Obtaining Plantlets by Flower Bud Culture and Scape Culture in *Primula kisoana*©

Hiroaki Ohashi and Kaoru Akaboshi
Faculty of Agriculture, Ehime University, 3-5-7, Tarumi, Matsuyama, Ehime 790-8566, Japan
Email: Ohashi@agr.ehime-u.ac.jp

Yoshinori Ochi
Seiko-en, 1-8-10, Kokubu, Imabari, Ehime 799-1533, Japan

INTRODUCTION

Primula kisoana Miq. is a hardy perennial distributed in a part of Shikoku province. This species has wide variation, especially in diameter and color of the flower. For example, 'White Flower', 'Lyo-beni', and 'Birodo' were on the market as garden cultivars.

We obtained a number of variations through breeding and selected some superior clones (Fig. 1), and are planning to propagate by root segment culture and micropropagation. However, the culture methodology and conditions have not been established for obtaining plantlets from the in vitro culture of this plant. We studied the effects of plant growth regulators and difference among individuals on flower bud and scape culture.

Figure 1. Setected clones of *Primula kisoana* showing variations resulting from hybridization.

MATERIALS AND METHODS

Collected scapes were sterilized with sodium hypochlorite solution (1% available chlorine) and rinsed with sterilized water three times. Flower buds and scape tips were cut from scapes and used for the following experiments.

Experiment 1: Difference among Individuals. Selected superior clones (31 total) were assigned to this experiment. Explants were put on ½ strength Murashige and Skoog basal medium (to which was added 3% sucrose and 2.5 $g \cdot L^{-1}$ gellan gum; pH 5.8) and supplement with 0.2 $mg \cdot L^{-1}$ benzyladenine (BA) for scape tip culture, and 0.2 $mg \cdot L^{-1}$ 1-naphthylacetic acid (NAA) and 2 $mg \cdot L^{-1}$ BA for flower bud culture. In the case of flower bud culture, flower buds were classified as L, M, S, and SS, depending on size. Cultures were incubated under a 16-h light photoperiod at 20 °C. Cultures were observed 30 and 75 days after culture for growth of apical bud, adventitious shoot, and root formation, on a 0 to 4 scale according to the following scales. In apical bud and adventitious shoot, 0: no organogenesis, 1: only shoot primordium, 2: shoots with undeveloped leaves, 3: shoots with developed leaves, 4: shoots with developed leaves and roots. In adventitious root, 0: no organogenesis, 1: only root primordium, 2: roots without lateral root, 3: roots with few lateral roots, 4: roots with some lateral roots.

Experiment 2: Effects of Plant Growth Regulators. Six clones were assigned to this experiment carried out much the same as Experiment 1 expect for the following plant growth regulator (PGR) conditions. For scape-tip culture five PGR combinations and for flower bud culture three PGR combinations were assigned to each.

RESULTS AND DISCUSSION

After 30 days of culture, most of explants were enlarged or elongated, and organogenesis and growth of scape-tip buds were observed on some explants. In 75 days after culture organogenesis and growth of shoots were observed on many explants.

Experiment 1: Difference among Individuals (after 75 Days of Culture).
Flower Bud Culture: Adventitious shoot and root formation were observed in 24 clones and 30 clones, respectively; '99C060-6' clone didn't show any organogenesis. There was a large difference between each clone in rate of adventitious shoot formation and its index.

Scape-Tip Culture: Rate of shoot formation (including adventitious shoot) and its index and their relationships between each clone were similar to results in flower-bud culture. We concluded that influence of difference among individuals was smaller than flower-bud culture, because total rate of shoot formation (92.4%) was higher than that of flower-bud culture (Table 1).

Experiment 2: Effects of Plant Growth Regulator after 75 Days of Culture. In the same way as Expt. 1, shoot formation in scape-tip culture was better than in flower-bud culture (Table 2).

In scape-tip culture, shoot formation was mainly adventitious shoots on the surface and section of scapes. We concluded that suitable PGR combination is 2 $mg \cdot L^{-1}$ BA alone for growth of scape-tip buds, 0.2 $mg \cdot L^{-1}$ NAA and 2 $mg \cdot L^{-1}$ BA for adventitious shoot formation. In flower bud culture, we concluded that suitable PGR combination with a low level of NAA to 2 $mg \cdot L^{-1}$ BA.

LITERATURE CITED

Murashige, T., and **F. Skoog.** 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15:473–497.

Table 1. Organogenesis in flower-bud and scape-tip culture of *Primula kisoana* (on 75 days after culture).

						Adventitious root formation			
	No. of explants	Callus formation		Shoot formation					
		Rate (%)	Index [1)]	Rate (%)	Index [2)]	Rate (%)	Index [3)]	No. of root/explant	Average of length (mm)
Flower-bud culture	212	4.7	0.30	48.8	0.63	74.1	1.53	4.9	17.9
Scape-tip culture	46	8.7	0.41	92.4	1.88	76.1	1.83	8.6	21.4

Number of explants were total of 31 clones. Media = $^1/_2$ MS basal medium (added 3% sucrose and 2.5 g·L^{-1} gellan gum, pH 5.8) supplement 0.2 mg·L^{-1} BA for scape-tip culture, 0.2 mg·L^{-1} NAA and 2 mg·L^{-1} BA for flower bud culture.

1) The means of values were based on the following scale, 0: no transformation, 1: enlargement of cut end, 2: few callus formation.

2) The means of values were based on the following scale, 0: no organogenesis, 1: only shoot primordium, 2: shoots with underdeveloped leaves, 3: shoots with developed leaves, 4: shoots with developed leaves and roots.

3) The means of values were based on the following scale, 0: no organogenesis, 1: only root primordium, 2: roots without lateral roots, 3: roots with few lateral roots, 4: roots with some lateral roots.

Table 2. Effects of plant growth regulators on organogenesis in flower-bud and scape-tip cultures of *Primula kisoana* (75 days after culture).

Plant growth regulator combination (mg/1)	No. of explants	Shoot formation		No. of buds/explant		Adventitious root formation			
		Rate (%)	Index [1]	Adventitious bud	Definite bud	Rate (%)	Index [2]	No. of root/ explant	Average of length (mm)
BA 2	10	80.0	1.60	4.75	2.55	60.0	1.00	2.6	20.8
BA 2+NAA 0.2	10	100	1.80	9.60	0.30	100	2.35	9.1	35.7
BA 1	11	100	2.32	6.05	1.91	45.5	0.95	2.3	19.5
BA 2	23	52.2	0.33	1.41	—	4.3	0.02	0.0	0.0
BA 1+NAA 0.1	24	62.5	0.63	4.08	—	45.8	0.38	0.9	10.4
BA 2+NAA 0.2	24	62.5	0.65	4.31	—	37.5	0.50	1.4	10.6
BA 4+NAA 0.4	23	60.9	0.87	5.98	—	65.2	1.20	4.0	17.5
BA 2+NAA 1	26	57.7	0.62	4.54	—	92.3	1.83	7.8	24.0

Upper three rows = scape-tip culture, lower five rows = flower-bud culture. Number of explants from total of 6 clones.

1) The means of values were based on the following scale, 0: no organogenesis, 1: only shoot primordium, 2: shoots with undeveloped leaves, 3: shoots with developed leaves, 4: shoots with developed leaves and roots.

2) The means of values were based on the following scale, 0: no organogenesis, 1: only root primordium, 2: roots without lateral roots, 3: roots with few lateral roots, 4: roots with some lateral roots.