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Original Research Article

Comparison of Yield and Growth Characteristics of *Platycodon grandiflorum* According to the Ploidy Levels and Growing Conditions

Hee-Doo Lee[1], Dong-Yeon Seo[2], Soo-Jeong Kwon[3], Hyun-Jeong Kim[3], Gab-Yeon Cho[4], Hee-Ock Boo[5], Swapan Kumar Roy[6] and Hag-Hyun Kim[3]*

[1]Chungbuk Agricultural Research and Extension Services, Ochang 28130, Korea
[2]Department of Hotel and Restaurant Culinary Art, Kunjang University, Gunsan 54045, Korea
[3]Department of Food Nutrition and Cookery, Woosong College, Daejeon 34606, Korea
[4]Department of Food Science and Biotechnology, Woosong University, Daejeon 34606, Korea
[5]WELLPHYTO Co. Ltd., BI Center, GIST, Gwangju 61005, Korea
[6]Department of Crop Science, Chungbuk National University, Cheong-ju 28644, Korea

Abstract - The present study aimed to compare and investigate the morphological characteristics and yield components according to ploidy level of diploid and tetraploid *Platycodon grandiflorum* under vinyl-greenhouse and open field conditions. Plant height of diploid and tetraploid *P. grandiflorum* was 51.3 ㎝, 54.0 ㎝, respectively. The results revealed that the plants grown in the vinyl-greenhouse showed significantly higher growth compared to those grown in the open field. Regardless of the growing place, diploid and tetraploid of *P. grandiflorum* showed the rapid elongation of internodes after 4 and 3 internodes respectively and elongation tends to be decreased as entering the flower-bud differentiation period. The starting day of flowering in vinyl-greenhouse cultivation was found to be faster than that of the open field cultivation by 2 ~ 3 days and tended to be delayed by about 5 ~ 6 days in tetraploid *P. grandiflorum* compared to diploid. Fresh weight of roots from the vinyl-greenhouse cultivation showed a high quantity as 34.2g and 49.4g in diploid and tetraploid *P. grandiflorum*, respectively and especially tetraploid *P. grandiflorum* was found to be increased by approximately 44.4% compared to other plots.

Key words - Tetraploid, Flowering date, Stomata size, Fresh weight, *Platycodon grandiflorum*

Introduction

Platycodon grandiflorum belonging to *Campanulaceae* is a perennial taprooted plant and it contained saponin, inulin, phytosterin and platycodinin in its roots that are effective for hematolysis, cough, discharge of phlegm and removal of fever etc. (Lee, 1974; Takagi and Lee, 1972). In particular, bronchial diseases due to environmental contamination and pollution caused by the development of industrialization is on the rise and growing. Cultivation area of *P. grandiflorum* are increasing with a rising demand for the preventive aspect or therapeutic purpose. With this increasing demand for *P. grandiflorum*, basic research has been carried out on the cultivation method such as soil, sowing time, fertilization conditions and herbicide use during *P. grandiflorum* growing (Lee *et al*., 1999; Shon *et al*., 2001). In addition, recently, studies on the composition analysis of sterol (Chung, 1985), proximate components (Cho, 1988), saponin content (Cho and Chang, 1989), volatile components (Chung *et al*., 1987), antioxidant enzyme activity (Boo *et al*., 2013) and platycodin D (Kim *et al*., 1990) content in the roots of *P. grandiflorum* have been also conducted actively.

Polyploidization is thought to be a powerful tool for improving desirable plant characteristics and is an effective breeding method to induce variation in many plants (Nakano *et al*., 2006; Oh *et al.* 2015; Stanys *et al*., 2006). In addition, polyploidy may increase the amounts of secondary metabolites in medicinal plants (Thao *et al*., 2003) which functional compounds accumulate in the vegetative parts in plants (Gao *et al.,* 1996; Wu *et al.,* 2011). *P. grandiflorum* is

*Corresponding author. E-mail : hkyushu@hanmail.net
Tel. +82-42-629-6988

needed a mass production of natural materials and the breeding of superior varieties. Previous reports revealed that the tetraploid induction of *P. grandiflorum* have also been available for breeding (Wang *et al*., 2005; Wu *et al.,* 2011).

However, it is crucial to develop varieties regarding genetic characteristics of resistance to various diseases and environmental stress and high yield ability. Because, *P. grandiflorum* cultivation in most farmhouses generally use seeds gathered from series collected in hills or seeds received from other growers. To this end, studies on excellent seeds breeding and cultivation methods including growing seedling skills of *P. grandiflorum* are needed.

Therefore, in order to obtain the basic data for improving the cultivation methods and yield ability of excellent seeds, the present study was conducted to compare and investigate the morphological characteristics and yield components of diploid and tetraploid *P. grandiflorum* using vinyl-greenhouse and open field conditions.

Materials and Methods

Plant materials and cultivation conditions

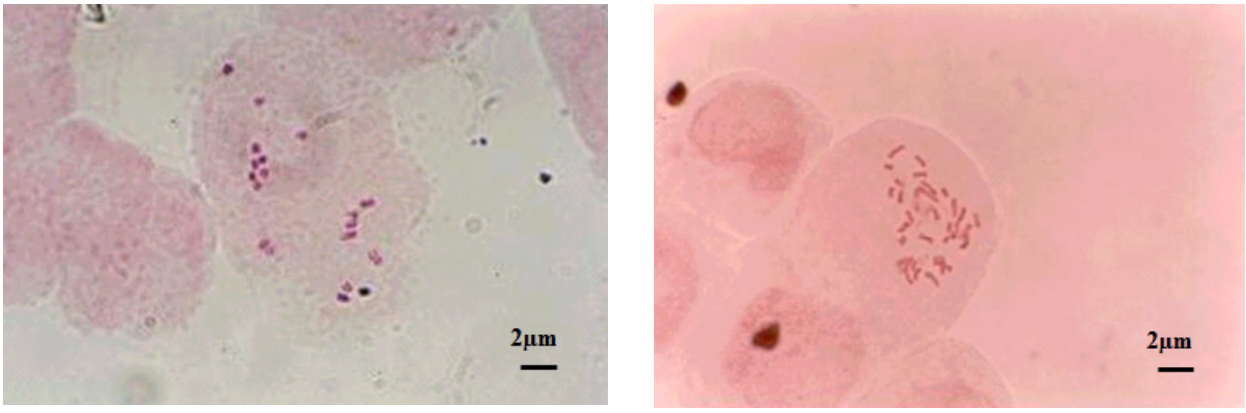
Field productivity was tested by using native *P. grandiflorum* seeds (2n=2x=18) that were collected in the fall season of 2013 and tetraploid *P. grandiflorum* seeds (2n=4x=36) were obtained by processing these seeds with colchicine as testing materials (Fig. 1).

In early April of 2014, leaves wide and height were observed 120 ㎝ and 15～20 ㎝ respectively in the open field. Until emergence, the maximum, average, minimum temperature and no. of precipitation in vinyl-green house condition were maintained at 32.4℃, 20℃, 12.7℃ respectively. In the case of open field cultivation condition, the maximum, average, minimum temperature and no. of precipitation were maintained at 21.3℃, 16.5℃, 11.7℃, and 7day respectively. However, to compare the cultivation methods, vinyl-greenhouse were made and diploid and tetraploid seeds were sown by maintaining 10×10 ㎝ planting distance. In order to identify the characteristics accurately among individuals, fertilizer was applied in the non-fertilized condition and watering was supplied periodically by installing a drip-watering facility in the vinyl-greenhouse cultivation.

In early September of 2015, the growth parameters such as plant height, leaf length and leaf width, leaf area, number of branches and internode length were investigated and leaf area was measured by 20 plants per repeat by using Li-3100 Area meter (Li-Cor, Inc. USA). At the end of September, the plants were harvested to examine the yield of lateral roots weight, root length, root diameter and fresh weight. Other cultivation methods were managed according to the wild edible greens standard cultivation method of Rural Development Administration.

Determination of number of chromosomes

The fresh root apex (1 ㎝) of *P. grandiflorum* was collected and pre-treated with 0.05% colchicine solution for 2-3 hours at room temperature, before fixing it with 95% ethanol and



Diploid Tetraploid

Fig. 1. Somatic chromosomes in diploid (2n=2x=18) and tetraploid (2n=4x=36) of *Platycodon grandiflorum.*

glacial acetic acid mixed solution (3:1) in a refrigerator. The sample was soaked again in 1N HCI solution, and then hydrolysis in a tank water. The hydrolyzed sample was then adjusted to 60°C at 120 rpm. Finally, it was soaked in 2% aceto-orcein solution before examining the number of chromosomes by using an optical microscope.

Measurement of stoma size

The number and size of stoma was investigated by using a optical microscope after removing the inner epidermis (0.5 ㎠) of leaves collected at the middle portion of diploid and tetraploid *P. grandiflorum* plants and the average of the number and size of stoma was calculated from the leaves of 30 individuals.

Number of chloroplasts determination in a guard cell

To observe the number of chloroplasts, the back of the leaves were collected from the middle part of the plant and the hypodermis was separated carefully. The sample was then soaked in iodine-potassium solution [1% (w/v) iodine, 2% (w/v) potassium iodide] for two to three hours for dyeing, and the dyed hypodermis was inspected by using a microscope. The number of chloroplasts in a guard cell was averaged after examining 30 cells in leaves of three entities per repetition.

Statistical analysis

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

Results and Discussion

Growth characteristics of diploid and tetraploid *P. grandiflorum* under vinyl-greenhouse and open field conditions

The differences of the growth characteristics of diploid and tetraploid *P. grandiflorum* under various cultivation methods are presented in Table 1.

Plant height of diploid and tetraploid *P. grandiflorum* grown in the vinyl-greenhouse was 51.3 ㎝, 54.0 ㎝, respectively. The results obtained from the vinyl-greenhouse showed significantly higher growth compared to the open field condition. However, significance between polyploids by cultivation method was not recognized. Regardless of the growing condition, diploid and tetraploid *P. grandiflorum* showed the rapid elongation of internodes after 4 and 3 internodes respectively, and elongation tends to decrease as entering the flower-bud differentiation period (Fig. 2). Based on these results, it was determined to be significant to start treatment of the growth regulator for lodging reduction in *P. grandiflorum* cultivation when vital elongation begins after 4-5 internodes. The results showed similarity to that of plant length, leaf length and leaf width that were better in the vinyl-greenhouse compared to open field cultivation. Especially in the case of leaf width, polyploidy was recognized significantly and leaf width increased by approximately 47% and 31% in

Table 1. Effect of cultivation method on growth characteristics in diploid and tetraploid of *Platycodon grandiflorum*

Cultivationmethods	Ploidy	Plant height (㎝)	Leaf		No. of leaves	Stem diameter (㎜)	No. of branches	No. of flowers	Flowering day
			length (㎝)	width (㎝)					
Vinyl- greenhouse	2x	51.3az	9.0b	5.3b	69.9a	2.6b	8.2a	9.1a	Jul. 25
	4x	54.0a	10.2a	7.8a	41.9b	3.3a	6.0b	7.5a	Jul. 30
Open field	2x	28.8b	5.9c	3.5d	28.0c	1.7c	4.1c	5.3a	Jul. 27
	4x	31.2b	6.4c	4.6c	21.8c	2.7b	3.1d	5.0a	Aug. 2
				Significance					
Cultured place (A)		**	**	**	**	**	**	NS	
Ploidy (B)		NS	NS	NS	NS	*	*	NS	
A×B		NS	NS	NS	NS	NS	NS	NS	

[z]Values followed by common letters in the same column are not significantly different.
[NS, *, **]Nonsignificant and significant at p=0.05 and 0.01, respectively.

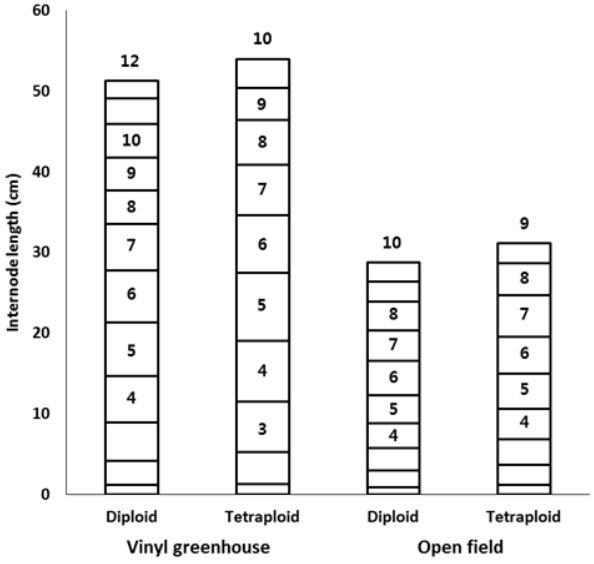


Fig. 2. Comparison of internode length between diploid and tetraploid of *Platycodon grandiflorum* depending on the differences in cultivation methods.

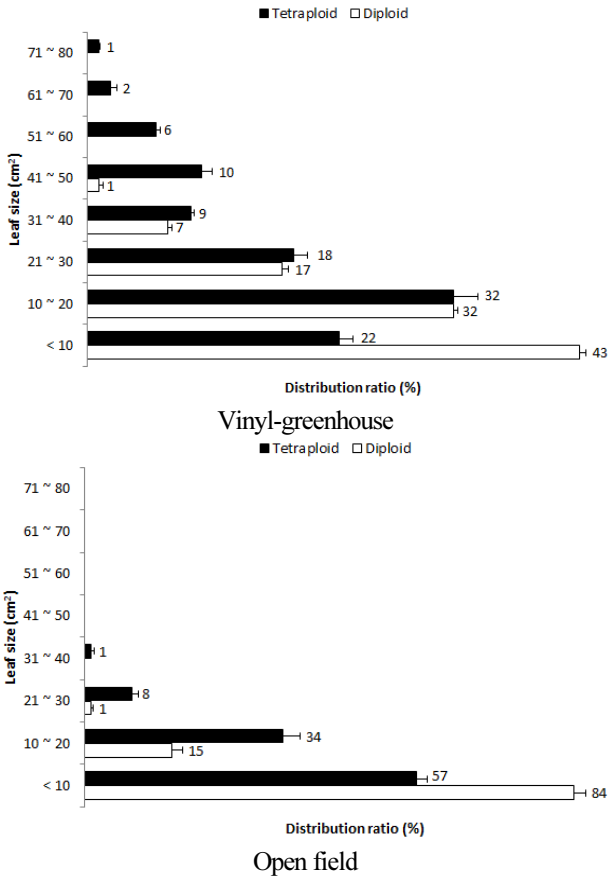


Fig. 3. Distribution of leaf size between diploid and tetraploid of *Platycodon grandiflorum* depending on the differences in cultivation methods.

tetraploid *P. grandiflorum* than diploid and open field cultivation, respectively, showing the result that the form of leaves is changed from lanceolate type to eggshape. For the leaf area, in the case of vinyl-greenhouse cultivation, less than 20 ㎠ accounted for the most, approximately 75% in diploid *P. grandiflorum* and more than 40 ㎠ was hardly distributed in diploid *P. grandiflorum* while less than 30 ㎠ was approximately 72% and more than 40 ㎠ was also found to be distributed approximately 19% in tetraploid *P. grandiflorum*. In the open field cultivation, less than 10 ㎠ was the most (84%) and less than 30 ㎠ was found to be distributed by more than 90% in diploid and tetraploid *P. grandiflorum*in respectively (Fig. 3). Leaf number was also found to be increased in vinyl-greenhouse cultivation than the open field cultivation and the diploid *P. grandiflorum* showed the highest number of leaves compared to tetraploid regardless of the cultivation method. Stem diameter was found to be somewhat thicker in vinyl-greenhouse cultivation in tetraploid. Branching showed more formation in the vinyl-greenhouse cultivation and was found to be more significantly in diploid *P. grandiflorum* compared to tetraploid regardless of the growing place. The number of flowers was in the range of 5.0 to 9.1 regardless of cultivation method and polyploidy and no significance was recognized.

The flowering start day of the vinyl-greenhouse cultivation was found to be faster than that of the open field cultivation by 2～3 days and tended to be delayed by about 5～6 days in tetraploid *P. grandiflorum* compared to diploid.

In tetraploid plants, several studies have demonstrated that organs or stems get larger and stems get thicker and longer and leaves and flowers also get larger (Cockerham and Galletta, 1976; Lapins, 1975). However, the present study showed that the growth of leaves and hypertrophy of stems of tetraploid *P. grandiflorum* showed higher results compared to diploid but plant length, leaf number and flower number were found to be the same level or lowered than those of diploid. In addition, the morphological characteristics of *P. grandiflorum*, the difference regarding cultivation methods was found to be even greater than that of the polyploidy.

The characteristics of flower of diploid and tetraploid *P. grandiflorum* under vinyl-greenhouse and open field conditions

The characteristics of the flower of diploid and tetraploid *P. grandiflorum* according to various cultivation methods are demonstrated in Table 2.

Regardless of the cultivation methods, flower length of tetraploid *P. grandiflorum* was significantly longer than that of diploid. Flower width in tetraploid *P. grandiflorum* was found to be a little larger under vinyl-greenhouse cultivation than diploid with open field cultivation. Flower height showed the similar result as the flower length and tetraploid *P. grandiflorum* was observed significantly longer than the diploid regardless of the cultivation methods. Flowers exhibited thicker in size under the open field cultivation compared to vinyl-greenhouse cultivation. Especially, tetraploid *P. grandiflorum* of the open field cultivation showed the highest result (0.60 ㎜). Style length and style diameter tetraploid *P. grandiflorum* was found to be bigger than diploid *P. grandiflorum* and showed no differences regarding the cultivation methods. The size of flowers was found to get bigger by about 30% compared to diploid according to polyploidy in tetraploid '*Bletilla striata*' variety (Yun *et al*., 2004) and *Platycodon grandiflorum* did not show the increase amount of the same rate as *Bletilla striata* but showed a tendency that all flower structures get bigger or thicker, showing the trend similar to the study results of '*B. striata*'.

The size of stoma and chlorophyll content of diploid and tetraploid *P. grandiflorum* under vinyl-greenhouse and open field conditions

The results of the characteristics of stoma and content of chlorophyll of diploid and tetraploid *P. grandiflorum* regarding the differences in the cultivation methods are presented in Table 3. The alteration of the size of stoma was not observed regarding the differences in the cultivation methods, but tetraploid *P. grandiflorum* was longer than diploid. However, stoma width of tetraploid *P. grandiflorum* was 29.1 ㎛ and 28.3 ㎛ in vinyl-greenhouse and open field cultivation, respectively and was also found to increase by 50% compared to diploid. This result was consistent with the report that the size of stoma was significantly increased according to doubling in potatoes (Cho *et al*., 1994) and tobacco (Bae *et al*., 2001). The number of stoma showed a tendency to increase in diploid *P. grandiflorum* with open field cultivation compared to tetraploid with vinyl-greenhouse cultivation respectively. Especially, diploid *P. grandiflorum* of the open field cultivation is 413/㎟, increasing by 1.5 to 2.3 times compared to diploid and tetraploid *P. grandiflorum* of the vinyl-greenhouse cultivation and tetraploid *P. grandiflorum* of the open field cultivation.

According to the results of this experiment, the number of stoma per ㎟ in tetraploid *P. grandiflorum* have been reduced, since the density of stoma decreased due to greater stoma length and stoma width. The number of chloroplasts was found to be significantly doubled in tetraploid *P. grandiflorum* compared to diploid regardless of the cultivation methods,

Table 2. Effect of cultivation method on flower characteristics between diploid and tetraploid *Platycodon grandiflorum*

Cultivation methods	Ploidy	Flower				Style	
		length (㎝)	width (㎝)	height (㎝)	thickness (㎜)	length (㎝)	diameter (㎜)
Vinyl- greenhouse	2x	4.8b[z]	6.5b	3.4c	0.28d	1.9b	1.0b
	4x	5.1a	7.1a	4.3a	0.44b	2.2a	1.6a
Open field	2x	4.8b	6.1c	3.6b	0.36c	2.0b	1.0b
	4x	5.1a	6.6b	4.2a	0.60a	2.2a	1.6a
Significance							
Cultured place (A)		NS	NS	NS	**	NS	NS
Ploidy (B)		NS	NS	**	NS	NS	**
A×B		NS	NS	NS	NS	NS	NS

[z]Values followed by common letters in the same column are not significantly different.
NS, *, **Non-significant and significant at p=0.05 and 0.01, respectively.

Table 3. Effects of cultivation methods on stoma characteristics between diploid and tetraploid of *Platycodon grandiflorum*

Cultivation methods	Ploidy	Stomata		Number of stomata (per ㎟)	Number of chloroplasts (per guard cell)	Chlorophyll content (mg·g fresh wt.)
		length (㎛)	width (㎛)			
Vinyl- greenhouse	2x	31.4c[z]	22.2b	268b	17.6a	13.7b
	4x	49.6a	29.1a	180c	35.7b	16.7a
Open field	2x	30.7c	21.4b	413a	18.0a	13.9b
	4x	46.6b	28.3a	272b	36.8b	17.2a
Significance						
Cultured place (A)		*	NS	**	NS	NS
Ploidy (B)		**	**	*	**	**
A×B		NS	NS	NS	**	**

[z]Values followed by common letters in the same column are not significantly different.
NS, *, **Non-significant and significant at p=0.05 and 0.01, respectively.

Table 4. Effects of cultivation methods on root characteristics between diploid and tetraploid of *Platycodon grandiflorum*

Cultivation methods	Ploidy	Root length (㎝)	Root diameter (㎜)	No. of lateral roots	Fresh weight (g)
Vinyl-greenhouse	2x	21.4b[z]	19.7b	3.2a	34.2b
	4x	24.5a	24.1a	2.9a	49.4a
Open field	2x	16.9c	16.3c	2.1b	17.8d
	4x	19.2bc	19.2b	1.5b	22.9c
Significance					
Cultured place (A)		**	*	**	**
Ploidy (B)		*	NS	*	NS
A×B		NS	NS	NS	NS

[z]Values followed by common letters in the same column are not significantly different
NS, *, **Non-significant and significant at p=0.05 and 0.01, respectively.

and increase or decrease in the number of chloroplasts according to the difference in the cultivation method could not be seen. This result showed a similar trend to the report that the average number of chloroplasts increases as doubling like potatoes (Cho *et al*., 1994), tobacco (Bae *et al*., 2001) etc. The content of chlorophyll is also a similar trend to the number of chloroplasts and was found to be slightly higher in tetraploid *P. grandiflorum*. However, the content of chlorophyll according to the difference in the cultivation methods was found to be slightly higher in the open field cultivation than in vinyl-greenhouse cultivation regardless of polyploidy, but there was no significance between these two groups.

The root characteristics of the diploid and tetraploid using vinyl-greenhouse and open field conditions

The root characteristics of diploid and tetraploid *P. grandiflorum* according to the differences in the cultivation methods are presented in Table 4. Root length and root diameter in tetraploid *P. grandiflorum* showed higher growth significantly in vinyl-greenhouse cultivation than diploid with open field cultivation. In particular, root length and root diameter of tetraploid *P. grandiflorum* in the vinyl-greenhouse cultivation were 24.5 ㎝ and 24.1 ㎜, respectively, and the obtained results significantly higher than other plots. The number of lateral roots also showed higher results in vinyl-greenhouse cultivation than in open field cultivation. Unlike the results of root length and root diameter, however, the formation of lateral roots was found to be a little more in diploid *P. grandiflorum* than tetraploid. Fresh weight of roots showed a high quantity in diploid (34.2 g) and tetraploid (49.4 g) *P. grandiflorum* under vinyl-greenhouse cultivation. However, especially tetraploid *P. grandiflorum* showed the

result of increasing by approximately 28.7～44.4% compared to other plots. *P. grandiflorum* is known to be capable of harvesting when two to three years has elapsed after sowing and root weight is more than 25 g (Rural Development Administration, 1999).

The results obtained from the present study revealed that the fresh weight of roots of diploid and tetraploid *P. grandiflorum* showed a pronounced growth characteristics in vinyl-greenhouse cultivation compared to open field condition. The fresh weight was found to significantly exceed 25 g when it was grown under the vinyl-greenhouse condition both in diploid and tetraploid *P. grandiflorum* regardless of polyploidy. Taken together, the result suggest that vinyl-greenhouse condition is considered as a potential cultivation method for shortening the harvest period.

References

Bae, C.H., Y.H. Lee, D.C. Yang, K.S. Min, H.I. Kim and H.Y. Lee. 2001. Changes of chloroplast number per guard cell pairs of leaves by ploidy level in *Nicotiana tabacum* L. cv. BY-4. Korean J. Plant Tiss. Cult. 28(4):179-184 (in Korean).

Boo, H.O., J.H. Shin, Y.S. Kim, H.J. Park, H.H Kim, S.J. Kwon and S.H.Woo. 2013. Comparative antioxidant enzyme activity of diploid and tetraploid *Platycodon grandiflorum* by different drying methods. Korean J. Plant Res. 26(3):3 89-396.

Cho, H.M., H.Y. Kim and I.J. Mok. 1994. Stomatal cell characters as an indicator for haploid identification from progeny plants derived by 4x×2x interspecific crosses in potatoes. Korean J. Breed. 26(1):66-73 (in Korean).

Cho, K.S. 1988. Comparison of chemical components of cultivated *Platycodon grandiflorum* and *Codonopsis lanceolata*. I. General constituents and saponin content. Research Report of Anseong Agricultural Junior College 20:426-439 (in Korean).

Cho, K.S. and Y.S. Chang. 1989. Comparison of chemical components of cultivated *Platycodon grandiflorum* and *Codonopsis lanceolata*. II. Inorganic elements and amino acid composition. Research Report of Anseong Agricultural Junior College 21:170-181 (in Korean).

Chung, T.Y. 1985. Studies on the sterols in the roots of bellflower (*Platycodon grandiflorum* Nakai). II: Steryl glycoside in the roots of bellflower. Research Report of College of Human Ecology. Pusan Nat'l. Univ. 11:7-16 (in Korean).

Chung, T.Y., J.L. Kim, B. Hayase and H. Kato. 1987. Flavor components in the bellflower roots (*Platycodon glaucum* Nakai). J. Kor. Soc. Food Nutr. 16(2):136-146 (in Korean).

Cockerham, L.E. and G.J. Galletta. 1976. A survey of pollen characteristics incertain *Vaccinium* species. J. Amer. Soc. Hort. Sci. 101(6):671-676.

Gao, S.L., D.N. Zhu, Z.H. Cai and D.R. Xu. 1996. Autotetraploid plants from colchicine-treated bud culture of *Salvia miltiorrhiza* Bge. Plant Cell Tiss. Org. 47(1):73-77.

Kim, T.J., S.I. Lee, T.H. Lee and J.S. Ko. 1990. Isolation and determination of platycodin D from *Platycodi radix*. J. Kor. Soc. Anal. Sci. 3(3):399-404 (in Korean).

Lapin, K.O. 1975. Polyploidy and mutations induced in apricot by colchicine treatment. Can. J. Genet. Cytol. 17(4):591-599.

Lee S.T., J.S. Ryu, M.B. Kim, D.K. Kim, H.J. Lee and J.S. Heo. 1999. Crude saponin contents of *Platycodon grandiflorum* (Jacq.) A. DC. Kor. J. Med. Crop 7(3):172-176 (in Korean).

Lee, E.B. 1974. Pharmaceutical studies on *Platycodon grandiflorum*. J. Med. Plants 5:49-60.

Nakano, M., T. Nomizu, K. Mizunashi, M. Suzuki, S. Mori, S. Kuwayama and M. Asano. 2006. Somaclonal variation in *Tricyrtis hirta* plants regenerated from 1-year-old embryogenic callus cultures. Sci. hortic. 110(4):366-371.

Oh S. A, K.H. Min, Y.S. Choi, S.B. Park, Y,C, Kim and S.M. Cho. 2015. Development of tetraploid watermelon using chromosome doubling reagent treatments. Korean J. Plant Res. 28(5):656-664 (in Korean).

Rural Development Administration. 1999. Edible wild plant cultivation (Farming standards handbook). p. 154 (in Korean).

Shon M.Y., JK. Seo, H.J. Kim and N.J. Sung. 2001. Chemical compositions and physiological activities of Doraji (*Platycodon grandiflorum*). J. Kor. Soc. Food Sci. Nutr. 30(4):717-720 (in Korean).

Stanys, V., A. Weckman, G. Staniene and P. Duchovskis. 2006. *In vitro* induction of polyploidy in Japanese quince (*Chaenomeles japonica*). Plant Cell Tiss. Org. 84(3):263-268.

Takagi, K. and E.B. Lee. 1972. Pharmacological studies on *Platycodon grandiflorum* A. DC. II. Anti-inflammatory activity of crude Platycodin, its activities on isolated organs and other pharmacological activities. Journal of the Pharmaceutical Society of Japan 92(8):961-986.

Wang, X., L. Xiong, Y. Qu, J. Yang and Z. Gu. 2005. The ploypoid induction and identification of *Platycodon grandiflorus*

(Campanulaceae) in China. Acta Botanica Yunnanica 28(6): 593-598.

Wu, Y., F. Yang, X. Zhao and W. Yang 2011. Identification of tetraploid mutants of *Platycodon grandiflorus* by colchicine induction. Caryologia 64(3):343-349.

Yun, J.S., E.Y. Hong, I.H. Kim, S.K. Shin, T. Yun, C.H. Lee, T.S. Kim and K.Y. Paek. 2004. Breeding of a new tetraploid *Bletilla striata* Reichb. fil., 'Chungbuk Jaran'. Kor. J. Hort. Sci. Technol. 22(4):495-498 (in Korean).

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