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Short note

Influence of 6-benzylaminopurine spray time after pinching on growth and flowering of *Veronica dahurica* Steven

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Key words: Cytokinin, foliar application, foliar spray, multiple shoots.

Abstract: *Veronica dahurica* Steven (family *Scrophulariaceae*) is an ornamental plant from Korea. The aim of the present study was to produce multiple-branched plants by using 6-benzylaminopurine (BA) spray application at several time intervals after pinching. All 10 cm long plants were sprayed with 0, 500, 1000, and 2000 mg·L^{-1} BA at 0, 7, and 14 days after pinching. Growth characteristics were examined 10 weeks after pinching and flowering time was recorded. The number of branches was highest in the group sprayed with 1000 mg·L^{-1} BA at 0 d after pinching. The greatest plant height was observed in the group treated with 1000 mg·L^{-1} BA 14 d after pinching and the minimum plant height was observed in the group sprayed with 500 mg·L^{-1} BA at 0 d after pinching. The groups sprayed with higher BA concentrations and with longer intervals between pinching and spraying showed greater delay in the time to first flower. The flower length was decreased in the pinched and BA-treated group compared with the control. Thus, BA application and pinching could promote multiple branch induction and control flowering time in *V. dahurica* Steven.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

The genus *Veronica* L. (family *Scrophulariaceae*) comprises about 500 species. It is distributed across most of the Northern Hemisphere and in many parts of the Southern Hemisphere and has a wide ecological range, from alpine to coastal vegetation and dry to aquatic vegetation (Albach *et al*., 2004; Albach *et al*., 2005). *Veronica* spp. have the characteristics of graceful and bountiful flowers, long-blooming, easy-care perennials, and they are commercially used in gardens and as cut-flowers (Areal *et al*., 2008; Hawke, 2010).

Veronica dahurica Steven is distributed across East Asia, in Siberia, Far East Russia, China, North and South Korea, and Mongolia (Choi, 2016). The stems of *V. dahurica* are erect and upright, with simple hairs, and occur individually or in small groups. The plant grows to 30-50 cm in height. The opposing leaflets are 18-25 mm in length and 15-22 mm in width and have a deltoid shape with deeply cut tips on the edge of the leaf. The flowers are racemule, white or pink colored, with short hairs on

the apical part. The corolla is 5-7.5 mm in length (KPNI, 2019).

Multiple branching has commercial advantages for flower production, because it improves yield and quality, controls plant size, and inhibits loathing (Zieslin *et al.*, 1975; Barbier *et al.*, 2017; Kim *et al.*, 2020). Pinching and BA foliar spray are the most useful methods for production of multiple-branched plants (Lee *et al.*, 2006).

Pinching is the removal of the apical bud to release the lower axillary buds from apical dominance. Pinching increases branching and stimulates axillary bud development by decreasing auxin production in the apical bud and inhibiting lateral bud growth (Barbier *et al.*, 2017). Pinching is commonly used to improve yield by inducing branches, decreasing plant height to produce dwarf plants, and controlling flowering time. This method also increases the cytokinin content. Exogenously applied cytokinin, like foliar application, has the same effect as pinching.

Plant growth regulators (PGRs), such as cytokinins, play important roles in the control of herbage type and flowering. Cytokinin regulates gynoecium formation and female gametophyte development, embryo development and seed size, pavement cell morphogenesis, axillary bud release, and nutrient uptake. This promotes vascular cambial development, nodulation, chloroplast development, cell division, and phloem development. It also inhibits lateral root formation, senescence, and cell proliferation in the root apical meristem (Kieber and Schaller, 2018).

In the present study, the use of foliar sprays of different concentrations of BA and pinching to improve the branching and flowering of *V. dahurica* are evaluated for commercial use on ornamental plants. In addition, the interaction and use of different growing environments is examined to further manipulate the production of *V. dahurica*, particularly for use as flowering pot plants.

2. Materials and Methods

Plant materials

Seeds of *V. dahurica* were collected from the Useful Plant Resources Center, Korea National Arboretum on 12th September 2018 and stored in a 4°C chamber. *V. dahurica* seedlings were grown on 128-cell trays filled with a commercial soilless substrate (Baroker; Seoul Bio, Eumseong, Korea) on 7th May 2019, and transplanted into 11.5 cm diameter containers filled with the same substrate on 26th June 2019. The Baroker consisted of 68% coir dust, 15% peat moss, 7% perlite, 6% vermiculite, and 4% zeolite, at pH 5.5-6.0. Two weeks after transplanting, plants were fertilized with 1000 mg·L^{-1} Peters Professional 20-20-20 (Everris, Geldermalsem, The Netherlands). All plants were grown in a greenhouse.

Pinching and BA foliar spray application

V. dahurica plants of 10 cm length, with 2-3 nodes and 2 leaves, were selected. All plant materials were pinched at every second internode from the basal stem, and then foliar-sprayed with 10 mL of 0, 500, or 2000 mg·L^{-1} BA (Duchefa, Haarlem, The Netherlands), using a hand-pump sprayer, at 0, 7, or 14 spraying days after pinching (SDP). The BA solutions were prepared in 10 mL of 99 % ethanol by diluting with distilled water.

Growth conditions

The experiment was conducted between 5th July 2019 and 13th September 2019. Pinching and BA treatments were applied once at 10 weeks of culture under greenhouse conditions. The mean values of relative humidity and temperature in the greenhouse are shown in figure 1. Irrigation was carried out by drip watering at 500 mL daily in September and twice daily from July to August.

Plant growth and flowering

The variables considered consisted of height and width of plant, number of nodes, length and width of leaf, number of leaves, lateral branch length, number

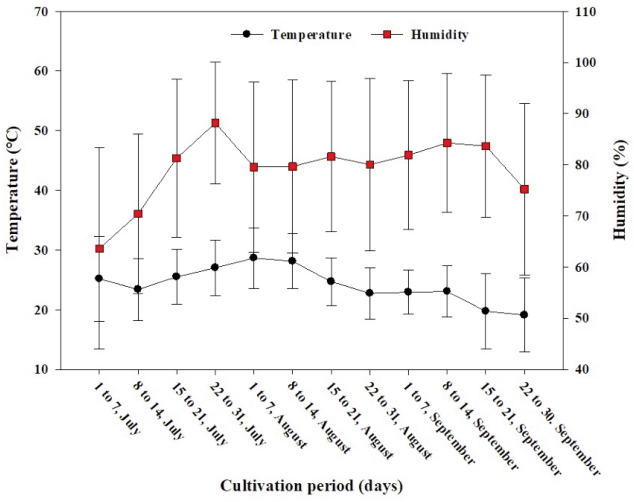

Fig. 1 - Principal component loading pattern of six traits of *Amaranthus* accessions.

of lateral branches and flower characteristics. These parameter were evaluated 10 weeks after treatment. The time to first flowering was recorded.

Statistical analysis

The experiment was conducted with twelve plantlets per three replicates and three replications per treatment, in a completely randomized block design, unless specified otherwise. Significant differences were determined using Duncan's multiple range test (DMRT) with SAS software (Version 9.4; SAS Institute, Cary, NC, USA).

3. Results and Discussion

Effects of BA and SDP on growth characteristics

V. dahurica was cultivated with 0, 500, 1000, or 2000 mg·L^{-1} BA foliar spray application at 0, 7, or 14 SDP, and plant growth was evaluated 10 weeks after treatment. BA foliar application groups, which were co-treated with pinching, successfully formed lateral branches, while the effect of pinching treatment alone was similar to that of the control. Maximum lateral branch formation was observed in the group treated with 1000 mg·L^{-1} BA spray on 0 day after pinching, with a mean of 13.92 secondary branches per plant and a branch production efficiency approximately 2.93-fold that of the control (Table 1). Both pinching and BA foliar spray application can promote branch production by controlling apical dominance through cytokinin signaling (Barbier *et al*., 2017; Kieber and Schaller, 2018). BA foliar spray application is recommended to promote branch production in many ornamental plants such as *Ardisia pusilla* (Lee *et al*., 2006), *Echinacea* cultivars (Latimer and Freeborn, 2009), and Kalanchoe species (Currey and Erwin, 2012). Pinching is also recommended for branch production in *A. pusilla* (Lee *et al*., 2006), *Targets exeta* L. (Meena *et al.*, 2015), and *Elsholtzia* (Sohn and Kim, 2003). However, even when the concentration of BA was increased with increasing SDP, the multiple-branch production effect was not greater than that of the treatment immediately after pinching. Lee *et al*. (2006) reported that 0 to 7 SDP increased branch production in *Ardisia pusilla*, but that branch production decreased at SDP>7. In our treatments, plant height was similar to or less than that in the control; however, lateral branch length was decreased in all treatment groups compared with that in the control (Table 1, Fig. 2). Leaf characteristics were also investigated in our experiments. Compared with the control, leaf number and leaf width were increased in almost all or all treatment groups, respectively, and leaf length was decreased in almost all treatment groups (Table 1). All treatments showed a decrease in the number of nodes compared with that in the control. Pinching and cytokinin application can be economically used to enhance production of plant stems, flowers, and

Table 1 - Growth effects of pinching and spray application on *V. dahurica* 10 weeks after treatment

SDP (days)	BA (mg·L^{-1})	Plant		No. of nodes (per/plantlet)	Leaf			Secondary branch	
		Height (cm)	Width (cm)		Length (cm)	Width (cm)	Number (per/plantlet)	Length (cm)	Number (per/plantlet)
Control		29.08 ab[z]	38.42 a	14.00 a	6.27 a	3.57 c	177.94 d	29.43 a	4.75 f
Pinching		28.69 ab	37.39 a	11.67 bc	6.33 a	3.76 bc	197.17 cb	28.52 a	6.78 ef
0	500	20.65 c	23.87 c	10.53 bc	6.99 a	4.24 ab	298.97 b	12.44 e	11.64 abc
	1000	24.86 bc	30.54 b	11.61 bc	7.08 a	4.48 a	407.36 a	15.60 cd	13.920 a
	2000	27.25 ab	23.44 c	7.00 d	4.71 b	3.86 bc	175.67 d	18.86 bcd	11.06 abc
7	500	26.59 ab	29.56 b	6.69 d	4.61 b	3.76 bc	215.61 cd	16.47 b	10.59 bcd
	1000	24.60 bc	27.68 bc	7.8 d	5.01 b	3.79 bc	191.08 cd	16.11 bc	11.48 bcd
	2000	28.22 ab	31.97 b	13.00 ab	4.23 b	3.75 bc	239.28 bcd	12.56 bcd	8.00 de
14	500	24.76 bc	30.57 b	12.28 ab	4.70 b	4.34 ab	256.72 bc	13.37 e	8.92 cde
	1000	30.56 a	32.53 b	12.77 ab	4.82 b	4.28 ab	432.14 a	12.86 de	12.86 ab
	2000	24.55 bc	30.98 b	11.36 bc	7.04 a	4.13 ab	370.58 a	18.10 bc	12.67 ab

SDP= spraying days after pinching;
BA= 6-benzylaminiopurine;
[z] Mean separation within columns by Duncan´s multiple range test at $p<0.05$.

seeds by decreasing the planting density and increasing the number of branches. Small dwarf plants were grown as pot plants by reducing the plant height (Table 1, Fig. 2).

Effects of BA and SDP on flowering

Pinching treatment alone resulted in the highest flowering percentage per plantlet after 10 weeks of culture (86%), followed by BA 2000 mg·L^{-1} spray application at 0 SDP, BA 1000 mg·L^{-1} at 0 SDP, BA 1000 mg·L^{-1} at 7 SDP, BA 1000 mg·L^{-1} at 14 SDP, BA 500 mg·L^{-1} at 7 SDP, and the control.

There were significant differences in days to first flowering among treatment groups. Pinching delayed the first flowering (24.69 days after pinching) compared with the control (14.14 days after pinching). BA 500 mg·L^{-1} (0 SDP: 30.94 days/ 14 SDP: 29.17 days) and 1000 mg·L^{-1} (0 SDP:30.72 days/ 14 SDP: 26.06 days) at 0 SDP and 14 SDP increased the number of days to first flowering compared with the control and BA 0 mg·L^{-1} with pinching groups. However, other treatments decreased the number of days to first flowering compared with the BA 0 mg·L^{-1} with pinching and control groups. In the flower development stage, the first increase in cytokinin occurred in the bud induction stage, followed by a dramatic increase in the transition stage. Exogenous cytokinin treatment can promote floral transition through transcriptional induction of the floral activators TWINSISTER OF FT (TSF), FLOWERING LOCUS D (FD), and SOCI (D'Aloia *et al*., 2011; Winterhagen *et al*., 2020).

The number of flowers and buds also differed among treatments. The BA 2000 mg·L^{-1} at 0 SDP treatment produced the highest flower number, followed by BA 1000 mg·L^{-1} at 14 SDP. However, the highest number of buds was observed in the BA 2000 mg·L^{-1} at 14 SDP treatment group, followed by BA 1000 mg·L^{-1} at 7 SDP, pinching, and BA 1000 mg·L^{-1} at 14 SDP (Fig. 2, 3). The observed increases in flower number and buds are due to the increase in branch number and the promotion of flowering by cytokinin (Kumar *et al*., 2002; D'Aloia *et al*., 2011).

Both the flower length and the peduncle length decreased with BA foliar spray application and pinching treatments. The effect of the interaction between pinching and BA on the flower length was found to be significant. The smallest flower length (10.49 cm) was recorded with application of BA 1000 mg·L^{-1} at 7 SDP compared with a flower length of 21.62 cm in the control group. With BA 2000 mg·L^{-1} treatment at

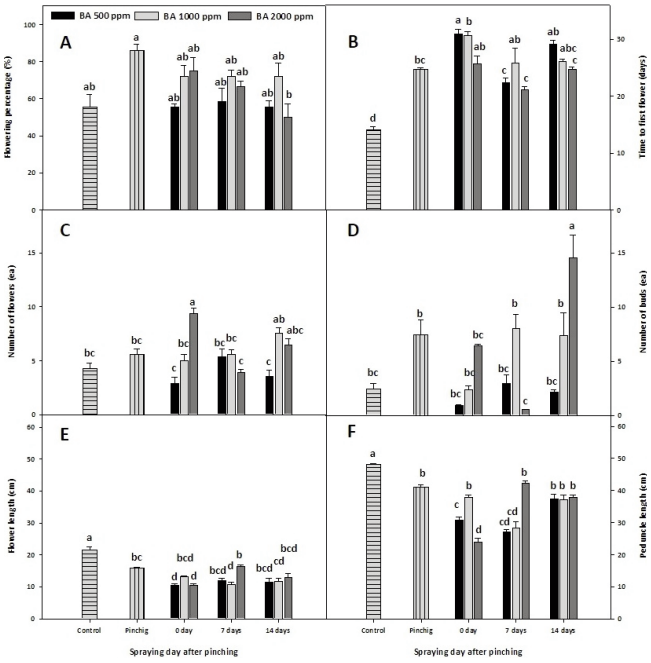

Fig. 2 - Flowering characteristics of *V. dahurica* with pinching and BA spray application.

0 SDP, the peduncle length of *V. dahurica* Steven was significantly lower than that of the control (Fig. 3). Ferrante *et al.* (2006) reported that a 500 µM BA spray application reduced inflorescence length in *Salvia splendens* Kerr Gawl 'Flamex 2000'. However, the effect of BA on flower size depends on BA concentration, method of application, and plant species (Pobudkiewicz, 2008) and can also affect other variables such as tepal length, flower diameter, and inflorescence length (Pobudkiewicz and Nowak, 1994; Ferrante *et al*., 2006; Pobudkiewicz and Treder, 2006).

4. Conclusions

The present study revealed the commercial utility of pinching and BA foliar spray application for induction of multiple branching and control of flowering in *V. dahurica.* Treatment with 1000 mg·L^{-1} BA at 0 days after pinching produced the highest branch number after 10 weeks. Pinching delayed the first flowering time compared with that in the control, but some BA foliar applications accelerated the first flowering time. Pinching and BA foliar application also had the effect of reducing flower length and peduncle length. We anticipate that this knowledge will be beneficial for enhancing branching and controlling flowering

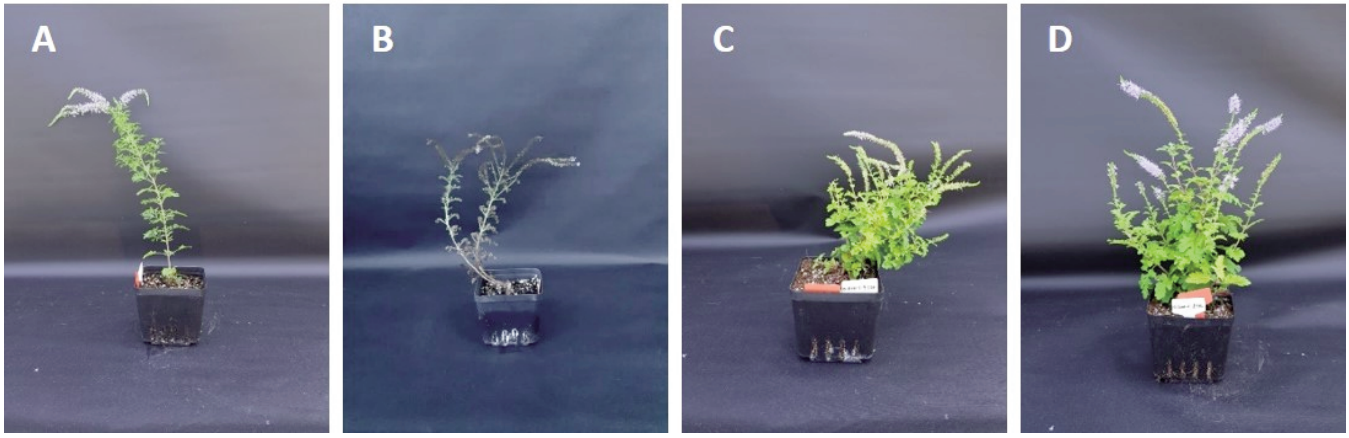

Fig. 3 - Growth and flowering characteristics in pinching and BA application groups 10 weeks after treatment. A) Control. B) Pinching. C) BA 500 ppm 0 week after pinching. D) BA 1000 ppm 0 week after pinching.

time and characteristics of *V. dahurica* in commercial applications.

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