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Growth and Flower Bud Induction in Strawberry 'Sulhyang' Runner Plant as Affected by Exogenous Application of Benzyladenine, Gibberellic Acid, and Salicylic Acid

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Abstract. Strawberry (*Fragaria* × *ananassa*) is one of the most important and popular fruit crops in the world, and 'Sulhyang' is one of the principal cultivars cultivated in the Republic of Korea for the domestic market. The growth and flower induction in strawberry is the process which influences directly on fruit bearing and yield of this crop. In this study, effect of benzyladenine (BA), gibberellic acid (GA_3), and salicylic acid (SA) on growth and flower bud induction in strawberry 'Sulhyang' was investigated. The 3-week-old runner plants, grown in 21-cell propagation trays, were potted and cultivated in growth chambers with 25°C/15°C (day/night) temperatures, 70% relative humidity (RH), and light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD) provided by white light emitting diodes (LEDs). The runner plants were treated with one of three concentrations, 0 (control), 100, and 200 $mg \cdot L^{-1}$ of BA, GA_3, or SA solution. The chemicals were sprayed two times on leaves of runner plants at an interval of two weeks. After 9 weeks the results showed that the application of all chemicals caused reduction of root length and chlorophyll (SPAD) content as compared to the control. The lowest chlorophyll (SPAD) content was recorded in plants treated with GA_3. However, the treatment of 200 $mg \cdot L^{-1}$ GA_3 promoted leaf area, leaf fresh weight, and plant fresh weight. The greatest flower induction (85%) and number of inflorescences (4.3 inflorescences per plant) were observed in the treatment of 200 $mg \cdot L^{-1}$ SA, followed by 100 $mg \cdot L^{-1}$ SA. Overall, results suggest that foliar application of GA_3 solution could accelerate plant growth, while foliar application of SA solution could induce hastened flowering. Further studies may be needed to find out the relationship between GA_3 and SA solutions treated in a combination, and the molecular mechanism involved in those responses observed.

Additional key words : floral differentiation, foliar spray, *Fragaria* × *ananassa*, plant growth regulator

Introduction

Photoperiod and temperature are two main factors affecting the floral induction and runner production in strawberry plants (Durner et al., 1984; Piringer and Scott, 1964). Besides, the application of extra nitrogen (Kim et al., 2013), restricted water supply, or exogenous plant growth regulators (PGRs) can also influence the timing of flowering (Guttridge, 1985; Guttridge and Thompson, 1963; Kim et al., 2017).

The PGRs that are capable of inducing flowering in plants are limited and different from species to species. Among them, cytokinins (Gupta and Maheshwari, 1970; Maheshwari and Venkataraman, 1966), gibberellins (Heide et al., 2013), and salicylic acid (Raskin, 1992) are all documented to induce flowering in some particular species. Benzyladenine (BA), gibberellic acid (GA_3), and salicylic acid (SA) are endogenous PGRs of a phenolic nature. They stimulate cell division and expansion, and control and modulate the initiation and development of shoots and roots.

The BA is a synthetic cytokinin that stimulates cell division in plants, promotes vegetative growth, stimulates flowering in some species, and improves fruit quality (Newton and Runkle, 2015). In previous studies, exogenous application of BA proved to be useful for increasing the number of inflorescences and flowers per inflorescence of orchids (Blanchard and Runkle, 2008; Kim et al., 2000; Lee et al., 1998).

The GA_3 has various effects on plant growth and development. It can stimulate stem and root growth, induce mitotic division in the leaves of some plants, and increase seed germination rate (Guttridge and Thompson, 1963). In short day plants, GA_3 takes part in many physiological pro-

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Plant growth regulator	Week								
	1	2	3	4	5	6	7	8	9
SA	x		x						
BA	x		x						
GA_3	x		x						

Short day (10 hours per day) Long day (14 hours per day) x Weeks of PGR sprayed

Fig. 1. Experimental design used in this study.

cesses such as increasing vegetative development, inhibiting flower bud formation, and enhancing runner growth (Porlingis and Boynton, 1961; Thompson and Guttridge, 1959). Moreover, GA_3 at a certain concentration could cause the internode elongation of the main stem, which is rarely induced by the effect of day length.

The SA is an endogenous PGR which plays a role as a natural inductor of thermogenesis, induces flowering in many plant species, and regulates ion uptake and stomatal conductivity (Raskin, 1992). The flowering induction capability of SA has been well known for decades. The effect of SA on this aspect was first reported in a tobacco tissue culture added with indole acetic acid and kinetin (Lee and Skoog, 1965). Other studies have demonstrated that flowering in many plant species has involved SA, such as stimulated flowering in several genera of the Lemnaceae family (Khurana and Cleland, 1992), restored flowering capability in the nutrient-stressed short day species *Pharbitis nil* (Wada and Takeno, 2010; Wada et al., 2010), and increased numbers of flowers in *Gloxinia* (Martín et al., 2003) and *Calendula officinalis* (Pacheco et al., 2013). Although SA can influence flowering in the short day plants, and some long day plant species, its effect still depends on photoperiod, and therefore, selection of the proper daylength might be crucial for induction of flowering (Cleland and Tanaka, 1979).

Strawberry (*Fragaria* × *ananassa*) is a perennial stoloniferous plant belonging to the family Rosaceae. With the highly desirable taste and a rich source of phytochemicals (Perez et al., 1997), strawberry is one of the most widely cultivated and consumed fruits in the world (Zobayer et al., 2011). Numerous studies have been published regarding cultivation conditions. The environmental factors and their interactions affect the growth, development, and flowering of strawberry plants. In this study, the effect of exogenous applications of PGRs such as BA, GA_3, and SA on growth and flower bud induction at the vegetative growth stage in strawberry 'Sulhyang' was investigated.

Materials and Methods

1. Plant materials and exogenous applications of plant growth regulators

Runner plants of strawberry 'Sulhyang' were collected from a strawberry farm (Sugok-myeon, Jinju, Gyeongsangnam-do, Korea) and rooted in 10cm plastic pots filled with a BVB medium (Bas Van Buuren Substrates, EN-12580, De Lier, The Netherlands). These runner plants were irrigated daily with a greenhouse multipurpose nutrient solution [in $mg \cdot L^{-1}$ $Ca(NO_3)_2 \cdot 4H_2O$ 737.0, KNO_3 343.4, KH_2PO_4 163.2, K_2SO_4 43.5, $MgSO_4 \cdot H_2O$ 246.0, NH_4NO_3 80.0, Fe-EDTA 15.0, H_3BO_3 1.40, $NaMoO_4 \cdot 2H_2O$ 0.12, $MnSO_4 \cdot 4H_2O$ 2.10, and $ZnSO_4 \cdot 7H_2O$ 0.44 and an electrical conductivity 0.8 $mS \cdot cm^{-1}$].

After 3 weeks, the plants were placed in growth chambers and the culture condition was set at 25°C/12°C (day/night) temperature, the light intensity of 300μmol·m^{-2}·s^{-1} PPFD, and 70 ± 5% relative humidity. A 14 h photoperiod (LD, long day) was given at two periods during the experiment, first 3 weeks and from week 7 to the end of the experiment. During weeks 4 to 6, a 10 h photoperiod (SD, short day) was given (Fig. 1).

The runner plants were treated with one of 3 concentrations, 0 (control), 100, and 200$mg \cdot L^{-1}$ of BA, GA_3, or SA solution. These solutions were applied by foliar spray for two times, each time with 10mL per plant, at an interval of 2 weeks (Fig. 1).

2. Measurements of growth and morphological parameters

After 9 weeks, shoot length (from the crown to longest leaf tip), root length, leaf area, chlorophyll index (SPAD), number of runners, runner fresh weight, and plant fresh and dry weights were measured. Leaf area was taken from all leaves detached by using a leaf area meter (LI-3100, LI-

COR Inc., Lincoln, NE, USA). Chlorophyll was measured using a chlorophyll meter (SPAD-502 Plus, Konica Minolta Sensing Inc., Osaka, Japan). Dry weight was measured after drying the samples for 72 hours in a dry oven (FO-450M, Jeio Technology Co., Ltd., Seoul, Korea) at 80ºC.

3. Statistical analysis

The experiment was set up in a completely randomized design with 6 plants per treatment. Data collected were analyzed for statistical significance by the SAS (Statistical Analysis System, V. 9.1, Cary, NC, USA) program. The experimental results were subjected to an analysis of variance (ANOVA) and Duncan's multiple range tests. Graphing was performed with OriginPro 9.0 (OriginLab Corporation, Northampton, MA, USA).

Results and Discussion

The BA, GA_3, and SA are plant growth regulators that play important roles in controlling growth and development of plants (Beyl, 2016; Rivas-San Vicente and Plasencia, 2011). After cultivation for 9 weeks in the control environment, the effect of type and their concentrations of PGRs on the vegetative growth of strawberry plants was significantly different (Fig. 2). The enhancement of shoot length was observed on the plants that were treated with all PGRs at a concentration of 100mg·L^{-1} (Table 1). The greatest shoot length (27.6cm) was recorded in the GA_3 treatment, followed by BA and SA at a concentration of 100mg·L^{-1}. The higher concentrations of these PGRs caused a reduction in shoot length. The shoot length of plants treated with a concentration of 200mg·L^{-1} was 3-6cm shorter as compared to that of plants treated with a lower concentration (Table 1). There were no significant differences in root length among the treatments. However, the application of PGRs in this study caused a reduction in root length as compared to the control, and the greatest root length (14.5cm) was obtained in the control. In all treated plants, the leaf area was considerably larger than that in the control, especially in a higher concentration (200mg·L^{-1}) of PGRs (Table 1). At the same level of PGRs, the greatest leaf area was obtained in the GA_3 treatment, followed by SA and BA. Nonetheless, the SPAD index of chlorophyll showed that the application of BA or GA_3 to the plants at both concentrations caused the decrease in chlorophyll content (Table 1). The leaf area of the plants treated with GA_3

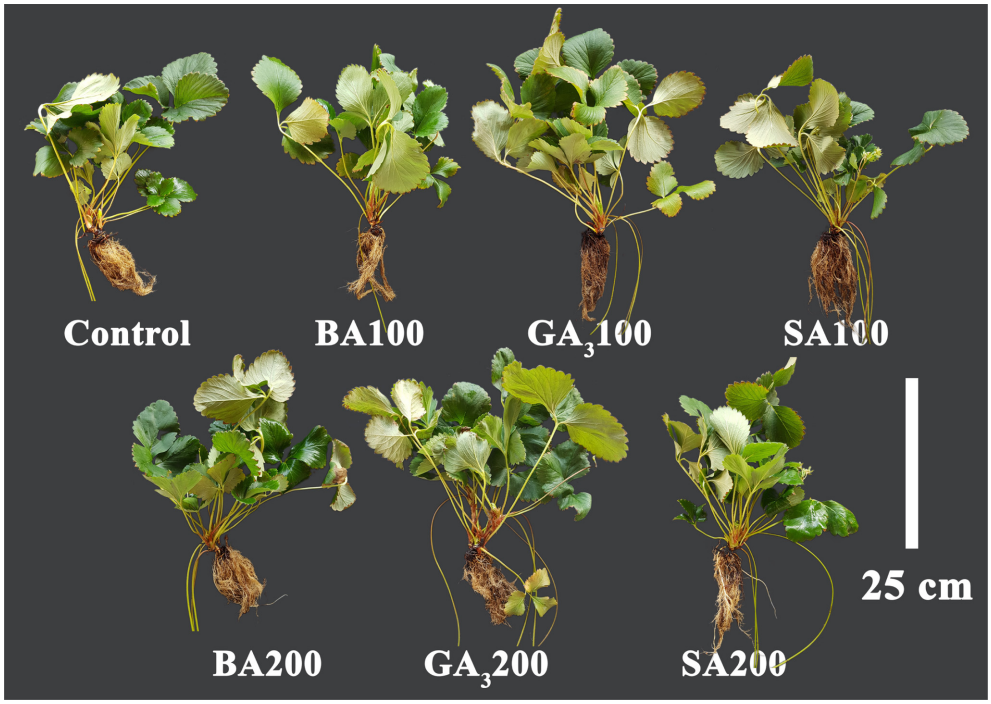

Fig. 2. The growth and development of strawberry 'Sulhyang' after 9 weeks as affected by concentration of foliar-sprayed benzyladenine (BA), gibberellic acid (GA_3), and salicylic acid (SA). Two first letters denote the plant growth regulators, and the last digitals denote the concentration in mg·L^{-1}.

Table 1. Effect of benzyladenine (BA), gibberellic acid (GA_3), and salicylic acid (SA) on growth and development of strawberry 'Sulhyang' after 9 weeks.

PGR (A)	Concentration ($mg \cdot L^{-1}$) (B)	Shoot length (cm)	Root length (cm)	Leaf area (cm^2)	Chlorophyll index (SPAD)	No. of runners	Runner fresh weight (g)	Plant fresh weight (g)	Plant dry weight (g)
0 (Control)		24.7 b[z]	14.5	626 c	44.8 a	1.6 d	9.4 c	31.1 d	7.5 d
BA	100	25.6 b	11.8	723 cb	43.6 ab	3.3 bc	21.3 a	38.5 bc	8.4 bc
	200	22.6 c	11.8	742 cb	40.6 c	3.3 bc	22.5 a	33.7 cd	7.6 cd
GA_3	100	27.6 a	11.6	909 a	44.7 a	4.0 abc	18.4 ab	46.1 a	11.3 a
	200	21.3 c	13.6	978 a	42.7 b	5.0 a	14.2 b	45.2 a	10.4 a
SA	100	25.0 b	13.1	759 b	40.6 c	4.3 ab	18.3 ab	35.1 bcd	8.7 bcd
	200	21.3 c	11.8	781 b	43.6 ab	2.6 cd	18.3 ab	40.5 ab	9.6 ab
F-test[y]	A	***	NS	NS	***	NS	NS	NS	NS
	B	*	NS	***	NS	*	***	***	***
	A x B	**	NS	NS	NS	*	NS	*	NS

[z]Mean separation within columns by Duncan's multiple range test at p=0.05.
[y]NS, *, **, **, Non-significant or significant at $p \leq 0.05$, 0.01, 0.001, respectively.

was enhanced by 56% as compared to the control, and this value in the SA treatment was 25%. Martín et al. (2003) reported that foliar spray in *Gloxinia* at the concentration of 10^{-8} M SA resulted in the increase of leaf area by 49%.

The application of those PGRs and their concentrations in this study also affected the formation and development of runners. The number of runners was greatly increased in the PGRs treatments. The greatest number of runners (5.0 runners per plant) was observed in the treatment of 200$mg \cdot L^{-1}$ GA_3 as compared to that in plants treated with BA (3.3 runners per plant) and SA (2.6 runners per plant). Interestingly, the number of runners in plants treated with BA or GA_3 increased with the increase in their concentration (Table 1). A similar finding by Thompson and Guttridge (1959) reported that the number of runners per strawberry crown increased with the increase in GA_3 concentration. On the other hand, the increase in the concentration of SA resulted in decrease in the number of runners. Weight of the runners separated from the mother plants after 9 weeks of cultivation, showed that the BA strongly enhanced the runner fresh weight, either at low or high concentration, by at least two times as compared to the control. However, an abnormal phenomenon was observed in the plants treated with GA_3. The first effects of those PGRs on strawberry plants appeared at about two weeks after the treatment with PGRs. The youngest leaf of the treated plants was slightly paler and larger as compared to that of other treatments. The internodes of mother plants and new daughter plants in these plants were oddly elongated (Figs. 2 and 3). Kang et al. (2018) and Thompson and Guttridge (1959) also observed the same phenomenon in strawberry plant treated with GA_3. This phenomenon may cause some troubles in utilizing and growing of the daughter plants as well as the mother plants.

Fresh weight of strawberry plants was significantly affected by PGRs treatments combined with their concentrations (Table 1). The greatest plant fresh weight was observed in the treatment of GA_3 regardless of its concentration. Plant fresh weight was 46.1 and 45.2g per plant in the treatment with GA_3 at a concentration of 100 and 200$mg \cdot L^{-1}$, respectively, as compared to the plant in the control treatment having only 31.1 g per plant. Plant dry weight showed the same trend as the fresh weight (Table 1). In the agreement with this results, a previous study demonstrated that the application of 100$mg \cdot L^{-1}$ GA_3 on strawberry plants only promoted in the initiation of runners, shoot length and petiole length, but not flower initiation (Thompson and Guttridge, 1959). In the long day condition, the vegetative growth of strawberry plants, such as the development of runners in the leaf axils, elongation of petiole and upright growth, was remarkably enhanced. Therefore, GA_3 would be considered as a substitute for a growth-promoting hormone which was typically produced naturally under long day condition in strawberry plants.

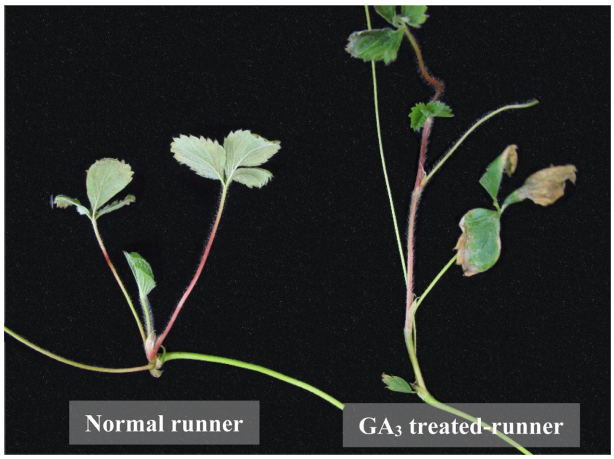

Fig. 3. The abnormal runner observed in the gibberellic acid (GA_3) treatment as compared to the normal runners observed in the other treatments.

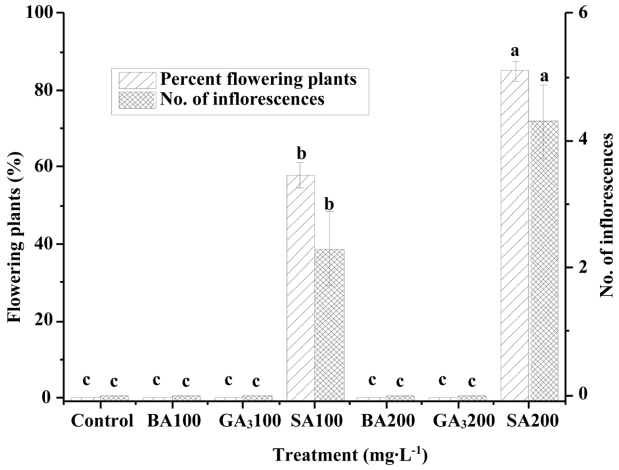

Fig. 4. Effect of concentration of foliar-sprayed benzyladenine (BA), gibberellic acid (GA_3), and salicylic acid (SA) on the number of flowers in strawberry 'Sulhyang' measured after 30 days. Means accompanied by different letters are significantly different ($p < 0.05$) according to the Duncan's multiple range test. Vertical bars indicate the standard error (n=6).

The flowering plants and number of inflorescences per plant were only observed in the treatment of SA. They were increased with the increase of SA concentration (Fig. 4). Results showed that up to 85% of plants treated with 200mg·L^{-1} SA flowered after 6 weeks from the beginning of short day period. However, only 58% of plants flowered in the treatment of 100mg·L^{-1} SA and there were no flowers observed in the other treatments. The number of inflorescences also showed the same trend. The greatest number of inflorescences (4.3 inflorescences per plant) was obtained in the 200mg·L^{-1} SA, followed by 100mg·L^{-1} SA (2.3 inflorescences per plant). Similar results were also observed in *Lemna gibba* G3, in which the exogenous application of SA dramatically induced flowering while having a slight effect on subsequent development of flowers (Cleland and Tanaka, 1979; Conn, 1984). The mechanism of flower induction effect of SA in the plant is mysterious. Research suggested that to induce flowers, SA might behave as a chelating agent in the plant (Oota, 1975). And this hypothesis has been reinforced by the fact that chelating agents could induce flowering in Lemnaceae (Oota, 1972; Seth et al., 1970).

Overall, the results indicated that the application of BA, GA_3, or SA could change various growth traits in strawberry plants. In particular plant species, BA displayed as the ideal factor for increasing percentage plants with inflorescence, and inducing earlier flowering. However, the present study demonstrated that the BA did not have the same effect in strawberry plants tested. Vegetative growth of strawberry plants treated with GA_3 was significantly enhanced. However, the abnormal elongation of internodes of main stem and daughter plants with the 200mg·L^{-1} GA_3 could be disadvantages. The recommended concentration of GA_3 would be 100mg·L^{-1} accounting both normal development and high growth traits. It should be noted that the GA_3 accelerated the vegetative growth, and consequently the flowering time may be delayed. As expected, SA played an important role in increasing the number of inflorescences in strawberry plants. At a concentration of 200mg·L^{-1} SA, not only the number of inflorescences, but also many growth traits were virtually enhanced as compared to the control. However, because of the limitation in time of this study, the yield and fruit quality were not assessed. A possibility may arise that despite hastening in flowering in the SA treatment, more vigorous plants in the GA_3 treatment may induce more number of flowers to give increased fruit yield. Therefore, further studies are still needed, especially, to reveal the hypothesis that the combination of GA_3 and SA may improve by not only increased vegetative growth, but also shortened time to flower induction.

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벤질아데닌, 지베렐린산, 살리실산이 '설향' 딸기묘의 생장과 화아 유도에 미치는 영향

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적 요. 딸기는 세계적으로 중요하고 인기있는 과채류이며 '설향'은 국내 시장에서 재배되고 있는 주요 품종 중 하나이다. 딸기의 생장과 화아 유도는 이 작물의 과실에 수량에 직접적으로 영향을 미치는 과정이다. 본 연구에서는 벤질아데닌(BA), 지베렐린 산(GA_3), 살리실 산(SA)이 '설향' 딸기의 생장과 화아 유도에 미치는 영향을 조사하였다. 21구 트레이에서 번식된 지 3주가 경과한 런너묘를 온도는 25ºC/15ºC(주간/야간), 상대습도는 70%, 광원은 백색 LED, 광도는 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD로 유지되는 생장 챔버에서 재배하였다. BA, GA_3 및 SA를 각 0(대조구), 100, 200 $mg \cdot L^{-1}$로 런너묘에 처리하였다. 이러한 생장조절제를 런너묘의 잎에 2주 간격으로 2회 엽면살포하였다. 9주 후의 생육을 비교한 결과, 생장조절제를 엽면살포한 모든 처리에서 대조구에 비해 근장과 엽록소함량(SPAD)이 감소하는 경향을 보였다. GA_3 처리에서 엽록소함량(SPAD)이 가장 낮았다. 하지만 GA_3 200 $mg \cdot L^{-1}$ 처리에서 엽면적, 잎 생체중, 식물 생체중이 증가하였다. 화아유도율과 화수는 SA 200 $mg \cdot L^{-1}$ 처리에서 각 85%와 식물체당 4.3개로 가장 높았고 그 다음으로 SA 200 $mg \cdot L^{-1}$ 처리에서 높았다. 전체적으로 GA_3 처리에서 식물 생장을 향상시켰고, SA 처리에서는 개화를 촉진하였다. 더 나아가 GA_3와 SA를 혼합한 처리를 추가한 연구를 수행하여 생장조절제간의 관계를 구명하고 그 결과에서의 분자 메커니즘과 관련된 반응을 조사하는 것이 필요하다고 판단된다.

추가주요어: 화아분화, 엽면살포, 딸기, 식물생장조절제