In vitro shoots formation by inflorescence apex culture of *Primula ×polyantha*©

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INTRODUCTION

Primula ×polyantha hort. selections are important pot flowering plants in Japan, however, homogeneous seed production is difficult on account of cross-fertilization (allogamous) plant. On the other hand, commercial vegetative propagation is also not possible because of low reproduction rates. Micropropagation is also difficult. In shoot apex culture, contamination occurs frequently because the shoot apexes occur close to the surface level of the soil.

In primulas, adventitious shoots were obtained by the flower-bud culture. We tried flower-bud culture, using *P. veris* L., *P. vulgaris* Hudson, and *P. juliae* Kusnetsow and obtained a few adventitious shoots from only *P. vulgaris* and *P. juliae* (Matsumoto and Ohashi, 2014).

In primulas, the inflorescence is the only elongated stem and those are an indefinite inflorescence; there is an apical meristem in the apex. Actually, a bud is formed on the tip of the inflorescence after flowering, in primulas such as *P. malacoides* franchet, Bull., *P. obconica* Hance, *P. sinensis* Sabine ex Lindley, and *P. modesta* Bisset & Moore.

In the present study, we tried in vitro shoots formation by inflorescence apex culture of *P. ×polyantha*.

MATERIALS, METHODS AND RESULTS

Inflorescence elongation instruction experiment

The selections of *P. ×polyantha* are distributed between the following three types by inflorescence elongation.

1) Polyanthus Type (PT): elongated both inflorescence and pedicel in flowering
2) Acaulis Type (AT): non-elongated inflorescence and elongated pedicel in flowering
3) Sham Acaulis Type (SAT): elongated inflorescence by temperature conditions

First, we examined the inflorescence elongation induction condition because the important cultivars of *P. ×polyantha* are distributed in the AT or SAT elongation types.

The six plug seedling forms of *P. ×polyantha* "Claudia" were purchased from Sakata Seed Corporation in the autumn of 2011 and 2012. These were planted in plastic pots (diameter 12 cm) containing a mix of equal parts of bark compost and pumice for gardening called "kanuma" soil, and cultured on bottom water supply trays in a greenhouse, and treated with gibberellin water solution mist from the flower bud appearing stage. Gibberellin Meiji (Meiji Seika Pharma Co., Ltd., Japan) was used as the gibberellin, and the concentration of water solutions were 50 mg L^{-1} in 2012 and 100 mg L^{-1} in 2013. The treatments were carried out monthly in 2012 and every 2 weeks in 2013, spraying 1, 3, or 5 times per plant (Tables 1 and 2) to the cluster of flower buds.

Table 1. Amount of gibberellin (mg $plant^{-1}$) in mist treatment to *Primula ×polyantha.*

Year	Gibberellin concentration (mg L^{-1})	1 spray	3 spray	5 spray
2012	50	0.031	0.039	0.156
2013	100	0.062	0.187	0.311

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Table 2. Day of carried out gibberellin mist treatment to *Primula* × *polyantha*.

Year	Cycle of spray treatments	Day of carried out treatment				
2012	Every 1 month	January 4	January 19	February 25	May 20	April 20
2013	Every 2 weeks	December 25, 2012	January 7	January 23	February 5	February 19

The length of elongated inflorescences more than 5 mm were measured at the treatments carried out after second treatments.

Figure 1 shows the results of the 2012 gibberellin treatment experiment. Most of elongated inflorescences were not observed on 19 January and 25 February, then, observed all treatments including control on 20 March. It is thought that the elongation of inflorescence was caused by the rises in temperature not an effect of gibberellin and "Claudia" forms are SAT types.

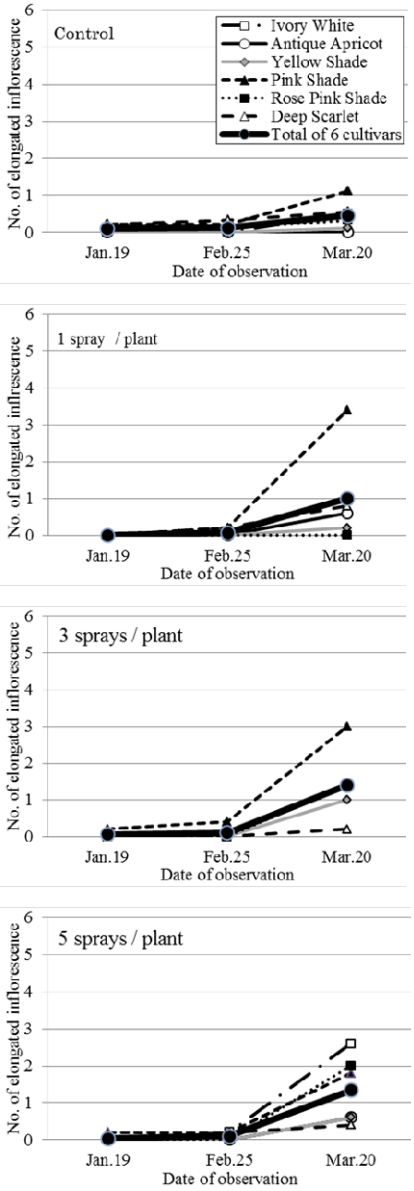

Figure 1. Effect of gibberellin mist treatments on inflorescence elongation in *Primula* ×*polyantha* (2012).

However, Figure 2 shows the results of the 2013 experiment, in which most of the elongated inflorescences were observed on the treated plants and not control plants on 19 February. It is thought that the elongation of inflorescence was caused by an effect of gibberellin, and increased on 8 March; observed elongated inflorescences per plant were 2.75-2.95 on average.

It will be necessary to investigate the following things in the future, optimum concentration point and interval of gibberellin treatments, and plant type difference.

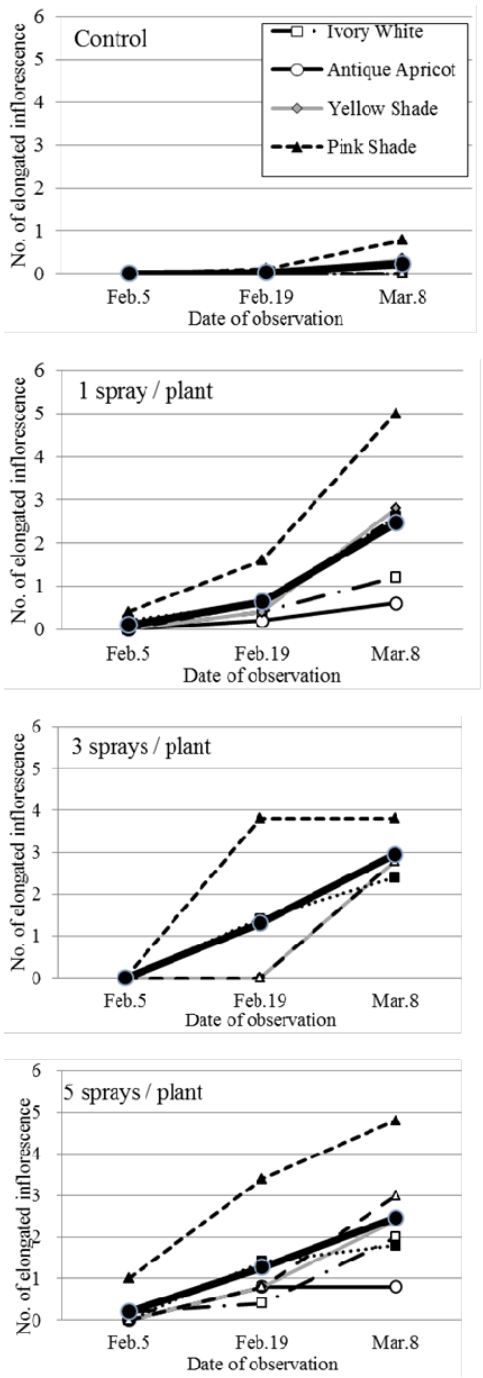

Figure 2. Effect of gibberellin mist treatments on inflorescence elongation in *Primula* ×*polyantha* (2013).

Inflorescence apex culture

Basal medium for inflorescence apex culture was MS medium (Murashige and Skoog, 1962) supplemented with 30 g L^{-1} sucrose and 1, 2, or 4 mg L^{-1} of 6-benzylaminopurine (BA) alone and in combination with 0.1 mg L^{-1} 1-naphthylacetic acid (NAA) as plant growth regulators (Table 3). The pH were adjusted to 5.8±0.1 and 2.5 g L^{-1} gellan gum (Wako pure Chemical Industries, Ltd., Japan) was added before dispensing 10 mL per test tube (25 mm diameters; 120 mm height).

The harvested elongated inflorescences consisting of flowers and flower buds were dipped in sodium hypochlorite solution (1% available chlorine) for about 8 minutes and rinsed with sterilized water. The inflorescence apexes were removed and put and placed one per testtube on each medium.

These were incubated under 20±2°C and16 h per day white fluorescent lamp illumination (about 2,000 Lux) condition, and then observed for 45 and 90 days after inoculation for shoot formation.

After 90 days the rate of contaminated explants was under 10% in spite of the simple method of surface sterilization. By addition of NAA, the rates of explant survival rose, and the rates of shoot formed on explants was 50%, and we obtained 1.8 shoots per inflorescence apex (Table 4).

Table 3. Combination of plant growth regulators for inflorescence apex culture of *Primula* ×*polyantha*.

		BA (mg L^{-1})		
		1	2	4
NAA (mg L^{-1})	0	O	O	O
	0.1	-	O	-

NAA = 1-Naphthylacetic acid, BA = 6-Benzylaminopurine, O = added grow regulator.

Table 4. Contamination rate and effect of plant growth regulators for callus and shoot formation in inflorescence apex culture of *Primula* ×*polyantha*.

Combination of plant growth regulators		Explants (no.)	Non contami-nated explants (no.)	Contamination rate (%)	Surviving explants (no.)	Rate of survival explants (%)	Rate of callus formed explants (%)	Amount of callus per explant	Rate of shoot formed explants (%)	No. of shoots per explant
NAA (mg L^{-1})	BA (mg L^{-1})									
0.1	2	48	44	8.3	30	68.2	65.9	1.5	50.0	1.8
0	1	49	45	8.2	12	26.7	4.4	0.1	20.0	0.7
0	2	51	47	7.8	13	27.7	4.3	0.1	14.9	0.6
0	4	52	50	3.8	9	18.0	2.0	0.0	10.0	0.3

CONCLUSIONS

In this study, we understood that we could lengthen an inflorescence by gibberellin treatment, and could obtain shoots by the inflorescence apex culture at a high rate. It will be necessary to define more closely the optimum point about the above points. In that case, it may be possible to perform the micropropagation of selected primula polyanthus plants.

Literature cited

Matsumoto, Y., and Ohashi, H. (2014). Adventitious shoots formation by flower bud culture of *Primula veris, Primula vulgaris*, and *Primula juliae.* Comb. Proc. Intl. Plant Prop. Soc. *64*, 383–387.

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. *15* (*3*), 473–497 http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x.