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손태권* · 이상철** · 정일경***†

*(주)이즈텍 기술연구소, **경북대학교 농업생명과학대학, ***대구가톨릭대학교 생명공학과

Colchicine-Induced Polyploidy and It's Agronomic Characters in *Bupleurum falcatum*

Tae Kwon SON*, Sang Chul Lee, and Il Kyung Chung***†**

**R&D Center, ISTECH, Inc., CU Techno Center, Catholic University of Daegu, Gyeongsan-si, Gyeongbuk 712-702, Korea.*

***Division of Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 702-701, Korea.*

****Department of Biotechnology, Catholic University of Daegu, Kyeongsan, Kyeongbuk 712-702, Korea.*

ABSTRACT : The effect of colchicine treatment on the agronomic performance and polyploid formation of *Bupleurum falcatum* using flow cytometry technique was investigated. The roots of 4-leaf stage plants were treated with colchicine (0.5%) for 3, 6, 12, and 24 hours and then transplanted in the field. Agronomic characters (survival rate, plant height, chlorophyll content, bolting rate) were recorded at 4 weeks and 6 months after transplanting while flow cytometry technique was conducted for determination of polyploid formation. Flow cytometry technique revealed polyploid nuclear DNA formation in colchicine treated plants. The highest number of polyploids was obtained at the shortest colchicine treatment time indicating an inverse relationship between colchicine treatment time and polyploid formation. Results also showed that survival and bolting rates were inversely related with the treatment time while plant height and chlorophyll were not significantly affected by the treatment. This study showed a convenient method for determination of colchicine-induced polyploid in *B. falcatum* and its superior agronomic performance at shorter treatment time.

Key Words : Agronomic characters, *Bupleurum falcatum*, colchicine, Flow cytometry, Polyploid

INTRODUCTION

Colchicine ($C_{22}H_{25}O_6N$) was found out to induce polyploidy in plants (Blakeslee & Avery, 1937). Since then, colchicine-induced polyploidy had become an important method for genetic manipulations in plants (Eigsti, 1938; Navarro-Alvarez *et al.*, 1994) and had been utilized in ploidy manipulation in many crops (Madon *et al.*, 2005). In medicinal plants, studies (Gao *et al.*, 2002; De Jesus-Gonzalez & Weathers, 2003; Kim *et al.*, 2003) showed that polyploid medicinal plants not only grew faster and stronger but also yielded higher oil content. *Bupleurum falcatum* L. (Umbelliferae) is a rich source of saikosaponin making it another strong candidate for genetic manipulation studies. It has been widely used in China, Japan and Korea as traditional medicine (Shon *et al.*, 1997) and has attracted attention for local studies (Choi *et al.*, 2006; Kim *et al.*, 2006). Studies (Otsuka *et al.*, 1977; Shimokawa *et al.*, 1980; Hosoda *et al.*, 1992; Kim *et al.*, 2005) were conducted to determine cultivation factors affecting the saikosaponin production. In general, both environmental and genetic conditions affect the saikosaponin production in *B. falcatum* (Shon *et al.*, 1997; Shon & Yoshida, 1997). Nowadays, polyploidy investigations in plants can be conveniently undertaken using flow cytometric analysis. It was proven effective for quantitative and qualitative studies of cell replication (Arumuganathan & Earle, 1991; Bino *et al.*, 1993). Therefore, this study aimed to determine the effect of colchicine treatment on polyploidy formation and growth characteristics of *Bupleurum falcatum*, and show

†Corresponding author: (Phone) +82-53-850-3638 (E-mail) hexa20@hanmail.net
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rapid determination of polyploidy formation by using flow cytometry analysis.

MATERIALS AND METHODS

1. Colchicine treatment

B. falcatum seedlings were grown in the field and harvested at 4-leaf stage. Harvested plantlets were moved to the laboratory and washed thoroughly to remove soil particles from the root. The roots were then treated with colchicine solution (0.5%) by wrapping the roots in a paper towel soaked with colchicine. The treatment durations were 3, 6, 12 and 24 hours. Each treatment was replicated 3 times (60 seedlings per treatment). After treatment, the roots were washed with water, planted in a flowerpot (5 × 12 ㎝) and kept in the greenhouse for acclimatization (2 weeks) before transplanting in the field. Untreated control plants were subjected to the same conditions except for colchicine treatment. Survival rate was recorded at 4 weeks and 6 months after colchicine treatment. Plant height, chlorophyll content, and rate of bolting were surveyed 6 months after transplanting. Chlorophyll content was measured using SPAD instrument (SPAD-502, Minolta, Ramsey, NJ).

2. Flow cytometry analysis

Polyploid formation was investigated using seedlings at 4 weeks after transplanting by flow cytometry analysis following the procedures described by Arumuganathan and Earle (1991). The prepared material was analyzed with a standard flow cytometer (BD FACSCalibur). Healthy young leaf tissue (1 ㎠) was excised, placed in a plastic petri dish (60 × 10 ㎜), and added with 400 uL DNA extraction solution (high resolution DNA staining kit containing 10 mM Tris-HCl (pH 7.5), 2 mM MgCl; 0.1% triton X-100. The fresh leaf tissue was chopped using razor blade. Suspended nuclei were recovered using a pipettor, filtered through 30-㎛ nylon mesh into an analysis tube, and allowed to stand in room temperature for 5 mins. For each measurement, the propidium iodide fluorescence area signals (FL2-A) from 1000 nuclei were collected and analyzed by CellQuest software (Becton Dickinson Immunocytometry System, San Jose, CA). The mean position of the G0/G1 (nuclei) peak of the sample and the internal standard were determined by CellQuest software. The mean nuclear DNA content of each plant sample, measured in picograms, was based on 1000 scanned nuclei.

RESULTS AND DISCUSSION

1. Effects of colchicine treatments on agronomic characters

Polyploid plants are often desired since they give taller and bushier plants with more leaf mass and bigger flowers (Lapins, 1975; Ramachandran, 1982; Hassan & Wazuddin, 2000). To determine this effect on the colchicine-induced polyploid *B. falcatum*, its agronomic characters were recorded (Table 1). Data showed that as the treatment time increased, the survival rate of the plants decreased. Survival rate at 6 hours treatment and above was already very low at 4 weeks after treatment (WAT) which further decreased by 50% at 6 months after treatment (MAT). It also severely decreased (90%) from 3 to 6 hours treatment at both 4 WAT and 6 MAT showing high mortality effect at higher colchicine treatment time. Growth inhibition and decreased survival rate of plants treated with colchicine had been confirmed and shown in several studies (Blakeslee & Avery, 1937; Navarro-Alvarez *et al.*, 1994; Kim *et al.*, 2003; Obute *et al.*, 2005; Rauf *et al.*, 2006). However, studies (Yan, 2001; Rauf *et al.*, 2006) also showed that growth retardation in plants treated with colchicine is only transient and later the plants recovered again. The result indicated that soaking *B. falcatum* in 0.5% colchicine for more than

Table 1. Effects of colchicine treatment on the agronomic characters of *Bupleurum falcatum*

Treatment time (hours)	Number of colchicine-treated plants	Number of surviving plants		Rate of bolting plant (%)	Plant height	Chlorophyll content (SPAD value)	Number of polyploidy plants
		4 WAT[¶]	5 MAT	6 MAT	6 MAT		
Control	60	–	–	31.3±9.03	33.3±2.22	50.1±1.92	-
3	60	41±5.35 (68.3)	22±4.55 (36.6)	24.6±5.79	33.2±4.15	50.0±2.24	22
6	60	4±2.36 (6.7)	2±1.70 (3.3)	–	–	–	2
12	60	1±1.41 (1.7)	1±0.94 (1.7)	–	–	–	2
24	60	1±0.47 (1.7)	1±0.82 (1.7)	–	–	–	1

[¶]WAT: Weeks after treatment, MAT: Months after treatment, number inside ( ) is the survival rate. Values are means of 3-replicated experiments.

3 hours was already toxic to the plant. Effect of colchicine treatment was also manifested on the shapes of the leaves. Colchicine-treated plants showed uneven rough bulging on the leaf surface while leaf surface of control plants were smooth and did not have bulging. The bulging were indications of uneven distribution of polyploid cells on the leaf tissues formed by colchicine treatment.

The plant height recorded 6 MAT showed that there was no significant difference between control, 3 and 6-hour treatment indicating that it was not affected by the treatment. This was contrary to the observed effect of colchicine treatment on cultures of *Artemisia annua* L. wherein colchicine actually promoted rapid growth (De Jesus-Gonzalez & Weathers, 2003). Also, no difference was observed on the chlorophyll content of plants at 3 hours treatment and control. However, chlorophyll content was not recorded in plants treated at 6 hours and above. This indicated that though some plants survived at 6 hours treatment, growth was severely inhibited. The standard deviation recorded in the plant height and chlorophyll content also showed lower values in the control compared to the treated indicating a more stable and uniform growth in the control plants than the treated ones.

In the case of rate of bolting, colchicine caused a decrease in the rate of bolting of *B. falcatum*. At 6 MAT, rate of bolting was lower at 3 hours treatment by 21% compared with that of the control and was not completely observed in plants treated for 6 hours and above. Bolting can be considered a major determinant of quality in *B. falcatum* roots. Tadato *et al.* (1987) showed that non-bolting *B. falcatum* plants had superior quality compared to the bolting plants in terms of histological and chemical characteristics. Non-bolting roots had a tendency to contain greater concentrations of tissue that can store bioactive saikosaponins and was found out to contain higher concentrations of magnesium and phosphorous (Minami *et al.*, 1995). These results agreed with the observed effect of colchicine treatment on other crops (Gao *et al.*, 2002; De Jesus-Gonzalez & Weathers, 2003; Kim *et al.*, 2003).

2. Effects of colchicine treatments on polyploidy

Colchicine treatment has been an important tool in chromosome doubling and polyploidy study (Stebbins, 1984). In fact, review of recent references showed that ploidy manipulations on several crops such as pomegranate, *Solanum*, *Miscanthus sinensis*, *Allium cepa*, etc. have been done already (Madon *et al.*, 2005). Studies conducted on medicinal plants included *Platycodon grandiflorum* (Jacq.) (Kim *et al.*, 2003a), *Cadonopsis lanceolata* (Sieb. et Zucc.) (Kim *et al.*, 2003b), *Scutellaria biicalensis* (Gao *et al.*, 2002), and *Artemisia annua* (De Jesus-Gonzalez & Weathers, 2003). Colchicine treatment successfully induced polyploid formation on these crops that resulted in superior quality and better oil-producing plants.

The effect of colchicine treatment on polyploid formation in *B. falcatum* was investigated by flow cytometric analysis. Flow cytometric analysis is a fast and effective method for nuclear DNA analysis (Valkonen *et al.*, 1994). Compared to other methods, it is an accurate method for looking at changes on the cell nucleus during differentiation (Bino *et al.*, 1993; Dolezel & Bartos, 2005). Fig. 1 showed the result of flow cytometric analysis of *B. falcatum* confirming polyploidy inducement in the treated plants samples from the 3 hours treatment. Comparison of these two showed the formation of new bands that was reflected as two distinct peaks M1 and M2 (Fig. 1c). M1 was the diploid in the untreated plants while M2 was the polyploid in the treated plants.

Flow cytometric analysis confirmed the polyploid inducement in *B. falcatum* by colchicine treatment. Polyploid formation was induced in all treated plants regardless of

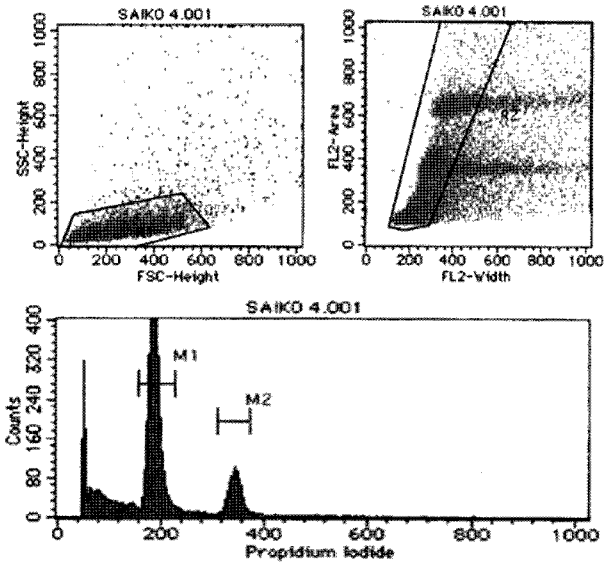

Fig. 1. Flow-cytometric analyses of propidium-iodide stained nuclear DNA of *Bupleurum falcatum*. FSC, forward scatter; SSC, side scatter; FL2, fluorescence parameters 564~606 nm.

treatment time. This could be attributed to the relatively older growth stage (4 leaves) of the plants. The highest number of mixoploid (22) obtained at 3 hours treatment was higher (by 91~95%) than those obtained at 6, 12 and 24 hours treatment which were 2, 2, 1 mixoploids, respectively. The result showed that for polyploid formation in 4-leaf plant stage, 3 hours is the most ideal treatment time using 0.5% colchicine concentration. Data also clearly showed an inverse relationship between treatment time and polyploid formation. Thus, colchicine concentration and treatment time can be adjusted depending on the plant stage and vice versa.

CONCLUSION

Polyploid inducement of colchicine (0.5%) in *Bupleurum falcatum* was confirmed by flow cytometric analysis. Polyploid was formed in the treated plants regardless of treatment time. Highest number of polyploid was formed at 3 hours treatment. Colchicine treatment caused significant reduction in survival rates of the plants and was deadly if applied for more than 3 hours treatment.

Plant height and chlorophyll content of polyploid *Bupleurum falcatum* were contrary to the commonly observed positive effects on other colchicine-induced polyploid crops. Though there was a very high increased in polyploid number at 3 hours treatment, growth was unstable and did not show significant increase in growth parameters compared to the untreated plants. Bolting decreased at 3 hours treatment indicating production of superior quality roots with higher saikosaponin concentrations. The result showed that polyploid inducement by colchicine treatment improved quality of *Bupleurum falcatum*.

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