Effect of Colchicine on Chromosome Doubling in *Codonopsis lanceolata*

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Abstract - The present study was performed to investigate the effects of the colchicine concentrations on chromosome doubling for producing of tetraploid plants of *Codonopsis lanceolata*, and its effect on plant morphology. A total of 180 individuals germinated from 16 treatment groups, were exposed to various concentrations (0.05-1.0% w/v) of colchicine for different soaking duration (3-24 hour). The highest numbers of tetraploid plants (3) were observed from the lowest concentration of colchicine (0.05%), and one (1) tetraploid plant was obtained from the 0.5% concentration group with a 6 hour treatment. However, no tetraploid individual was observed in any other treatment groups. The plant height of the diploid (18.1 cm) was slightly shorter than that of the tetraploid (13.4 cm). The fresh weight of the main root in the diploid (0.5 g) was four-fold higher than the tetraploid (2.2 g). The colchicine-treated plant regeneration rate in *C. lanceolata* was decreased when the plants were subjected to high concentration of colchicine. In particular, the highest number of tetraploid plants (5 and 3) was obtained from the lower concentration (0.05% and 0.1%) of colchicine for 6-hour treatment, which were a higher rate (29.4% and 30%) of regenerated tetraploid plants than other regenerated plants. As in the seed treatment result, the plant height of the diploid was significantly higher (10.4 cm) than tetraploid. The higher morphological changes were observed comparatively from tetraploid plants than the diploid.

Key words - Concentration, Soaking duration, Explant, DNA content, Morphological changes

Introduction

*Codonopsis lanceolata* is a perennial creeper of the Campanulaceae, that is used for food or medicine. The root of *C. lanceolata* contains substances such as saponin, vitamin, B1, B2, and inulin, which are reported to have effects on vigor, cough, and blood pressure (Moon, 1984) and also used as a crude drug substitute for ginseng (Chang et al., 1987). Recently, functional substances of medicinal plants have been attained increasing attention as a result of economic development and health-oriented lifestyle, but research on breeding and cultivation method to increase yields is still limited.

Polyploid organisms have multiplied sets of the same genomes, and in comparison to diploids, there is no qualitative change in genetic information, but, because of the quantitative increase of genetic materials, formative and physiological characters of the plant change (Kwon et al., 2013; Lapin, 1975). Artificial induction of tetraploid plants is generally achieved based on colchicine concentration, treatment time, and treatment method (Kim et al., 2003; Suh, 2005).

Colchicine is an alkaloid (phytotoxic) component contained in the seed or bulb or *Colchicum autumnale*, which is a plant of the Liliaceae, and, when united with tubulin during mitosis of a plant. It suppresses formation of spindle fibers and, in the middle stage of cell division, interferes with chromosome movement to poles and microtubules formation, inducing...
polyplloidization of chromosomes (Hadlaczky et al., 1983). In addition, colchicine has been used to good effects in numerous plants that have been reported earlier (Hancock, 1997). The effect of colchicine for in vitro chromosome doubling is different regarding to its concentration, method and duration of treatment, and also genetic factors of the treated plants (Aina et al., 2012; Dhooghe et al., 2011; Moghbelt et al., 2015).

In general, tetraploid plants have larger organs or stems (Cockerham and Galletta, 1976; Lapins, 1975) with thicker and longer stems and larger leaves and flowers. As a result of polyploidization, contents of secondary metabolites such as saccharide in sugar canes, vitamin C in tomato and apple, and nicotine in tobacco leaf, change, and, sometimes, physiological characteristics such as virus resistance in radish (Hahn, 1969) and freezing resistance in mulberry tree (Park, 1994) have attempted to improve prominently.

Polyploidy provides the opportunity for selection to sculpt a variety of new gene functions, traits, and lineages and polyploidy may increase the amounts of secondary metabolites (Thao et al., 2003) in medicinal plants of which functional compounds are accumulated in the vegetative parts of plants (Gao et al., 1996; Nilanthi et al., 2009; Young et al., 2015). So polyploid breeding is an effective approach of germplasm development for medicinal plants. Previous study focused on doubling the chromosome number of diploid as well as haploid plants, and the regenerated tetraploid plants showed a pronounced growth characteristic when the plants were treated with colchicine (Nilanthi et al., 2009). Polyploids may also have enhanced ornamental characteristics in some cases (Kehr, 1996) and tetraploid plants exhibited significant differences regarding morphological and cytological alteration in Platycodon grandiflorus when the plants were exposed to colchicine treatment (Wu et al., 2011).

To the best of our knowledge, the effects of colchicine on chromosome double have not been performed in Codonopsis lanceolata, previously. In the present study, successful induction of tetraploid plants of C. lanceolata was reported and focused on potential methodology for the efficient recovery of such polyploid plants through the in vitro application of colchicine. As well as establishing the ploidy of these plants, the growth habit and morphological characteristics of the tetraploids were examined with the ultimate aim of improving productivity and breeding of C. lanceolata.

**Materials & Methods**

**Plant growth condition and colchicine treatment**

Seeds of C. lanceolata grown in Jeju and harvested in 2015 were sown in in vitro sterile environment, and an explant from the cultivated plant was used as an experimental material. For seed treatment, 20 ml of 0, 0.05, 0.01, 0.5, and 1.0% colchicine aqueous solutions were put in 9 cm-diameter petri dishes, on which two sheets of filter paper were placed each, and 50 grains of seeds were soaked in them. To accelerate germination, they were left for 3, 6, 12 and 24 hours at 5℃. The treatment was repeated three times each.

After the soaking treatment, each seed was cleaned three to four times by using sterilized water, and sown in ridging for which vermiculite and peat moss were mixed at 1:1 ratio before forcing the germination in a 25℃ thermostatic chamber. Germination was examined when a cotyledon appeared, and, when 6 or more foliage leaves appeared, the leaves were collected to check polyploidy. Also, for colchicine treatment of the in vitro embryoid, explants (1 cm) of C. lanceolata were soaked in 40 ml of 0.01, 0.05, and 0.1% colchicine aqueous solutions in 100 ml beakers for 1, 6, and 12 hours. After the soaking treatment, each explant was cleaned with sterilized water three to four times, and plated in the MS medium. The treatment was repeated nine times each. For cultivation, at 25±1℃, 40 μmol·m⁻²·s⁻¹ light intensity was applied for 16 hours.

**Determination of number of chromosomes**

About 1 cm of the root apex of milk white color in fresh, of C. lanceolata was collected and pre-treated with 0.05% colchicine solution for two to three hours at room temperature, before fixing it with 95% ethanol and glacial acetic acid mixed solution (3:1) in a refrigerator. The sample was soaked again in 1N HCl solution, and after hydrolysis in a water tank, it was adjusted to 60℃ with RPM 120. Then, it was soaked in 2% aceto-orcein solution before examining the number of chromosomes by using an optical microscope.
Effect of Colchicine on Chromosome Doubling in *Codonopsis lanceolata*

DNA content analysis by using flow cytometry

The leaves of each treatment group were cut around 0.5×0.5 cm in size, and after adding HR-A liquid (Patec Ltd, Germany), the tissues were mashed to extract DNA. With this solution, HR-B liquid (Patec Ltd, Germany) was added for dyeing. And then, the DNA content was examined by using flow cytometry (Patec PA-1, Germany), and polyploidy was determined based on the result.

Statistical analysis

Using the SAS program (SAS, 9.2, Institute Inc, USA), statistical analysis was conducted by Duncan’s multiple range test (p=0.05).

Results & Discussion

Influence of concentration of colchicine on chromosome doubling and seed germination

The effects of various concentrations of colchicine and its treatment duration on germination and induction of tetraploid *C. lanceolata* are presented in Table 1. The germination rate decreased at the high concentration of colchicine with longer treatment duration. In particular, after soaking treatment for 12 hours and 24 hours, there was no germination in 0.1% and higher concentrations groups. A total of 180 individuals was germinated from 16 treatment groups. The highest number of tetraploids (3) was observed from the concentration of 0.05% and one (1) tetraploid individual was obtained from the 0.5% concentration treatment group. However, no tetraploid individual was observed in any other treatment groups. In addition, even with oryzalin treatment, which is known to multiply chromosomes, no tetraploid individual was observed in any treatment group (data not shown). To examine the multiplication of DNA content in leaves, flow cytometry was used for fixing gain at 535. In the result, the DNA content peak in the diploid of G1 phase was 98.7, whereas the DNA content peak of the tetraploid was 182.4 (Fig. 2). Kim *et al.* (2003) suggested that the concentration of 0.01% and 0.05% colchicine treatments for 1 hour and 12 hours are suitable for obtaining the tetraploid in *Platycodon grandiflorum*. In addition, previous investigation demonstrated that the highest induction rate (82.2%) was occurred with the concentration

### Table 1. Effect of colchicine on chromosome doubling and germination of *Codonopsis lanceolata* seeds

| Conc. (%) | Soaking time (hrs) | No. of seeds treated | No. of seeds germinated | % of germination | No. of tetraploids |
|-----------|--------------------|----------------------|-------------------------|-----------------|-------------------|
| Control   |                    | 150                  | 90                      | 60              | 0                 |
| 0.05      |                    | 150                  | 45                      | 30              | 0                 |
| 0.1       | 3                  | 150                  | 33                      | 22              | 0                 |
| 0.5       |                    | 150                  | 15                      | 10              | 0                 |
| 1.0       |                    | 150                  | 0                       | 0               | 0                 |
| 0.05      | 6                  | 150                  | 36                      | 24              | 3                 |
| 0.1       |                    | 150                  | 24                      | 16              | 0                 |
| 0.5       |                    | 150                  | 15                      | 10              | 1                 |
| 1.0       |                    | 150                  | 3                       | 2               | 0                 |
| 0.05      | 12                 | 150                  | 6                       | 4               | 0                 |
| 0.1       |                    | 150                  | 0                       | 0               | 0                 |
| 0.5       |                    | 150                  | 0                       | 0               | 0                 |
| 1.0       |                    | 150                  | 0                       | 0               | 0                 |
| 0.05      | 24                 | 150                  | 3                       | 2               | 0                 |
| 0.1       |                    | 150                  | 0                       | 0               | 0                 |
| 0.5       |                    | 150                  | 0                       | 0               | 0                 |
| 1.0       |                    | 150                  | 0                       | 0               | 0                 |
of 1.0% colchicine in Citrullus lanatus (Oh et al., 2015). However, in the present study, none of the tetraploid individuals were obtained at 0.05% concentration with the same 12 hr treatment duration, and it is believed that suitable concentration and treatment duration vary widely according to species of plants.

A significant difference was noted between control and treated seeds of C. lanceolata when the seeds were subjected to colchicine. Colchicine application reduced significantly the germination percentage of C. lanceolata. Similar type of findings were obtained by Sourour et al. (2014) and Bakry et al. (2007) on Hordeum vulgare L. and Vicia narbonensis respectively. Previous study demonstrated that colchicine in high concentration had very effect on germination of seed and survival plants recovered in medicinal plant, Salvia hains (Grouh et al., 2011).

**Morphological changes between diploid and tetraploid Codonopsis lanceolata**

The growth characteristics of diploid and tetraploid plants during the early growth stage are presented in table 2. The morphological features of tetraploid plants such as leaf length, leaf width, stomata length and width were found to be increased in tetraploid plants compared to diploid plants. But, the plant height in tetraploid (13.4) was decreased dramatically than the diploid (18.1) plant.

Although, in general, tetraploid plants showed potential growth characteristics than diploid that have been reported earlier (Tan and Dunn, 1973; Esen et al., 1978), but, the observation of the present study is contradictory with that result. However, there was no difference in the leaf width and length between the tetraploid and diploid, but interestingly the number of leaves was halved in the tetraploid (Fig. 1, Table 2). The size of the stroma was about 1.3 times larger in the tetraploid than the diploid. This finding showed consistency with the results found in mulberry tree (Park, 1994), lettuce (Eenink and Alvarez, 1975), and other plants.

The root characteristics of the diploid and tetraploid C. lanceolata are summarized in Table 3. The fresh weight of the main root in the diploid (0.5 g) was found four-fold higher (2.2 g) in the tetraploid. Also, the root length and diameter of the tetraploid were 8.5 cm and 8.0 mm, respectively, that significantly about twofold larger than the diploid. However, no number of lateral root was formed both in diploid and tetraploid plants (Fig. 3).

![Diploid (2n=2x=16)](image1)

![Tetraploid (2n=4x=32)](image2)

**Fig. 1.** Chromosomes in diploid and tetraploid of Codonopsis lanceolate.

**Table 2. Comparison of growth characteristics between diploid and tetraploid of Codonopsis lanceolata**

| Ploidy     | Plant height (㎝) | Leaf Length (㎝) | Leaf Width (㎝) | No. of leaves | Stomata size Length (㎛) | Stomata size Width (㎛) |
|------------|------------------|-----------------|----------------|--------------|-------------------------|------------------------|
| Diploid    | 18.1b            | 4.4a            | 3.0a           | 17.0a        | 15.1a                   | 9.7a                   |
| Tetraploid | 13.4a            | 4.8a            | 3.1a           | 8.0b         | 19.9b                   | 13.0b                  |

*Values followed by common letters in the same column are not significantly different (P=0.05, Duncan’s multiple range test).*
Effect of Colchicine on Chromosome Doubling in *Codonopsis lanceolata*

Fig. 2. Comparison of DNA contents between diploid and tetraploid of *Codonopsis lanceolata*. Flow histograms showing DNA measurements of nuclei from leaves.

![Diploid and Tetraploid](image)

**Table 3. Comparison of root characteristics between diploid and tetraploid of *Codonopsis lanceolata***

| Ploidy    | Fresh weight (g) | Root length (㎝) | Root diameter (㎟) | No. of lateral roots |
|-----------|------------------|-----------------|-------------------|---------------------|
| Diploid   | 0.5a             | 5.3a            | 4.1a              | 0                   |
| Tetraploid| 2.2b             | 8.5b            | 8.3b              | 0                   |

*Values followed by common letters in the same column are not significantly different (P=0.05, Duncan’s multiple range test).*

Fig. 3. Comparison of morphology and root characteristics between diploid (A) and tetraploid (B) of *Codonopsis lanceolata*.

**Effect of Colchicine Concentration on Chromosome Doubling and plant regeneration of in vitro-cultured *Codonopsis lanceolata* explants**

The effects of concentration and treatment duration of colchicine on plant regeneration and induction of tetraploid *C. lanceolata* are presented in Table 4. Although there was no consistent tendency in plant regeneration rates, according to the treatment duration, it was found that, when the concentration is high, the regeneration rate is low. A total of 126 individual plants were regenerated in the entire treatment groups, and in
Table 4. Effect of colchicine on chromosome doubling and plant regeneration of *Codonopsis lanceolata* explants

| Conc. (%) | Soaking time (hrs) | No. of explants treated | No. of explants regenerated | % of regeneration | No. of tetraploids |
|-----------|--------------------|-------------------------|-----------------------------|-------------------|------------------|
| 0.01      | 1                  | 45                      | 19                          | 42.2              | 0                |
| 0.05      | 1                  | 45                      | 16                          | 35.6              | 0                |
| 0.1       | 1                  | 45                      | 11                          | 24.4              | 0                |
| 0.01      | 6                  | 45                      | 13                          | 28.9              | 1                |
| 0.05      | 6                  | 45                      | 17                          | 37.8              | 5                |
| 0.1       | 6                  | 45                      | 10                          | 22.2              | 3                |
| 0.01      | 12                 | 45                      | 18                          | 40.0              | 2                |
| 0.05      | 12                 | 45                      | 15                          | 33.3              | 2                |
| 0.1       | 12                 | 45                      | 7                           | 15.6              | 1                |

Table 5. Comparison of growth characteristics between *in vitro* cultured diploid and tetraploid of *Codonopsis lanceolata*

| Ploidy     | Plant height (㎝) | Stem diameter (㎜) | Leaf length (㎝) | Leaf width (㎝) | No. of leaves | Root length (㎝) |
|------------|-------------------|-------------------|-----------------|---------------|---------------|-----------------|
| Diploid    | 10.4b             | 0.9a              | 1.5a            | 1.3a          | 10.0a         | 5.5a           |
| Tetraploid | 7.3a              | 1.3b              | 2.4b            | 2.1b          | 12.0a         | 10.1b          |

*Values followed by common letters in the same column are not significantly different (P=0.05, Duncan’s multiple range test).

all concentration groups for 6 hours and longer. In particular, 5 individuals of tetraploid plant were obtained at the concentration of 0.05% with 6-hour treatment that showed higher rates (29.4%) of the number of tetraploid than other regenerated plants. However, regardless of the colchicine concentration, after 1-hour soaking treatment, no tetraploid plant was obtained.

**Growth characteristics between *in vitro*-cultured diploid and tetraploid *Codonopsis lanceolata***

The comparison of formative characteristics between the diploid and tetraploid plants are presented in Table 5. As in the seed treatment result, the plant height of the diploid was significantly higher with 10.4 ㎝. As for the stem diameter, the tetraploid was thicker than the diploid. The leaf length and width of the tetraploid were 2.4 ㎝ and 2.1 ㎝ respectively, that was significantly thicker than those of the diploid. However, the numbers of leaves were similar regardless of ploidy. Also, the leaf color of the tetraploid was darker than that of the diploid. The root length of the tetraploid was 10.1 ㎝, longer than the diploid, and the root was also thicker (Fig. 4).

This study suggests that in seed treatment using colchicine, the 6 hour 0.05% and 0.5% treatment groups induced 3 and 1 tetraploid individuals respectively, at a low, 2.2%, rate. However, colchicine treatment of the *in vitro* culture showed a high tetraploid induction rate (11.1%) than seed treatment (Table 4). This is similar to a research investigation on tetraploid induction of *Prunella vulgaris for. albiflora*, in which soaking treatment of explants obtained from *in vitro* culture induced 9.6 times as many tetraploid plant bodies as seed treatment using colchicine (Kwon *et al.*, 2015).

This study concluded that *C. lanceolata* is more effective...
to apply in vitro cultured colchicine treated explants, which has active cell division, than directly to seeds, in order to induce tetraploid plant bodies, and it can be used as an effective method for breeding tetraploid of *C. lanceolata*.

### Acknowledgement

This research was supported by High Value-added Food Technology Development Program (114036-04-2-HD030) of IPET, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

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(Received 4 May 2016 ; Revised 17 June 2016 ; Accepted 21 June 2016)