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Breeding of Tetraploid in *Platycodon grandiflorum* (Jacq.) A. DC. by Colchicine treatment

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ABSTRACT

Present studies were carried out to produce tetraploid plants by colchicine treatment using seeds, seedlings and shoot tips of *Platycodon grandiflorum* in Campanulaceae. The most successful colchicine treatment for tetraploid production in *P. grandiflorum* was soaking treatment using 0.01 and 0.5% colchicine solution for 1 hour and 12 hours, respectively. Morphological characteristics of both diploid and tetraploid were similar, but tetraploid plants had more leaves. Compared to diploid, tetraploid had the larger stomata, but less number of stomata. Fresh weight of tetraploids was 20~40% heavier than that of diploid.

Key words **:** colchicine, chromosome, stomata, root characteristic

INTRODUCTION

The family *Campanulaceae* encompasses many edible native species; 14 in the genus *Adenophora*, 6 in *Campanula*, and 3 in *Codonopsis* (Chung, 1955).

The plants of *Platycodon grandiflorum* (Jacq.) A. DC. are perennial with enlarged roots. They grow up to 40~100 cm high and leaves are oval-shaped with serrated perimeter. When wounded, plants excrete milky white liquid because they have laticifer. Single or several purple or white flowers bloom at the tips of main stems during July and August (Chung, 1955; Galston *et al.*, 1963). Like *Codonopsis lanceolata* mentioned in other experiment, underground tuberous roots are used for vegetable or medicinal purposes. They are known to remedy or alleviate symptoms of hemolysis, cough, phlegm, and fever, due to the presence of saponin, inulin, phytosterin, and platycodinin in the roots (Lee, 1974; Takaki and Lee, 1972). Although, presently, a great deal of roots of *P. grandiflorum* is consumed for root vegetables, studies on cultivation methods and varietal improvement are not many. The good soil for cultivation is clay loam or compost-rich sand loam (Lee, 1974). Various kinds of fertilizers are used in farms, due to lack of standard fertilization for the species, but usually chicken excrements and soybean mills are given as fertilizers. It was shown that nitrogen fertilization influences root

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growth of *P. grandiflorum* most profoundly, followed by phosphorus and potassium (Kuraita, 1974). Seeds can be sown on spring of fall (Chung, 1960), and use of herbicide is recommended in case of direct sowing (Lee and Cho, 1980). Transplanting seedlings 5~6 months after sowing is also desirable because it is better method in terms of weed control and seedling management (Lee, 1974). Cho (1984) demonstrated that 76% of total growth was obtained at the time of budding, and 79% of total root length and 58% of root diameter were obtained during the first year.

Pharmaceutical constituents of this species have been identified as triterpenoid saponins (Kubota *et al.*, 1969; Akiyama *et al.*, 1972) and Ishii *et al.* (1984) isolated 17 new saponins. Recently, studies were done on sterol (Chung, 1985), on general components (Cho, 1988), on saponin contents (Cho and Chang, 1989), on flavor components (Chung *et al.*, 1987) and on platycodinin D (Kim *et al.*, 1990). Chung *et al.* (1997a, 1997b) showed that number of years of cultivation made differences in the contents of pharmaceutical constituents and microelements, but not in the general components, inorganic substances and fatty acids.

It would be very desirable to have higher contents of pharmaceutical constituents as well as higher yield in this species. One of the ways to achieve this goal is production of tetraploid plants that were known to have bigger vegetative organs. So present studies were conducted to obtain tetraploidy in *P. grandiflorum* by colchicine treatment. Optimum concentration and materials for colchicines induction were investigated, and morphology and yield of resulting tetraploid plants were also examined.

MATERIALS AND METHODS

Seeds, seedlings, and adult plants of *P. grandiflorum* were treated with different concentrations of colchicines. In seed treatment, 2 sheets of filter papers were placed in 9 cm petri-dish, and 20ml of 0, 0.01, 0.1, or 0.5% colchicine solution were added to each dish. Then 100 seeds were placed on the each filter paper. Duration of the treatment were 1, 3, 6, or 12 hours, and each treatment has done with 3 replications. After the treatment, seeds were washed 3~4 times with sterile water, planted on horticultural soil mix, and kept in a greenhouse. When cotyledons emerged, germination rates were examined, and then seedlings were transferred to 12cm plastic pots with equal parts of perlite and coarse sand. To verify ploid levels of treated plants, root tips from each treatment were excised at the time of main leaf appearance. Then tips of about 1cm long were stored in 0.05% colchicine solution for 3 hours, and then fixed in the solution of 3 parts ethanol and 1 part acetic acid for 1 day. Next, the tips were hydrolyzed with 1 N HCl solution at 60°C, stained with 1% lacto-propionic-orcein solution and examined under light microscope within 48 hours. Measurement of stomates, and morphological characters were made 6 months after transplanting.

Colchicine treatment on seedlings were performed at the time of cotyledon emergence. Concentrations and duration of treatments were same as in seed treatment. After the treatment, seedlings were washed 3~4 times with sterile water and planted on 12 cm pots containing mix of equal part of perlite and coarse sand. Survival rate and chromosome numbers were counted 30 days after transplanting. After 4 months, measurement of stomates and morphological characters were performed. For chromosome doubling in adult plants, growing points were covered with cotton balls and sprayed with various concentration of colchicines solutions 3 times a day for 3 days. Concentrations and duration of treatment were same as earlier experiments. Chromosome numbers, stomata size, and morphological characters were investigated 30 days after colchicine treatment.

RESULTS AND DISCUSSION

Effects of colchicine on the seed germination of *P. grandifolium* were listed in the Table 1. Germination rate decreased at high concentration and by long soaking time. Especially, the highest (0.5%) and longest (12 hours) treatment resulted in only 1.3% survival rate. In spite of all the efforts, it was not possible to obtain tetraploids with seed treatment.

Colchicine treatment on seedlings reduced survival rate significantly (Table 2), indicating severe physiological toxicities of the colchicine. However, 2 tetraploid plants were obtained in this experiment: one by 0.5% for 1 hour and the other by 0.01% for 12 hours. Chromosome number of these plants is 36, while untreated plants it is 18 (Fig.1), indicating tetraploidy of

Table 1. Effect of colchicine on seed germination

Colchicine (%)	Soaking time (hrs.)	No. of seeds treated	No. of seeds germinated	% germination
Control		300	192	64.0 a[z]
0.01	1	300	129	43.0 b
0.05		300	115	38.3 c
0.1		300	95	31.7 d
0.5		300	50	16.7 fg
0.01	3	300	118	39.3 c
0.05		300	84	28.0 e
0.1		300	56	18.7 f
0.5		300	30	10.0 h
0.01	6	300	113	37.7 c
0.05		300	43	14.3 g
0.1		300	27	9.0 hi
0.5		300	29	9.7 h
0.01	12	300	86	28.7 e
0.05		300	23	7.7 hi
0.1		300	20	6.7 i
0.5		300	4	1.3 j

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

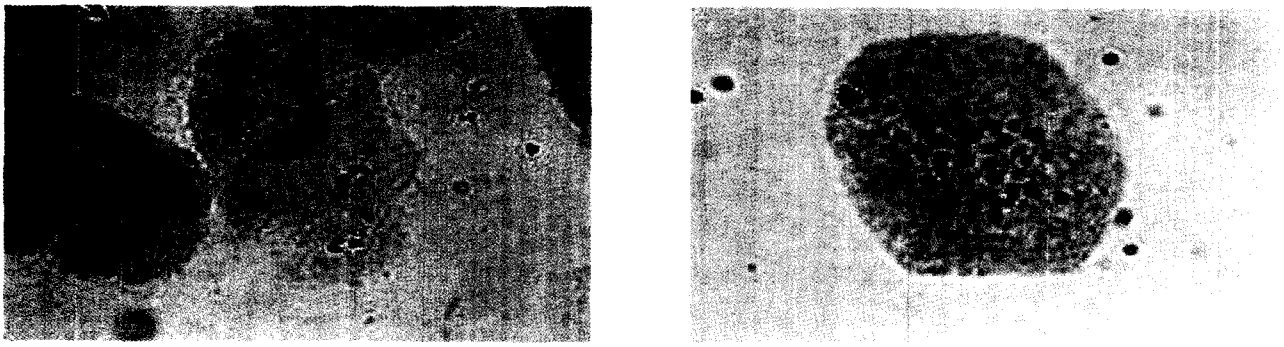

Fig. 1. Comparison of chromosome number between diploid (left, 2n=2x=18) and tetraploid (right, 2n=4x=36) plants.

Table 2. Effect of colchicine on survival rate of seedling

Colchicine (%)	Soaking time (hrs)	No. of seedling treated	No. of seedlings survived	% survivals
0.01	1	300	140	46.7 a[z]
0.05		300	95	31.7 b
0.1		300	85	28.3 bc
0.5		300	80	26.7 bcd
0.01	3	300	90	30.0 bc
0.05		300	95	31.7 b
0.1		300	80	26.7 bcd
0.5		300	70	23.3 cde
0.01	6	300	70	23.3 cde
0.05		300	60	20.0 def
0.1		300	55	18.3 ef
0.5		300	40	13.3 fg
0.01	12	300	60	20.0 def
0.05		300	55	18.3 ef
0.1		300	40	13.3 fg
0.5		300	25	8.3 g

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

Table 3. Comparison of growth characteristics between diploid and tetraploid plants

Ploidy	Line code	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	No. of leaves	Stem diam. (cm)
Diploid	21.7	5.4	2.9	20.3	1.9	
Tetraploid	Line A	22.5	5.3	3.0	33.0	2.4
	Line B	15.5	4.6	3.0	21.0	1.9

these plants.

Colchicine spray on adult plants resulted in death of growing points and subsequently did not produce tetraploid plants.

The comparison of growth characters between diploid and tetraploids are shown in Table 3. There were not much differences in growth characters except number of leaves in which one tetraploid plant had 33 leaves, compared to 20.3 of diploid. Tetraploid plants are known to show gigantism with thicker, longer stems, and larger leaves and flowers (Cockerham and Galletta, 1976; Lapins, 1975), but it was not observed in this experiment. To obtain reliable data on morphological traits, we definitely need more number of tetraploid plants.

The size and number of stomata is listed in Table 4. As reported in case of lettuce (Eenink and Alvarez, 1975), tetraploid plants tended to have larger stomata (Fig. 2), which indicated that stomata size could be used to identify tetraploidy (Wenzel and Foroughi-Wehr, 1984). Number of stomates per unit area was lower in tetraploid than in diploid. Still, however, the number of

Table 4. Size and number of stomata in diploid and tetraploid plants

Ploidy	Line code	Stomata size		No. of stomata /mm²
		Length (㎛)	Width (㎛)	
Diploid		21.7	13.9	387
Tetraploid	Line A	30.2	19.0	191
	Line B	25.9	15.8	375

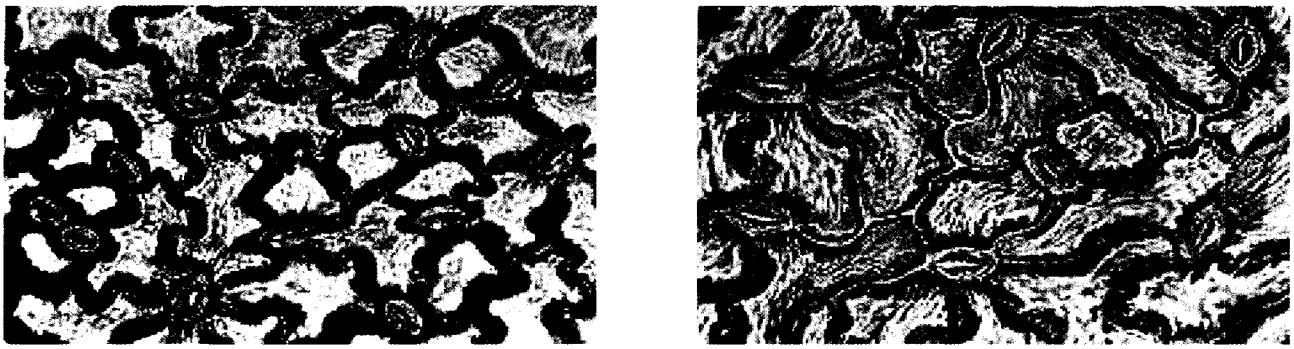

Fig. 2. Stomata of diploid (left) and tetraploid (right) plants

Table 5. Root characteristics of diploid and tetraploid plants.

Ploidy	Line code	Length (cm)	Diameter (cm)	Fresh wt. (g)
Diploid		6.1	1.0	4.2
Tetraploid	Line A	11.5	1.2	5.2
	Line B	11.0	1.0	5.9

a. Line A b. Line B

Fig. 3. Roots of diploid (left) and tetraploid (right) plants.

tetraploid plants is too low to get any solid information out of this experiment.

Table 5 shows that tetraploid plants had much longer roots than diploid. Root diameter was not different significantly, compared to diploid. Fresh weight was higher in tetraploid; 24~40% increase compared to diploid (Fig. 3). Similar results were reported by Han (1962) in case of radish. It can be said that tetraploid

plants might be used for better yielding *P. grandiflorum.*

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