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Original Research Article

Effect of Gibberellin Treatment on Growth and Flowering Characteristics in the Cultivation of *Aquilegia japonica* Nakai & H. Hara

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Abstract - This study was conducted to develop gibberellin treatment technique to enhance flower initiation in *Aquilegia japonica* Nakai & H. Hara. Seedlings were planted in 12㎝-diameter pots on October 2016 and grown in green house. Ambient temperature in the green house was set at minimum 15℃ during day and night to suppress flower initiation at cold temperature condition. Two different types of gibberellin, GA_3 and GA_{4+7}, at 4 different concentration levels 100, 200, 400 and 600 ㎎/L, were tested in this study. Gibberellin was sprayed first at planting and secondly at 1-week after planting. Ten to fifteen ㎖ of gibberellin was sprayed for each pot. Plant height and petiole length were elongated by both gibberellin types, flowering was more enhanced by GA_3 (91.7～100%) compared to of GA_{4+7}. However, abnormal flower was less observed in GA_3 treatment (0～16.7%) than GA_{4+7}. Number of flower stalks per plant ranged from 1.9 to 2.5. Number of flowers per plant ranged from 6.8 to 10.3. Differences in flowering characteristics between treatments were statistically significant. Optimal gibberellin treatment to enhance flower initiation in *A. japonica* Nakai & H. Hara substituting cold treatment was GA_3 at the concentration between 400 ㎎/L to 600 ㎎/L.

Key words − Floral differentiation, Plant growth regulator, Stem elongation

Introduction

Aquilegia belongs to Ranunculaceae which include about 70 perennial plant species. Natural habitats found in meadow and higher altitude area throughout the Northern Hemisphere. Three species, *A. japonica* Nakai & H. Hara, *A. buergariana* var. *oxysepala* and *A. buergariana* var. *oxysepala* f. *pallidiflora* reportedly distribute in Korean Peninsula (Lee, 2003), and Ha *et al.* (2016) reported that *A. buergariana* var. *oxysepala* distribute in Gyeonggi-do province, Korea. *A. japonica* Nakai & H. Hara is widely cultivated in domestic ornamental flower market. *Aquilegia* normally flowers from April to May. Cold temperature triggers floral differentiation and further flower development. Minimum 8 weeks of cold treatment at 4℃ at 12-leaves stage is needed to initiate flower development (Shedron and Weiler, 1982). White *et al.* (1990) reported that floral differentiation and further development of 13 species of *Aquilegia* was not observed after 7 months cultivation at 20℃ regardless of light condition. However, Zhang *et al.* (1991) reported that *Aquilegia* ‘Dove’ and ‘Purple’ flowered after 7~8 months cultivation at 20℃ day and 16℃ night temperature condition. They concluded gibberellin and light treatment accelerated flowering time by 2 weeks compared to control. Gibberellin is a plant physiological metabolism regulator. Lim *et al.* (2015) reported that gibberellin can facilitate germination of *Pinus pumila*. Gibberellin can facilitate floral differentiation and further flower development in some of angiosperm species which usually need long day and cold temperature condition to initiate floral differentiation and development (Ruth *et al.*, 1992). Anton (1957) studied effect of gibberellin treatment on flowering in 17 angiosperm species and GA treatment enhanced flowering of *Daucus carota* L. (biennial plant) and *Hyoscyamus niger* L. (long-day plant), while *Glycine max* L. (short-day plant) did not respond by GA treatment. However, physiological mechanism controlling GA induced flowering in angiosperm is not clear yet. Zeevaart (1983) reported that flowering on *Samolus parviflorus* was inhibited by gibberellin biosynthesis inhibitor treatment.

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This result suggested that the gibberellin biosynthetic pathway determined flowering in *Samolus parviflorus*.

In this study we tested different GA types at various concentrations and proposed optimal treatment enhancing flower initiation and further flower development of *Aquilegia japonica* Nakai & H. Hara.

Materials and Methods

Plant materials and treatment

Aquilegia japonica Nakai & H. Hara seedlings were planted in 12 ㎝-diameter pots on October 2016 and grown in green house. Ambient temperature in the green house was set at minimum 15℃ during day and night to suppress flower initiation at cold temperature condition. Two different types of gibberellin, GA_3 and GA_{4+7}, at 4 different concentration levels 100, 200, 400 and 600 ㎎/L, were tested in this study.

Gibberellin was sprayed first at planting and secondly at 1-week after planting. Ten to fifteen ㎖ of gibberellin was sprayed for each pot. Positions of 10 plants per treatment were completely randomized and experiments were repeated 3 times.

Growth characteristics and Chlorophyll content measurement

Growth characteristics including plant height, leaf length, leaf width and petiole length were measured according to agricultural examination research investigation standard (RDA, 2003) at 45-days after gibberellin treatment.

Chlorophyll content was measured by portable chlorophyll meter (JP/SPAD-502, Konica minolta). Flower characteristics including number of flower stalks, flower stalk length, number of flowers and corolla length were measured following agricultural examination research investigation standard (RDA, 2003) at 60-days after gibberellin treatment. Flowering percentage was calculated and abnormal flower morphology such as flower stalk dwarfness and fading flower color were monitored and frequency of abnormal flower was recorded.

Data analysis

Data were analyzed using CoStat (CoHort software, version 6.45, USA), and statistical significance between treatments were determined by Duncan's multiple range test.

Results and Discussion

Growth characteristics

The growth of *A. japonica* Nakai & H. Hara at 45 days after gibberellin treatment is summarized in Table 1. Plant height was statistically different by gibberellin treatments compared to control. There was no noticeable difference between GA treatment and treatment at 100 ㎎/L concentration. However, plant height was started show difference at higher GA concentrations compared to control. Plant height ranged between 8.2~14.2 ㎝ when GA_3 and GA_{4+7} were treated at 200, 400 and 600 ㎎/L compared to 8.0 ㎝ in control. Gibberellin is one of the plant growth regulator and can cause rice bakanae disease (Galston, 1961).

One of the major effect of gibberellin on plant physiology is plant stem elongation. Phinney *et al.* (1986) proved that dwarfed maize caused by inhibition of endogenous gibberellin biosynthesis could be overcome by synthetic gibberellin treatment. Similarly plant height of *A. japonica* Nakai & H. Hara was elongated by gibberellin treatment in this study. But *A. japonica* Nakai & H. Hara, differently from maize is a rosette type plant with dwarf stem. Because of this reason, plant height elongation of *A. japonica* Nakai & H. Hara is driven by leaf and petiole length elongation rather than stem elongation. In our study, leaf length was 5.5 ㎝ in control while leaf length ranged from 6.1 ㎝ to 7.1 ㎝ when GA_3 at 200~600 ㎎/L was treated and 6.6 ㎝ to 6.8 ㎝ when GA_{4+7} at 200~600 ㎎/L was treated. Petiole length was even greater than leaf length. Petiole length was 5.3 ㎝ in control while it ranged from 8.2 ㎝ to 13.0 ㎝ when GA_3 at 100~600 ㎎/L was treated, and 9.9 ㎝ to 13.2 ㎝ when GA_{4+7} at 100~600 ㎎/L was treated. Similarly, Tamotsu *et al.* (2005) also reported that the petiole of *Arabidopsis thaliana* was elongated by gibberellin treatment.

Gibberellin can elongate leaf or petiole as well as stem or stem node. We observed that leaf width was significantly elongated. Leaf width was 10.1 ㎝ in control while it was 12.2 ㎝ when GA_3 at 400 ㎎/L was treated, and 12.7 ㎝ when GA_3 at 600 ㎎/L was treated and similar trend was observed in GA_{4+7} treatments. Leaf width was 12.3 ㎝ when GA_{4+7} at 400 ㎎/L was treated and 11.8 ㎝ when GA_{4+7} at 600 ㎎/L was treated. Gibberellin can facilitate plant cell division and

Table 1. Effect of gibberellin treatment on growth characteristics in the cultivation of *A. japonica* Nakai & H. Hara

Gibberellin Type	Con. (mg/L)	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	No. of leaves/plant	No. of tillerings/plant	Chlorophyll content (SPAD)
Control		8.0 c[z]	5.5 cd	10.1 cd	5.3 e	28.9 ab	4.0 b	57.2 a
GA_3	100	10.2 c	5.2 d	8.9 d	8.2 d	33.2 a	4.6 ab	57.6 a
	200	19.0 ab	6.1 a-d	10.5 b-d	10.8 bc	18.3 c	3.6 c	54.6 ab
	400	21.1 a	7.0 a	12.2 ab	13.0 a	18.0 c	4.0 b	51.0 b
	600	22.2 a	7.1 a	12.7 a	12.4 ab	21.2 bc	4.2 b	54.6 ab
GA_{4+7}	100	11.5 c	5.6 b-d	9.6 d	9.9 cd	23.3 bc	3.4 c	56.3 ab
	200	16.2 b	6.6 a-c	11.7 a-c	11.6 a-c	27.6 ab	6.1 a	53.7 ab
	400	16.5 b	6.8 ab	12.3 ab	12.8 ab	24.6 bc	5.1 ab	54.7 ab
	600	19.6 ab	6.6 a-c	1.8 a-c	13.2 a	28.0 ab	6.1 a	51.5 b
Two-way ANOVA	Type (A)	*	ns	ns	ns	ns	*	ns
	Con. (B)	***	***	***	***	**	ns	**
	A×B	ns	ns	ns	ns	***	**	ns

[z]DMRT : 5%.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

elongation by increasing RNA synthesis in plant cell (Johri and Varner, 1968). Because of this reason, GA is expected to trigger elongation of leaf length, petiole length and leaf width of *A. japonica* Nakai & H. Hara.

Average number of leaves per plant was 28.9 in control while it ranged from 18.0 to 33.2 by GA_3 and 23.3 to 28.0 by GA_{4+7}. No remarkable difference was observed between GA types. Chlorophyll content was 57.2 in control while 51.0 by GA_3 at 400 mg/L and 51.5 by GA_{4+7} at 600 mg/L. Chlorophyll appeared to be decreased by gibberellin treatment. Physiological disorder on plant morphology by gibberellin has not been reported in *A. japonica* Nakai & H. Hara. But inhibition of chloroplast development and fading leaf color were reported on some plant, similar to our study.

Table 2. Effect of gibberellin treatment on flowering and abnormal flowering percentage in the cultivation of *A. japonica* Nakai & H. Hara

Gibberellin Type	Concentration (mg/L)	Flowering percentage (%)	Abnormal flowering percentage (%)
Control		-	-
GA_3	100	100.0	16.7
	200	91.7	9.1
	400	100.0	-
	600	100.0	-
GA_{4+7}	100	58.3	28.6
	200	66.7	25.0
	400	91.7	18.2
	600	83.3	20.0

Flowering characteristics

A. japonica Nakai & H. Hara started flowering at 60 days after gibberellin treatment while control plants did not flower. Flowering percentage was 91.7~100% by GA_3 and 58.3~91.7% by GA_{4+7} (Table 2). Flowering percentage was statistically different between GA_3 and GA_{4+7} treatment. Anton (1957) reported that gibberellin could induce flowering on some of angiosperm species which need long-day and low temperature condition to flower. We also confirmed that *A. japonica* Nakai & H. Hara could start flowering by gibberellin treatment replacing cold treatment. Gianfagna and Merritt (1998) reported that *Aquilegia* 'Rose−White' flowered after gibberellin treatment without low temperature condition, and flowering time was earlier by GA_{4+7} than GA_3. However in our study, days to flowering was similar between treatments regardless of GA types. Interestingly, flowering percentage was higher by GA_3 than GA_{4+7} (Fig. 1, Table 2). Abnormal flowers such as flower stalk dwarfness and fading flower

Fig. 1. Effect of gibberellin treatment on flower growth in the cultivation of *A. japonica* Nakai & H. Hara (A) Normal Flower, (B) Abnormal flower (Flower stalk dwarfness), (C) Abnormal flower (Fading flower color).

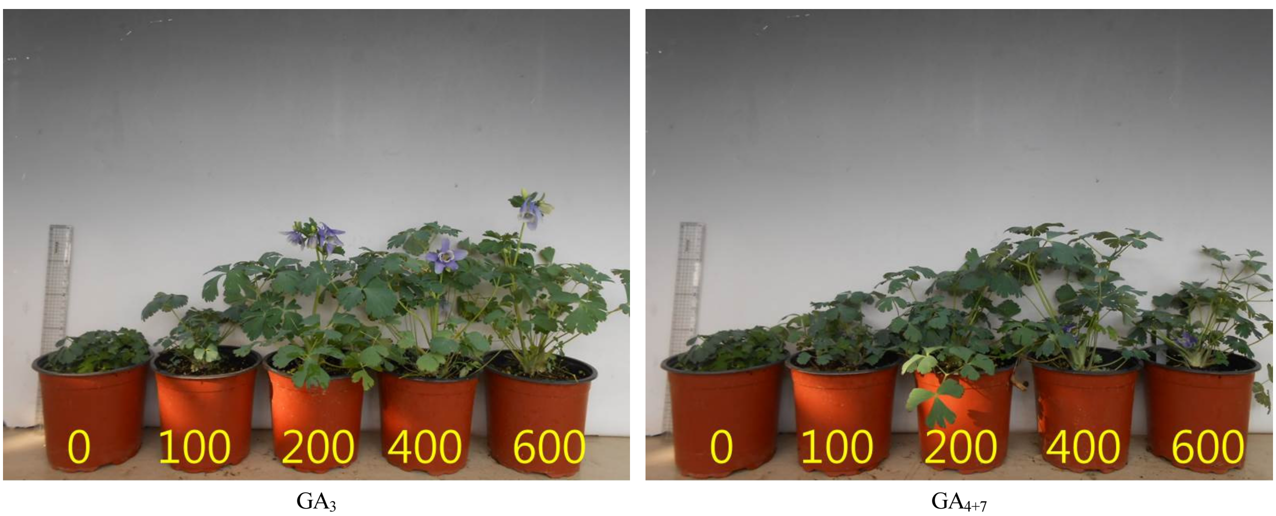

Fig. 2. Effect of gibberellin treatment on flowering characteristic in the cultivation of *A. japonica* Nakai & H. Hara.

color were observed at the frequency of 0~16.7% when GA_3 was treated, and this was lower than GA_{4+7} treatment (18.2~28.6%). Suh *et al.* (1992) reported that the flower color of tulip 'Apeldoorn' was clearer by GA_{4+7} than no treatment control. And Lee (1988) reported that gibberellin facilitated flowering time of *Camellia japonica*. However, our result was different possibly because of the difference in experimental design and condition. Respond of different plant materials by different GA types might result in difference in results as well.

Flowering characteristics of *A. japonica* Nakai & H. Hara were favorable by GA_3 treatment than GA_{4+7} (Fig. 2). Number of flower stalks per plant was higher by GA_3 ranging between 1.9 and 2.5 compared to 0.8 and 2.0 by GA_{4+7} (Table 3). Flower stalk length ranged from 7.2 ㎝ to 10.4 ㎝ by GA_3 compared to 4.5 ㎝ to 8.5 ㎝ by GA_{4+7}. Peduncle length ranged from 0.9 ㎝ to 1.3 ㎝ by GA_3 compared to 0.3 ㎝ to 0.8 ㎝ by GA_{4+7}. Flower stalk and peduncle length especially appeared to elongate longer by GA_3. Interestingly, petiole length was elongated regardless of gibberellin types. Flower stalk diameter was also longer by GA_3 ranging between 3.0 ㎜ to 3.6 ㎜ compared to 1.7 ㎜ to 2.6 ㎜ by GA_{4+7}. But statistical significance was not remarkable compared to other characteristics. Number of flowers per plant was significantly different between GA types and concentrations. Number of flowers per plant was higher by GA_3 than GA_{4+7}. In addition, number of flowers increased with increase in GA concentration. It was 2.0 by GA_{4+7} at 100 ㎎/L compared to 10.3 by GA_3 at 600 ㎎/L. Flower size was also bigger by GA_3 treatment. Corolla height ranged between 2.0 ㎝ and 2.8 ㎝ when GA_3 was treated compared to 1.1 ㎝ to 1.9 ㎝ by GA_{4+7} treatment. Corolla height was statistically different between GA types

Table 3. Effect of gibberellin treatment on flowering characteristics in the cultivation of *A. japonica* Nakai & H. Hara

Gibberellin		No. of flower stalks/plant	Flower stalk length (㎝)	Flower stalk diameter (㎜)	Peduncle length (㎝)	No. of flowers/plant	Corolla height (㎝)	Corolla width (㎝)
Type	Con. (㎎/L)							
GA_3	100	2.3 a[z]	8.1 a-c	3.0 ab	1.2 a	6.8 bc	2.0 bc	3.2 ab
	200	2.0 a	7.2 a-c	3.0 ab	1.3 a	8.8 ab	2.2 a-c	3.8 a
	400	1.9 ab	9.7 ab	3.5 a	1.2 a	8.9 ab	2.8 a	4.3 a
	600	2.5 a	10.4 a	3.6 a	0.9 a	10.3 a	2.6 ab	4.0 a
GA_{4+7}	100	0.8 b	4.5 c	1.7 b	0.3 c	2.0 e	1.1 d	1.9 c
	200	1.5 ab	5.9 a-c	2.0 b	0.4 bc	3.1 de	1.5 cd	2.2 bc
	400	2.0 a	8.5 a-c	2.6 ab	0.8 a-c	6.0 bc	1.9 cd	2.7 bc
	600	1.8 ab	5.7 bc	2.5 ab	0.8 ab	5.7 cd	1.9 b-d	2.4 bc
Two-way ANOVA	Type (A)	*	**	**	***	***	***	***
	Con. (B)	ns	ns	ns	ns	**	**	ns
	A×B	ns	ns	ns	*	ns	ns	ns

[z]DMRT : 5%.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

and concentrations. Corolla width ranged between 3.2 ㎝ and 4.0 ㎝ by GA_3 compared to 1.9 ㎝ to 2.7 ㎝ by GA_{4+7} treatment. There was statistical difference between GA types. In conclusion, GA_3 was found more would be effective in regulating cell division and elongation of *A. japonica* Nakai & H. Hara. Optimal gibberellin for flowering on *A. japonica* Nakai & H. Hara, replacing cold treatment was GA_3 ranging between 400 ㎎/L and 600 ㎎/L.

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