Adventitious Shoots Formation by Flower Bud Culture of *Primula veris*, *Primula vulgaris*, and *Primula juliae*©

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Selections of *Primula* × *polyantha* hort. are important pot flowers in Japan, which are complex hybrids of *P. elatior* (L.) Hill, *P. veris* L., and *P. vulgaris* Hudson, commonly called polyanthus. In addition, hybrids of polyanthus and *P. juliae* Kusnetsow were called Juliana hybrid or Julian, and they were produced as well as polyanthus. Homogeneous seed production is difficult because they are allogamous plant.

Callus induction and regeneration from vegetative organs has not been successful in polyanthus and Julian types, although in *P. juliae* callus was induce easily and a few adventitious shoots were abtained from flower bud culture.

In this study, we studied the induction of adventitious shoot formation by flower bud culture of *P. veris*, *P. vulgaris*, and *P. juliae*. *Primula veris*, *P. vulgaris*, and *P. juliae* plants were divided into into explants containing 2-3 buds, and planted in plastic pots (diameter 9 cm) containing pumice for growing (called "kanuma" soil), in the autumn of the year before flower bud culture. These were placed on subirrigation trays in February, and the flower buds (length: 10-15 mm) were harvested in late March to early in April.

These picked flower buds were dipping in sodium hypochlorite solution (1% available chlorine) for about 8 min and rinsed by sterilized water. Basal medium for flower bud culture was MS medium (Murashige and Skoog, 1962) supplemented with 30 $g \cdot L^{-1}$ sucrose and 2.5 $g \cdot L^{-1}$ gellan gum (Wako pure Chemical Industries, Ltd., Japan), and supplemented with six combinations of 1-naphthyl acetic acid (NAA) and 6-benzylaminopurine (BA) as plant growth regulators (PGR), and hormone-free as control (Table 1). The surface sterilized flower buds were put individually on medium (10 ml) in test tubes (25 mm diameters; 120 mm height), later on measured length of flower buds, divided into S (6-10 mm), M (11-13 mm) and L (14-17 mm).

These were incubated under 20±2°C, 16 h/day with white fluorescent lamp illumination (about 2,000 Lux) conditions, and then observed for callus formation and organogenesis by external observation at 45 and 100 days after inoculation.

Induced callus was cut and divided into approximately 5-mm squares and inoculated on the same fresh medium with *P. veris* and *P. vulgaris*, but *P. juliae* was inoculated on a different PGR combination (Table 2). At 2 and 4 months after inoculation callus formation and organogenesis were recorded by external observation.

Cultured flower buds developed callus in all species, especially in *P. juliae* which showed vigorous callus formation (Fig. 1). However, a relation between callus amount, size of flower buds and, plant growth regulators combinations was not observed. At 100 days after inoculation, adventitious shoots appeared on callus of *P. juliae*, but only one each on two combination of PGR that contained NAA and BA with 1 or 5 $mg \cdot L^{-1}$ each (Table 1) .

Two months after subculture, callus of *P. juliae* showed a high survival rate and vigorous callus proliferation; however, *P. veris* and *P. vulgaris* showed poor callus proliferation (Table 2). However, an adventitious shoot differentiated on callus of *P. vulgaris* for the first time and also on *P. juliae*.

In this study, flower buds of the three species formed callus with differentiated adventitious shoots on callus of only *P. juliae* and *P. vulgaris*. In conclusion, it was shown that those two species have plant regeneration ability. In the future, if the frequency of adventitious shoot formation on these parent species can be improved, it may be possible to establish a regeneration system for polyanthus and Julian primroses.

Table 1. Effects of plant growth regulators to organogenesis on flower bud culture of *Primula juliae*, *P. veris* and *P. vulgaris*, at 45 and 100 days after inoculation (DAI).

Combination of plant growth regulators (mg/L)	*Primula juliae*					*Primula veris*					*Primula vulgaris*				
	No. of flower buds	Rate of adventitious roots formation (%)		Rate of adventitious shoots formation (%)		No. of flower buds	Rate of adventitious roots formation (%)		Rate of adventitious shoots formation (%)		No. of flower buds	Rate of adventitious roots formation (%)		Rate of adventitious shoots formation (%)	
NAA:BA		45 DAI	100 DAI	45 DAI	100 DAI		45 DAI	100 DAI	45 DAI	100 DAI		45 DAI	100 DAI	45 DAI	100 DAI
0:0	20	0	0	0	0	6	0	0	0	0	14	0	0	0	0
1:1	24	0	20.8	0	4.2	9	0	22.2	0	0	24	0	0	0	0
1:3	25	0	0	0	0	12	8.3	25.0	0	0	25	0	0	0	0
1:5	24	0	4.2	0	0	12	0	8.3	0	0	24	0	0	0	0
5:1	24	8.3	20.8	0	0	12	8.3	8.3	0	0	25	0	34.8	0	0
5:3	23	0	4.4	0	0	11	0	0	0	0	24	0	17.4	0	0
5:5	24	0	0	0	4.2	11	0	0	0	0	24	0	4.2	0	0

Table 2. Effects of plant growth regulators to callus increase and organogenesis on subculture from flower bud culture of *Primula juliae*, *P. veris*, and *P. vulgaris*, at 2 months after subculture.

Combination of NAA:BA (mg/L)		*Primula juliae*					*Primula veris*					*Primula vulgaris*				
		No. of callus segments	Callus		Rate of organogenesis (%)		No. of callus segments	Callus		Rate of organogenesis (%)		No. of callus segments	Callus		Rate of organogenesis (%)	
Primary culture	Sub-culture		Survival rate (%)	Magnification of increase	Adventitious roots	Adventitious shoots		Survival rate (%)	Magnification of increase	Adventitious roots	Adventitious shoots		Survival rate (%)	Magnification of increase	Adventitious roots	Adventitious shoots
1:1	1:1	125	34.7	3.1	2.4	0	19	0	0	0	0	4	0	0	0	0
	2:1	86	60.3	2.9	1.2	0	—	—	—	—	—	—	—	—	—	—
1:2.5	1:3	255	37.3	2.8	1.6	0	30	0	0	0	0	4	25.0	1.0	0	25.0
	1:5	64	32.8	2.9	0	0	—	—	—	—	—	—	—	—	—	—
1:5	1:5	85	51.3	2.9	3.5	2.4	88	2.4	2.5	0	0	5	0	0	0	0
	1:3	199	39.2	2.7	2.5	0.5	—	—	—	—	—	—	—	—	—	—
5:1	5:1	99	66.3	3.6	3.0	0	6	0	0	0	0	58	29.2	2.1	0	0
	5:3	99	50.0	3.1	3.0	0	—	—	—	—	—	—	—	—	—	—
5:2.5	5:3	294	60.7	3.5	3.1	0	1	0	0	0	0	49	7.9	3.0	2.0	0
5:5	5:5	254	39.6	3.4	0.8	0	-	-	-	-	-	53	0	0	0	0

Survival rate (%) = 100 × Number of survival callus/Number of inoculated callus.
Magnification of increase = Volume of callus at 2 monthes after/Volume of callus at inoculation by external observation.
Rate of organogenesis (%) = 100 × Number of callus with organogenesis/Number of inoculated callus.

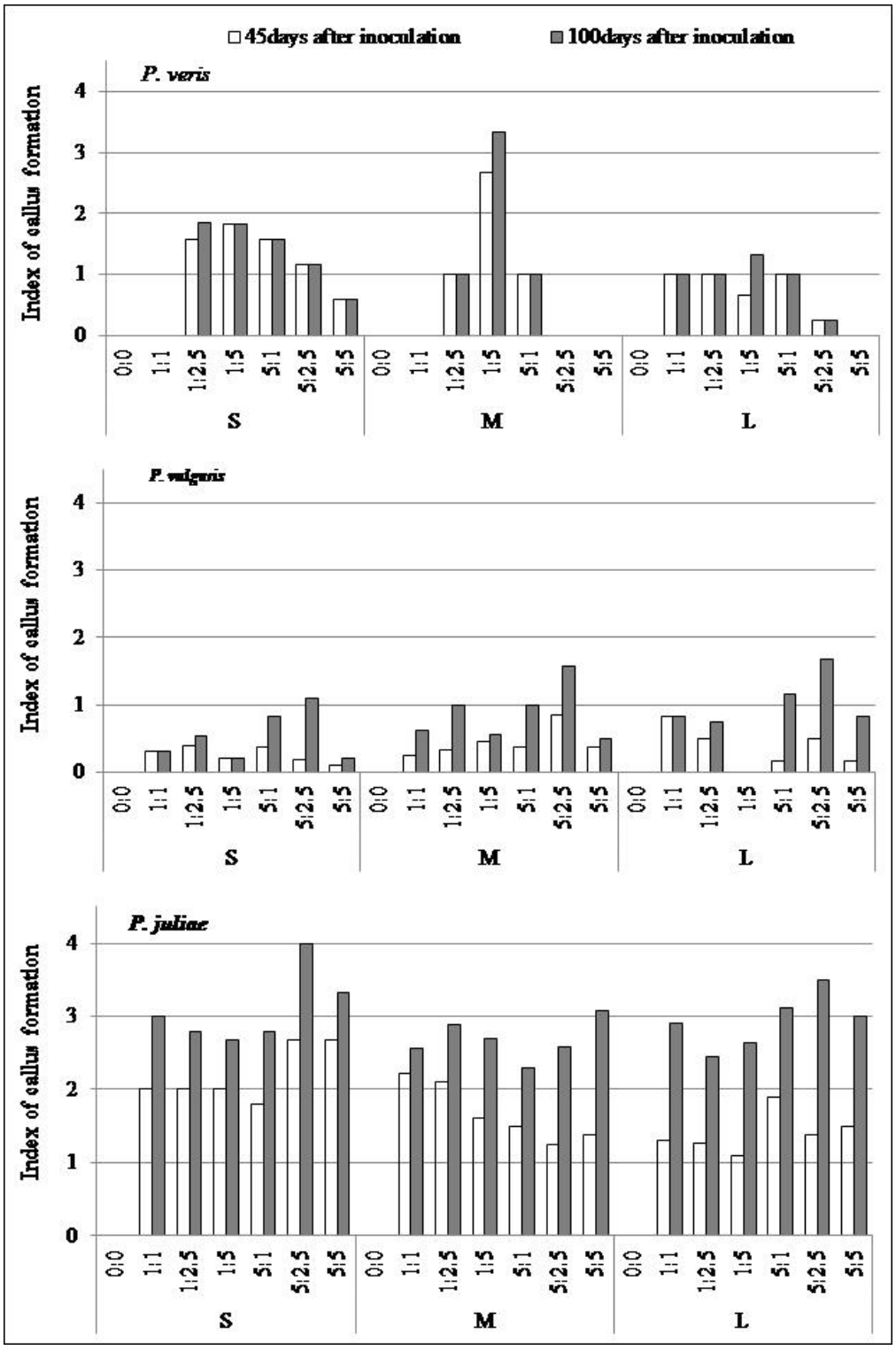

Fig. 1. Callus formation by external observation at 45 and 100 days after inoculation on flower bud culture of *Primula veris*, *P. vulgaris*, and *P. juliae*, each flower bud size. The horizonal axis shows combinations of 1-naphthylacetic acid and 6-benzylaminopurine combination ($mg \cdot L^{-1}$) and flower bud sizes were S (6-10 mm), M (11-13 mm) and L (14-17 mm). The vertical axis shows average callus formation index by external observation, valued 0 to 4.

Literature Cited

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473-479.