Breeding of Tetraploid in *Codonopsis lanceolata* (Sieb. et Zucc.) Trautvetter 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 *Codonopsis lanceolata*. Three tetraploid plants of *C. lanceolata* were produced from seeds which absorbed 0.1% colchicine solution for 12 hours, and 0.5% colchicine solution for 1 and 6 hours from seedlings, respectively. But tetraploid was not produced from shoot tips treated by colchicine solution. Compared to diploid, tetraploid plants had larger stomata, but less number of stomata. Fresh weight of tetraploid plants was 1.4~3.6 times heavier than diploid plants.

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

INTRODUCTION

Many species belonging to *Campanulaceae* family are used as root vegetables, for example, 14 species in genus *Adenophora*, 6 in *Campanula* and 3 in Codonopsis (Chung, 1955). Among these species, *Codonopsis lanceolata* (Sieb. et Zucc.) Trautvetter is distributed in Korea, Japan, and China. *C. lanceolata* plants, perennial vines, are growing under half-shade, and reach height of 2~3m. Purple, bell-shaped flowers bloom during August and September on panicles, and enlarged roots of this species are called tuberous roots (Lee, 1982). Roots contain many constituents such as saponin, vitamin B1 and B2, which are known to have beneficial effects on fatigue, blood pressure, lung cancer and cough (Moon, 1984).

Plants of *C. lanceolata* have been used traditionally as medicinal purpose or vegetables in Korea, but still wild plants are domesticated for the commercial cultivation. Though cultivation acreage is increasing, due to its cash crop value, the supply has not met the demand.

Therefore, improvements on cultivation methods, varieties and utilization are urgently needed. This species is vine type, so support system is needed for the cultivation. Mass production of roots is not possible without mechanization, which requires breeding of no support plants.

Kim and Lee (1979) reported that direct sowing resulted in higher yield than transplanting methods, and

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Kim (1985) demonstrated that in case of transplanting methods, larger seedlings produced better root growth, and consequently higher yields. Transplanting is recommended to be done on April with planting density of 60 × 10 cm (Chung and Cho, 1983).

Recommended fertilization for good root growth is 6kg of N, P, and K, respectively (Cho, 1984). Enlarged root growth was obtained with well-aerated (Russell, 1973), and compost-rich soil such as sandy loam (Lee, 1991). It was reported that spray of 10 mg · L^{-1} of growth regulators, uniconazol and paclobutrazol at the time of 12th node emergence, increased yield of *C. lanceolata* (Kim *et al.*, 1997).

As far as chemical components are concerned, 2 or 3 year old roots have financial advantage over 4 or 5 year old ones, because root growth is better during first 2 or 3 years. Wild plant roots have higher sugar contents than cultivated ones (Kim, 1985), but saponin content was same (Lee, 1984). Sugar contents increased as the years of cultivation of roots increased (Park *et al.*, 1985). Screen test of cell toxicity with roots collected at 14 regions in Korea revealed that there were no regional differences (Kim *et al.*, 1998). Present studies were carried out to induce tetraploid plants by colchicine treatment. Optimum concentrations for colchicine treatment were examined and morphology and yield of obtained tetraploids were also investigated.

MATERIALS AND METHODS

Seeds, seedlings, and adult plants of *C. lanceolata* 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 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℃, 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 treatment were same as 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 made. To induce polyploids 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 colchicines on the seed germination of *C. lanceolata* were listed in Table 1. Germination rate decreased at higher concentration and longer soaking time. Especially, no germination was observed at the concentration of 0.5% for 6 or 12 hours. One tetraploid

Table 1. Effect of colchicine on chromosome doubling and seed germination

Colchicine (%)	Soaking time (hrs.)	No. of seeds treated	No. of seeds germinated	% germination
Control		300	185	61.7 a[z]
0.01	1	300	163	54.3 b
0.05		300	138	46.0 c
0.1		300	57	19.0 g
0.5		300	38	12.7 h
0.01	3	300	126	42.0 d
0.05		300	89	29.7 f
0.1		300	20	6.7 i
0.5		300	6	2.0 jk
0.01	6	300	102	34.0 e
0.05		300	38	12.7 h
0.1		300	14	4.7 ij
0.5		300	0	0.0 k
0.01	12	300	98	32.7 e
0.05		300	21	7.0 i
0.1		300	11	3.7 j
0.5		300	0	0.0 k

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

plant was obtained with concentration of 0.1% for 12 hour treatment. Chromosome number was 32 while diploid was 16, indicating true tetraploid (Fig.1).

Table 2 showed the survival rate of seedlings by colchicine treatment. As in seed treatment, survival rate decreased with the increasing concentration and duration of treatment. Particularly, longest treatment, 12 hours, reduced the rate by 50%, at all concentration, except 0.01%, compared with control. Two tetraploid plants were obtained at the concentration of 0.5% for 1 and 6 hours.

No plant was obtained by colchicine treatment on the growing points of adult plants.

Table 3 showed the comparison growth characters

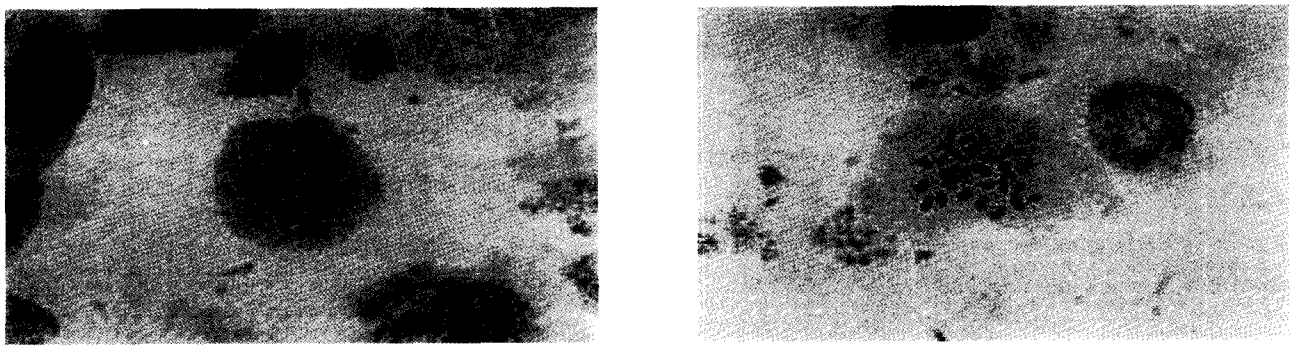

Fig. 1. Comparison of chromosome number between diploid (left, 2n=2x=16) and tetraploid (right, 2n=4x=32) plants.

Table 2. Chromosome doubling and survival rate of seedling as influenced by colchicine soaking treatment

Colchicine (%)	Soaking time (hrs.)	No. of seedling treated	No. of seedlings survived	% survivals
0.01	1	300	200	66.7 a[z]
0.05		300	195	65.0 ab
0.1		300	175	58.3 abc
0.5		300	165	55.0 bcd
0.01	3	300	170	56.7 bc
0.05		300	155	51.7 cd
0.1		300	155	51.7 cd
0.5		300	135	45.0 def
0.01	6	300	165	55.0 bcd
0.05		300	155	51.7 cd
0.1		300	120	40.0 efg
0.5		300	110	36.7 fg
0.01	12	300	145	48.3 cde
0.05		300	115	38.3 fg
0.1		300	105	35.0 fg
0.5		300	95	31.7 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 diameter (cm)
Diploid		33.5	5.0	3.0	26.1	1.4
Tetraploid	Line A	50.0	6.2	3.5	43.0	2.8
	Line B	24.0	4.2	2.4	32.0	1.5
	Line C	35.3	5.2	3.0	38.0	2.2

between diploid and tetraploid. Plant height was 33.5 cm in the former, but latter showed some variation. As in earlier experiment done by same authors, vigorous growth or gigantism was not observed. Generally, tetraploid plant grow more vigorously than diploid plants (Tan and Dunn, 1973: Esen *et al.*, 1978). Other growth characters, except number of leaves, showed no difference between diploid and tetraploid.

The size and number of stomata is shown in Table 4. The size was larger in tetraploid (Fig. 2), as observed in mulberry tree (Park, 1984) and lettuce (Eenink and Alvarez, 1975). Number of stomata was lower in tetraploid than diploid.

Measurements of root characters were shown in Table 5, and Fig.3. Fresh weight of tetraploid was increased by 1.4~3.6 folds, compared to diploid, and root length and width was also increased. In conclusion we have obtained tetraploid *C. lanceolata* successfully, and these tetraploid, due to its increased fresh weight, are expected to be used for materials for high yielding

Table 4. Comparison of stomata size between diploid and tetraploid plants

Ploidy	Line code	Stomata		No. of stomata / mm²
		Length (㎛)	Width (㎛)	
Diploid		20.6	15.3	264
Tetraploid	Line A	24.5	16.5	210
	Line B	25.5	18.0	187
	Line C	41.0	29.8	73

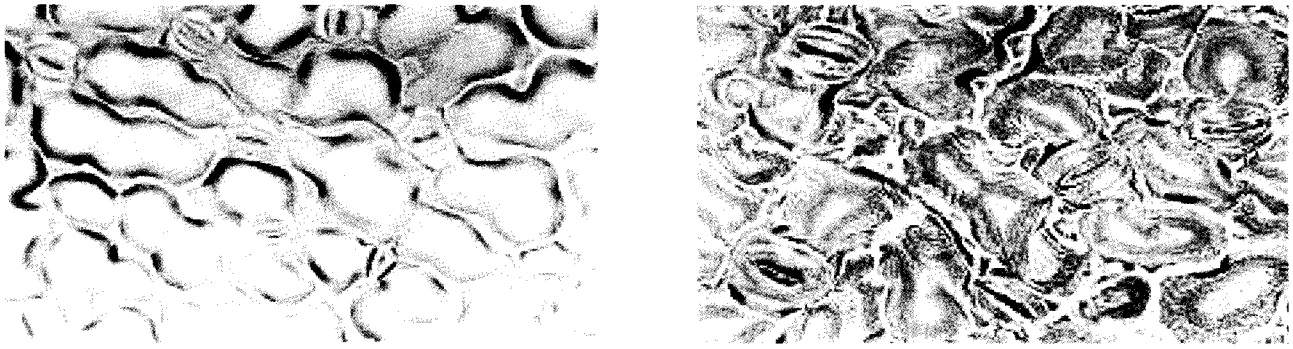

Fig. 2. Comparison of stomata size between diploid (left) and tetraploid (right) plants

Table 5. Comparison of root characteristics between diploid and tetraploid plants

Ploidy	Line code	Length (cm)	Diameter (cm)	Fresh wt. (g)
Diploid		5.7	0.8	2.4
Tetraploid	Line A	7.8	1.2	4.7
	Line B	7.5	1.2	3.3
	Line C	11.5	1.7	8.6

a. Line A b. Line B c. Line C

Fig. 3. Comparison of root characteristics between diploid (left) and tetraploid (right) plants.

crop in a future.

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