

MATERIALS AND METHODS

Seeds of two cultivars, 'Samidare-murasaki' and 'Sentimental Blue' (dwarf type), were obtained from Sakata Seed Corporation. The seeds were surface sterilized

with 70% ethanol for 10 sec, then with 5% sodium hypochlorite solution containing a drop of Tween 20 for 20 min. After being rinsed with sterilized water four times, the seeds were planted on the Murashige and Skoog medium without hormones. The medium containing 3% sucrose had been adjusted to a pH of 5.75 before autoclaving and solidified with 0.2% Gelrite®. After germination in the darkness at 20 °C, the plantlets were transferred onto hormone-free MS medium. The cultures were kept at 20 °C under a 16-h photoperiod with a light intensity of 3000 lux from cool fluorescent lamps. When the plantlets had expanded two cotyledons 15 days after germination, the cotyledons were excised from the plantlets and transversely divided into two halves, distal and proximal parts. At the same time, segments of hypocotyl (5 mm in length) were excised from the plantlets. The cotyledon halves were cultured with adaxial side in contact with the media supplemented with several combinations of different concentrations of BA and NAA. Hypocotyl segments were also cultured on the same media.

Another experiment was carried out to investigate the difference in the potential of shoot formation among the positions within the explant. Sixteen explants were cultured in a series and the experiments were replicated three times.

Several concentrations of IBA or NAA were added to the MS medium to compare the effect on rooting of adventitious shoots. All the cultures were kept in the same temperature and light conditions as those of germinated plantlets. For acclimatization, the regenerated plants were transferred to a vermiculite medium in a plastic pot (6 cm in diameter and 5 cm in height). The acclimatized plants were grown in a greenhouse to observe growth and development.

RESULTS AND DISCUSSION

Table 1 shows the effect of BA and NAA combinations on the formation of adventitious shoots from the cotyledon explants divided into two halves. In both cultivars, the combination of BA 1.0 mg·L^{-1} and NAA 0.1 mg·L^{-1} gave the best results for not only the frequency of shoot formation, which was expressed as percentage of shoot-forming explants to all the explants cultured but also the number of shoots per explant. In this case, more than 20 shoots were obtained from the half-divided cotyledon of both cultivars during 6 weeks of culture.

As shown in Table 2, the maximum values of frequency for shoot formation and number of shoots per hypocotyl segment were observed in the medium supplemented with BA 1.0 mg·L^{-1} and NAA 0.1 mg·L^{-1}. The number of shoots formed from hypocotyl explants in the medium (BA 1.0 mg·L^{-1} and NAA 0 mg·L^{-1}) was close to that in the medium (BA 1.0 mg·L^{-1} and NAA 0.1 mg·L^{-1}). From a comparison of the results in Tables 1 and 2, it is apparent that NAA (0.1 mg·L^{-1}) had a larger effect on shoot formation from cotyledon explant than from hypocotyl explant. There are many reports on the effects of combinations of cytokinins and auxins on the formation of adventitious shoots from cotyledon and hypocotyl explants. In most cases, the cytokinin resulting in the best results has been reported to be different for different kinds of plants. The most efficient combination of BA and NAA similar to the present experiment was reported with *Astragalus sinicus* (Cho et al., 1995) and with *Brassica oleracea* var. *tronchda* Bailey (Msikita and Skirvin, 1989).

Figure 1 shows shoot-forming potential in each cut end of divided cotyledons and hypocotyl segments. In cotyledon explants, adventitious shoots were highest at the proximal end of the proximal half and next at the proximal end of distal half. In

hypocotyl segments, adventitious shoots were higher at the distal end than at the proximal end. In both of the two types of explants, adventitious shoots were rarely observed on other parts except at the ends. It has been reported with geranium (Chang, et al., 1996), with *Vigna radiate* L. (Gulati and Jaiwal, 1990), with *Cucumis sativus* L. (Gambley and Dodd, 1990), and with *Farfugium japonicum* (Yamamoto et al., 2000) that basal region is most active in the formation of adventitious shoot within cotyledon. The distal end of hypocotyl segment of *B. carinatthan* is higher in the capacity of formation of adventitious shoots than proximal end (Yang, et al., 1991). The results obtained in the present experiments were consistent with the above-mentioned observations. The youngest cell and tissue of a cotyledon is located in the basal region close to petiole, while in the hypocotyl segment the distal end is the youngest region. These regions can be considered to be more active in cell division and therefore shoot primordial might be more easily formed. We may ascribe the reason for the difference in the shoot-forming potential shown in Fig. 1 to the degree of the activity of cell division in each region of explant.

When the shoots attained approximately 20 mm in length, the shoots were transferred to the rooting medium shown in Table 3. The highest rooting of both cultivars was observed in the medium supplemented with IBA 1 mg·L^{-1}, while NAA (0.05 and 0.1 mg·L^{-1}) had little promotive effect on the rooting (Table 3).

After acclimatization, the regenerated plants grew normally and flowered. An example of flowering plant regenerated by the method is shown in the picture of Fig. 2. No genetic variation has been observed.

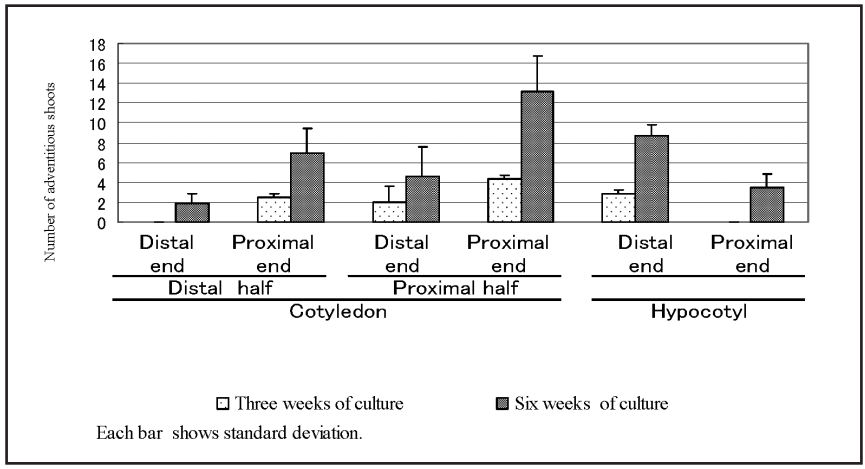

Figure 1. Shoot-forming potential in the each cut end of divided cotyledon and hypocotyl segment.

Figure 2. Flowering of the regenerated plant *Platycodon grandiflorum* 'Samidare-murasaki'.

Table 1. Effect of BA and NAA on the formation of adventitious shoots from cotyledon segments divided into two halves.

Cultivars	BA (mg·L^{-1})	NAA (mg·L^{-1})	Explant with shoots (%)	Shoots per explants (no.)
'Samidare-murasaki'	0	0	0	0
	0.5	0	42.9	2.9±2.3
	1	0	30	2.2±1.9
	1	0.1	100	20.6±7.4
	1	1	61.9	10.2±2.8
'Sentimental Blue'	0	0	0	0
	0.5	0	16.7	1.6±1.4
	1	0	25	1.1±1.5
	1	0.1	82.1	23.1±13.5
	1	1	14.3	7.4±12.8

Each value was scored 6 weeks after the beginning of culture.

*Mean ± standard deviation.

Table 2. Effect of BA and NAA on the formation of adventitious shoots from hypocotyl segments.

Cultivars	BA (mg·L^{-1})	NAA (mg·L^{-1})	Explant with shoots (%)	Number of shoots per explant
'Samidare-murasaki'	0	0	0	0
	0.5	0	16.7	3.8±3.4
	1	0	50	7.8±6.1
	1	0.1	100	9.3±3.5
	1	1	52.9	1.9±1.9
'Sentimental Blue'	0	0	0	0
	0.5	0	0	0
	1	0	83.3	7.8±2.8
	1	0.1	91.7	8.2±4.8
	1	1	0	0

Each value was scored 6 weeks after the beginning of culture.

*Mean ± standard deviation.

Table 3. Rooting of shoots formed from cotyledon explants.

Cultivars	IBA ($mg \cdot L^{-1}$)	NAA ($mg \cdot L^{-1}$)	Rooting (%)
'Samidare-murasaki'	0	0	31
	0.1	0	53.8
	0.5	0	81.6
	1	0	87.2
	0	0.05	0
	0	0.1	0
'Sentimental Blue'	0	0	11.5
	0.1	0	3.3
	0.5	0	58.8
	1	0	88.9
	0	0.05	5
	0	0.1	13.3

Medium for adventitious shoots formation; MS medium supplemented with BA (1.0 $mg \cdot L^{-1}$) and NAA (0.1 $mg \cdot L^{-1}$).

Each value was scored 6 weeks after the beginning of culture.

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