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Prohexadione-Calcium Application during Vegetative Growth Affects Growth of Mother Plants, Runners, and Runner Plants of Maehyang Strawberry

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Abstract: Strawberry (*Fragaria* × *ananassa* Duch.) is an important horticultural crop that is vegetatively propagated using runner plants. To achieve massive production of runner plants, it is important to transfer the assimilation products of the mother plant to the runner plants, and not to the runner itself. Application of prohexadione–calcium (Pro–Ca), a plant growth retardant with few side effects, to strawberry is effective in inhibiting transport of assimilates to runners. This study aimed to determine the optimum application method and concentration of Pro–Ca on the growth characteristics of mother plants, runners, and runner plants for the propagation of strawberry in nurseries. Pro–Ca was applied at the rate of 0, 50, 100, 150, or 200 mg·L^{-1} (35 mL per plant) to plants via foliar spray or drenching under greenhouse conditions at 30 days after transplantation. Petiole lengths of mother plants were measured 15 weeks after treatment; growth was suppressed at the higher concentrations of Pro–Ca regardless of the application method. However, the crown diameter was not significantly affected by the application method or Pro–Ca concentration. The number of runners was 7.0 to 8.2, with no significant difference across treatments. Runner length was shorter at higher concentrations of Pro–Ca, especially in the 200 mg·L^{-1} drench treatment. However, fresh weight (FW) and dry weights (DW) of runners in the 50 mg·L^{-1} Pro–Ca drench treatments were higher than controls. Foliar spray and drench treatments were more effective for runner plant production than the control; a greater number of runner plants were produced with the 100 and 150 mg·L^{-1} Pro–Ca foliar spray treatment and the 50 and 100 mg·L^{-1} drench treatment. The FW and DW of the first runner plant was not significantly different in all treatments, but DW of the second runner plant, and FW and DW of the third runner plant were greatest in the 50 mg·L^{-1} Pro–Ca drench treatment. These results suggested that growth and production of runner plants of Maehyang strawberry were greatest under the 50 mg·L^{-1} Pro–Ca drench treatment.

Keywords: drench; foliar spray; *Fragaria* × *ananassa*; runner length

1. Introduction

Plant growth retardants (PGRs), like anti-gibberellins, have been used in agricultural industries for decades to improve the quality and quantity of horticultural crops [1,2]. PGRs such as daminozide, paclobutrazol, chlormequat chloride, uniconazole, and prohexadione–calcium (Pro–Ca) are used to control plant size and shape, specifically to reduce vegetative growth [3–6]. Among them, Pro–Ca has various advantages over other PGRs; it has negligible toxicological effects on mammals and a short persistence period in plants and soil [7,8]. In addition, Pro–Ca application delays senescence by lowering ethylene production within plants [9], and enhances resistance to disease and insects by inhibiting the biosynthesis of phenol [10]. Pro–Ca is a gibberellin (GA) biosynthesis inhibitor, which is the co-substrate for dioxygenases catalyzing hydroxylations involved in the late stages of GA biosynthesis. The main target of Pro–Ca seems to be 3β-hydroxylase, an enzyme that catalyzes primarily the conversion of inactive GA_{20}/GA_9 into highly active GA_1/GA_4 in either the early 13-hydroxylated pathway or the early non-13-hydroxylation pathway, respectively [11–13]. Reducing plant height is an important effect of PGR application, leading to increased quality and yield, and decreases in cost, space, and labor [13,14]. Pro–Ca has been shown to reduce and regulate the growth of crops such as petunia, impatiens, rice, chrysanthemum, pear, and various vegetables without the negative effects of decline in fruit quality and yield [8,13–16]. Most studies on the influences of Pro–Ca have focused on seed-propagated crops, but there are few reports on the application of Pro–Ca to vegetatively-propagated crops [16–18].

In fruit and vegetable crops, the quality of seedlings and other propagules are known to be very important for the quality and quantity of subsequent production. Accordingly, the quality of strawberry (*Fragaria* × *ananassa* Duch.) propagules has a direct influence on the yield and quality of fruit after transplanting; propagule quality is estimated to account for 80% of the whole crop cultivation quality [19]. Unlike fruit and vegetable crops such as tomato, cucumber, and watermelon, strawberry is distinctive in that it requires a lot of time and labor for the production of runner plants from vegetative organs. Strawberries are cultivated nurseries from the end of March to the beginning of September in the Republic of Korea. Various processes, such as transplanting of the strawberry mother plants, occurrence of runners and runner plants, fixation of runner plants, removal of runner plants from mother plants, and induction of flower bud differentiation require a period of five to six months [20,21]. Initiation of runner and runner plants occurs from May to June. Previous studies have focused on nutrient uptake, such as management of calcium fertilization [22], phosphorus [23], bicarbonate [24], and sulfur [25], of the strawberry mother plant. In addition, strawberry is known to be more sensitive to salinity than the other crops [26]. For that reason, previous studies have been conducted to determine the optimum electrical conductivity (EC) levels of nutrient solutions for mother and runner plants during the nursery period [27,28].

Although previous studies have reported production of large numbers of runners and runner plants, there are insufficient studies on runner length. During runner production, runners that are overly long are difficult to manage and require a considerable labor force within the restricted space of nurseries. Furthermore, production of runners and runner plants within high planting densities can reduce their quality and yield. Thus, there is a need for effective research to improve the quality and quantity of runner plants by shortening the runner without negatively effecting physiology.

In the present study, we hypothesize that the application method and concentration of Pro–Ca will improve the quantity and quality of strawberry runner plants by promoting their growth and development. To test our hypothesis, we investigated the growth of mother plants and propagation of runners and runner plants, and measured the biomass of the first, second, and third runner plants of the Maehyang cultivar strawberry for export in the Republic of Korea under greenhouse conditions, as well as confirmed the feasibility of practical application of the technology.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The experiment was conducted in an even-span greenhouse (9 × 24 × 3 m) set up as a strawberry nursery with a hydroponic system and located at Gyeongsang National University in the Republic of Korea. Mother plants of strawberry (*Fragaria* × *ananassa* Duch. 'Maehyang') were planted at a density of four plants per pot using a strawberry cultivation container (61 × 27 × 18 cm, Hwaseong Industrial Co. Ltd., Okcheon, Korea) filled with commercial strawberry-growing medium (BC2, BVB substrates Co. Ltd., De Lier, the Netherlands) on 20 March, 2018. During the cultivation period, the temperature of the even-span greenhouse was maintained at 26 ± 5 °C during the day and 16 ± 5 °C at night, 60 ± 10% relative humidity, and a natural photoperiod of 12–14 h. Well-rooted mother plants were fertilized using drip tape with Bas Van Buuren (BVB) strawberry solution from the Netherlands (in $mg \cdot L^{-1}$: $Ca(NO_3)_2 \cdot 4H_2O$ 613.0, KNO_3 187.0, KH_2PO_4 227.0, K_2SO_4 114.0, $MgSO_4 \cdot H_2O$ 275.0, NH_4NO_3 84.0, Fe–EDTA 10.60, H_3BO_3 0.31, $MnSO_4 \cdot 5H_2O$ 2.54, $ZnSO_4 \cdot 7H_20$ 2.21, $CuSO_4 \cdot 5H_2O$ 0.16, and $Na_2MoO_4 \cdot 2H_2O$ 0.12, pH 5.8, and EC 1.5 $dS \cdot m^{-1}$). Chemical analysis of tap water revealed a composition of Ca^{2+} 0.40, Mg^{2+} 0.20, NH_4^+ 0.10, NO_3^- 0.10, HCO_3^- 0.71 $mmol \cdot L^{-1}$, pH 7.3, and EC 0.2 $dS \cdot m^{-1}$. During the cultivation period, 300 to 450 mL per culture pot was supplied two or three times (10 min per time), and the nutrient solution was adjusted to EC 1.5 $dS \cdot m^{-1}$ and pH 5.8. Prior to the treatment of strawberry mother plants with Pro–Ca, old leaves, axillary buds, and all runners were removed. Pesticides were applied every 7 days to control major diseases and insects, such as powdery mildew, anthracnose disease, *Bradysia agrestis*, aphids, and mites.

2.2. Application Methods and Concentration of Pro–Ca

Four different concentrations, 50, 100, 150, and 200 $mg \cdot L^{-1}$ of Pro–Ca (prohexadione–calcium, Sigma–Aldrich Co. Ltd., Saint Louis, MO, USA), were applied to plants via a foliar spray or drench. The Pro–Ca treatment for mother plants was applied one time under greenhouse conditions at 30 days after transplanting on 18 April, 2018. Foliar spray of Pro–Ca was applied using a hand sprayer, and the drench treatment of Pro–Ca was applied by slowly pouring it into the medium. The same volume of Pro–Ca solution (35 mL per plant) was applied in all treatments. Control plants were treated with tap water (35 mL per plant).

2.3. Measurements of Plant Growth Characteristics

Numbers of leaves, petiole length, soil plant analysis development (SPAD), number of runners, and runner length were measured each week after treatment with Pro–Ca for six weeks. The number of leaves and runners were counted by eye. Chlorophyll content was represented as the SPAD, which was measured using a portable chlorophyll meter (SPAD-502, Konica Minolta Inc., Tokyo, Japan). Growth parameters of mother plants, runners, and runner plants, such as petiole length, number of leaves, crown diameter, shoot fresh weights (FW), and dry weights (DW) of mother plants, leaf area, runner length, FW and DW of runners, number of runner plants, and FW and DW of the first, second, and third runner plants were measured at 15 weeks after treatment. The crown diameter was measured using a vernier caliper (CD-20CPX, Mitutoyo Co. Ltd., Kawasaki, Japan). Leaf area was measured using a leaf area meter (LI-3000, LI-COR Inc., Lincoln, NE, USA). The FW of the mother plant shoot, runner, and runner plants (first, second, and third) were measured using an electronic balance (EW220-3NM, Kern and Sohn GmbH, Balingen, Germany), and the DW of the mother plant shoot, runner, and the first, second, and third runner plant were measured the in a similar manner. Plant tissue was dried in an oven (Venticell-220, MMM Medcenter Einrichtungen GmbH, Planegg, Germany) at 70 °C for 72 h, and DW measured using an electronic balance.

2.4. Measurement of Chlorophyll Fluorescence (Fv/Fm)

For assessing photosystem II (PS II) performance, chlorophyll fluorescence measurements were taken from dark-adapted leaves of all treatments using a portable leaf fluorometer (FluorPen FP 100, Photon System Instruments, Drasov, Czech Republic). After dark adaptation for 30 min, chlorophyll fluorescence was measured on the upper surfaces of the leaves [29]. The minimum fluorescence (Fo) was obtained by measuring the light at 0.6 kHz and photosynthetic photon flux density (PPFD) below 0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ using a red LED light. The maximum fluorescence (Fm) was measured by irradiating saturation light of 7000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 20 kHz for 0.8 s. The variable/maximum fluorescence ratio

(Fv/Fm) was calculated by the formula Fv/Fm = (Fm − Fo)/Fm [30]. Fv/Fm represents the maximum quantum yield of PS II photochemistry measured in the dark-adapted state. To measure the Fv/Fm, leaves of six mother plants were used for each treatment.

2.5. Statistical Analysis

The experimental treatments were randomized in a split-plot design, assigning the Pro–Ca application methods to the main plots and the Pro–Ca concentrations to the sub-plots. Each treatment included four plants and was repeated three times. The statistical analyses were performed using an SAS program (SAS 9.4, SAS Institute Inc., Cary, NC, USA). The experimental results were subjected to analysis of variance (ANOVA) and Tukey's tests. Graphing was performed with the SigmaPlot program (SigmaPlot 12.0, Systat Software Inc., San Jose, CA, USA).

3. Results and Discussion

3.1. Growth Characteristics of Mother Plants and Runners after Pro–Ca Treatment for Six Weeks

Figure 1 summarizes the growth characteristics of mother plants and runners of Maehyang strawberry treated with two application methods of four different Pro–Ca concentrations for six weeks. The petiole length of mother plants significantly decreased in the Pro–Ca treatment groups compared to control plants, regardless of the application method. Further, the higher concentration of Pro–Ca showed an inhibition effect on petiole length extension (Figure 1A). In previous studies, it has been shown that PGR treatment in *Spathiphyllum*, rice, chrysanthemum, cucumber, apple, and tomato was associated with suppression of plant stretchiness [8,14,31–34]. In the present study, the same results were obtained in the Pro–Ca treatment groups. Additionally, there was no negative effect on the development of new leaves in Pro–Ca treatment groups, except for treatments at four and five weeks with 200 mg·L^{-1} Pro–Ca applied as a drench (Figure 1B). Similarly, Reekie et al. [17] observed that the number of leaves was not affected by 62.5 mg·L^{-1} Pro–Ca applied as a foliar spray on the strawberry cultivars Sweet Charlie and Camarosa. Plant height was inhibited in tomato by Pro–Ca without affecting the leaf number during the seedling growth period [2]. These results are explained by the fact that GA regulates cell elongation rather than cell division. The SPAD was significantly higher in Pro–Ca treatment groups than in the control group during the four-week period after treatment, especially in the foliar spray treatment with 200 mg·L^{-1} Pro–Ca (Figure 1C). Generally, PGRs inhibited plant size and biomass while increasing chlorophyll content. This effect is presumably because PGRs reduce GA biosynthesis in the plant, furthermore, cell elongation was decreased, which is the main physiological function of Pro–Ca [35]. According to Yoon and Sagong [4], the leaf area of apple trees decreased, while the specific leaf area and chlorophyll content were increased by Pro–Ca treatment. In addition, Chinese cabbage treated with Pro–Ca at 400 mg·L^{-1} exhibited significantly increased chlorophyll content compared to non-treatment [1]. Appropriate inhibition of plant vegetative growth may increase light use efficiency under high plant density. Furthermore, the increase of SPAD could improve the photosynthetic rate of individual leaves. The Fv/Fm of plants grown under normal conditions is generally in the range 0.80 to 0.84, indicating the stress index and maximum quantum yield of PS II photochemistry [29,36] (Figure 1D). In the present study, we confirmed chemical stress was caused by Pro–Ca application, but that the range of normal growth conditions (0.80 to 0.84) occurred in all treatment groups, except for plants in the first week following treatment. On the contrary, Fv/Fm was 0.778 and 0.815 at weeks two and five, respectively, for the control group, which was lower than Pro–Ca treatment groups. Similarly, Ilias et al. [7] reported that Fv/Fm was not decreased by Pro–Ca treatment in the okra cultivars Psalidati and Clemson Spineless. These results suggest that Pro–Ca treatment does not impose a negative stress on mother plants of strawberry. Runner length was significantly shorted in the higher treatment concentrations of Pro–Ca; notably, runner length was significantly inhibited at 200 mg·L^{-1} Pro–Ca with the drench treatment (Figure 1E). Pro–Ca blocks the conversion of physiologically inactive GA_{20}/GA_9 into highly physiologically active GA_1/GA_4. Pro–Ca remains in the plant and medium for 3–4 weeks after treatment and inhibits vegetative growth. After that period, inhibition of endogenous GA biosynthesis by Pro–Ca decreases and vegetative growth resumes [37,38]. Runner length tended to increase after treatment with50 and 100 mg·L^{-1} Pro–Ca as foliar spray and 50 mg·L^{-1} Pro–Ca as a drench 4–6 weeks after treatment. This result implied that the elongation of runners was due to the lower levels of residual Pro–Ca in the mother plant, which stimulated vegetative growth. The occurrence of runners did not differ significantly from the control treatment, except for 200 mg·L^{-1} Pro–Ca applied as a drench (Figure 1F).

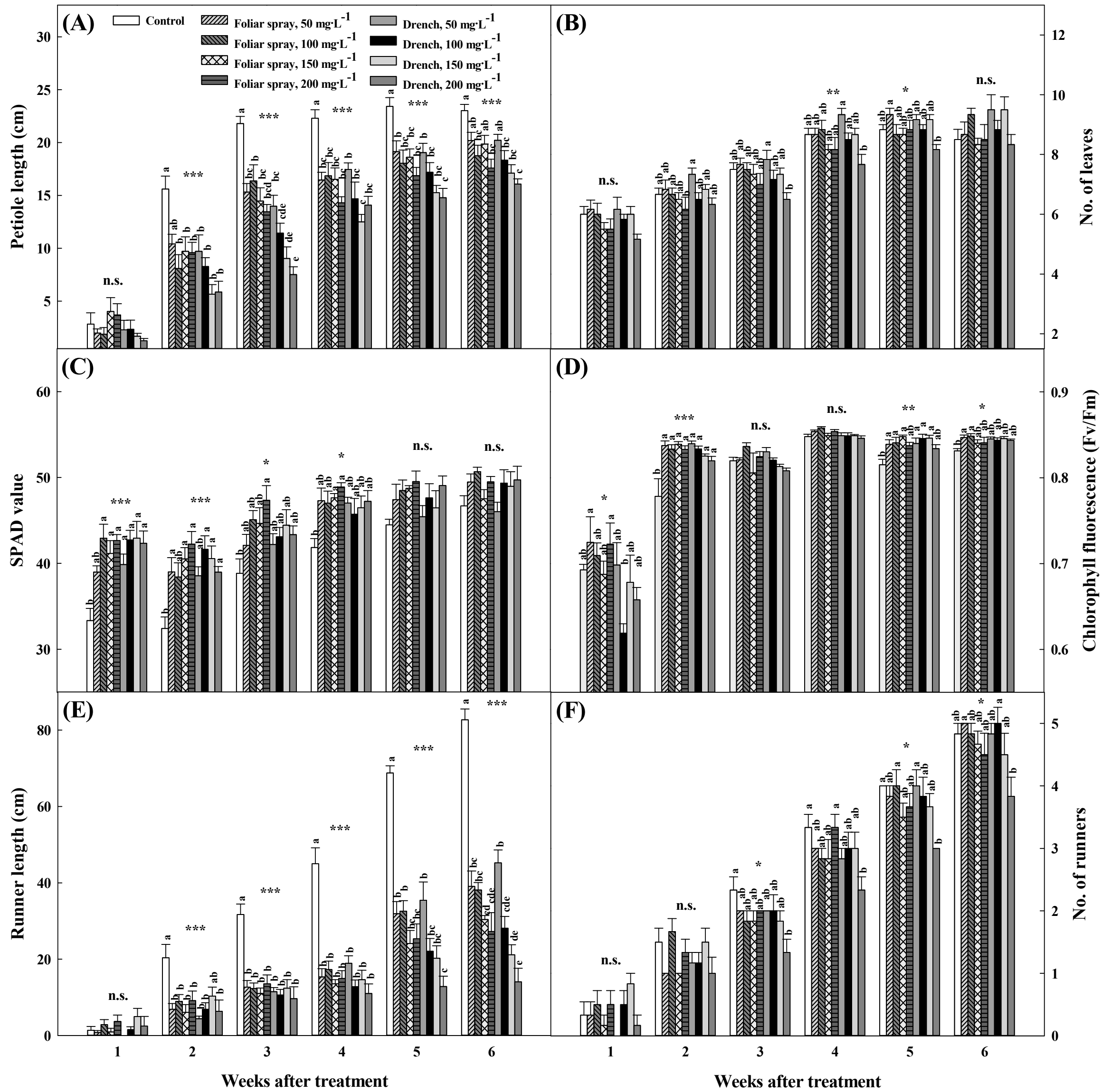

Figure 1. Petiole length (**A**), number of leaves (**B**), SPAD value (**C**), chlorophyll fluorescence (**D**), runner length (**E**), and number of runners (**F**) of strawberry cultivar Maehyang as affected by application method and concentration of prohexadione–calcium (Pro–Ca) at 1, 2, 3, 4, 5, and 6 weeks following treatment. Vertical bars represent standard deviation from the mean (n = 6). Different letters in the same column indicate significant differences based on Tukey's test ($p \leq 0.05$). n.s, *, **, *** no statistically significant difference or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

3.2. Growth Characteristics of Mother Plants at 15 Weeks after Treatment with Pro–Ca

The growth characteristics of the mother plant at 15 weeks following treatment with Pro–Ca, the method of application, and the concentration are shown in Table 1. The petiole length significantly inhibited drench application more than foliar spray. The combined FW and DW of leaves and petioles and leaf area were decreased more by the Pro–Ca drench application than the foliar spray, but there was no significant difference between the two application method treatment groups. In the treatment with PGRs, foliar spray was rapidly absorbed through the leaves and a larger amount of PGR was required. On the other hand, the effect of the drench application was slow but effective for a long time period even at low concentrations [39]. In the present study, the same amount (35 mL) of treatment was applied to all mother plants regardless of application method. Therefore, it is considered that the shorter petiole length was a function of drench application, but not foliar application. In terms of the Pro–Ca concentration, increasing the Pro–Ca concentration caused a reduction in the vegetative growth in the strawberry cultivar Maehyang. The petiole length was significantly inhibited at the 200 mg·L^{-1} concentration treatment. However, the crown diameter and the FW and DW of the crown were not significantly different between the treatment groups. In particular, the growth of the mother plant slowed at 200 mg·L^{-1} Pro–Ca applied as a drench, even when the residual period had passed. Reekie et al. [17] reported that in the strawberry cultivars Sweet Charlie and Camarosa, DW of leaf, stem, and root were similar to the control at 42 days after treatment with 62.5 mg·L^{-1} Pro–Ca as a foliar spray. However, in the present study, the lowest concentration of Pro–Ca (50 mg·L^{-1}) resulted in lower combined FW and DW of leaves and petioles, compared to the control. Generally, strawberry plants show different responses depending on the cultivar being treated [40,41], and these characteristics are controlled by genetic traits of the cultivars [42]. Barreto et al. [43] reported that concentrations of 200 and 400 mg·L^{-1} Pro–Ca markedly reduced vegetative growth indicators such as petiole length and leaf area in the strawberry cultivars Camarosa and Aromas. Thus, a concentration of 100 mg·L^{-1} Pro–Ca was suggested as the most appropriate concentration to apply to the cultivars Camarosa and Aromas. In the present study, however, the lower concentration 50 mg·L^{-1} Pro–Ca was sufficient to inhibit vegetative growth in the cultivar Maehyang. Therefore, it is likely that the effective concentration of Pro–Ca for vegetative growth inhibition will be different for each strawberry cultivar.

Table 1. Growth characteristics of the strawberry cultivar Maehyang mother plants as influenced by application method and concentration of Pro–Ca at 15 weeks after treatment.

Experiment Factor	Petiole Length (cm)	Crown Diameter (mm)	Fresh Weight (g/plant)		Dry Weight (g/plant)		Leaf Area (cm^2/plant)
			Leaves + Petioles	Crown	Leaves + Petioles	Crown	
Application method							
Foliar spray	18.6	16.5	72.6	6.9	17.2	1.4	1561.1
Drench	16.4	16.8	67.3	7.2	15.6	1.5	1449.3
	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Concentration (mg·L^{-1})							
Control (0)	23.9 ^{a}	17.0	87.2 ^{a}	6.8	26.9 ^{a}	1.4	1867.3 ^{a}
50	19.3 ^{b}	16.3	75.5 ^{ab}	7.4	18.0 ^{b}	1.5	1538.7 ^{b}
100	18.0 ^{bc}	16.4	71.5 ^{b}	7.3	16.3 ^{b}	1.5	1541.0 ^{b}
150	17.4 ^{bc}	16.8	66.1 ^{b}	6.3	15.6 ^{b}	1.3	1557.7 ^{b}
200	15.5 ^{c}	17.1	66.8 ^{b}	7.2	15.6 ^{b}	1.5	1383.4 ^{b}
		n.s.		n.s.		n.s.	

Within each column, * significant difference at $p \leq 0.05$; n.s. no statistically significant difference; means followed by different letters are significantly different according to the Tukey's test at $p \leq 0.05$.

3.3. Growth Characteristics of Runners and Runner Plants at 15 Weeks after Treatment with Pro–Ca

There was no significant difference in the number of runners irrespective of application method and concentration of Pro–Ca at 15 weeks after treatment (Figure 2A). Foliar spray and drenching of Pro–Ca were more effective for runner plant production than the control. The greatest number of runner plants were produced by applications of 100 and 150 mg·L^{-1} Pro–Ca by foliar spray, and

50 and 100 mg·L^{-1} Pro–Ca by drenching (Figure 2B). The number of runner plants per mother plant was the lowest (10.6) after application of 200 mg·L^{-1} as a drench, which was lower than the control. In previous studies, foliar spray application of GA produced large numbers of runners and runner plants of strawberries in the nursery period [42,44]. In the present study, however, Pro–Ca, an anti-gibberellin was more effective for the production of runner plants using both application methods and at concentration of 50 to 150 mg·L^{-1}, compared to the control. These results implied that the assimilation products used for the growth of mother plants were more effectively translocated for the development of runners and runner plants after application of Pro–Ca.

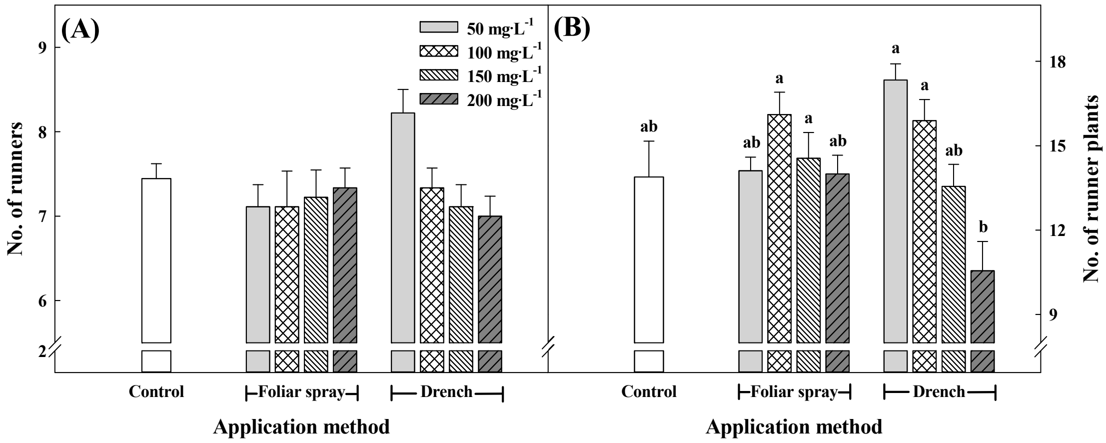

Figure 2. Number of runners (**A**) and runner plants (**B**) of the strawberry cultivar Maehyang as affected by application method and concentration of Pro–Ca at 15 weeks after treatment. Vertical bars represent the standard deviation of the mean (n = 9). Different letters in the same column indicate significant differences based on Tukey's test ($p \leq 0.05$).

The FW and DW of runners were similar to those of runner plants (Figure 3A,B). As the concentration of Pro–Ca increased, the FW and DW of runners decreased, and especially, negative correlations were observed from plants treated by drench application. In tomato, plant height was reduced more effectively by Pro–Ca applied as a drench application than as a foliar spray [2]. The results of this study also showed that reducing the FW and DW of the runners occurred more effectively as the concentration increased in the drench application rather than the foliar spray. According to Savini et al. [45], the runner acts as a transporter to translocate assimilates, nutrient elements, and water from the mother plant to the runner plant. Therefore, heavier biomass of the runners has a positive effect on plant-to-plant communication. Consequently, the increased FW and DW of the runner was a positive achievement of the application of 50 mg·L^{-1} Pro–Ca as a drench.

Total runner length and comparison of runner lengths are shown in Figure 4A,B. The total runner length was shorter in all Pro–Ca treatment groups than in the control except for 50 mg·L^{-1} applied as a drench. However, the comparison of runner length from the mother plant to the first runner plant, from the first runner plant to the second runner plant, and from the second runner plant to the third runner plant showed that they were shorter after application of 50 mg·L^{-1} Pro–Ca as a drench than in the control. Similar results were obtained by Hytönen et al. [16], who obtained reduced elongation of runners by application of 50 mg·L^{-1} Pro–Ca with foliar spray compared to the non-treatment in the strawberry cultivar Korona. The total runner length and comparison of runner length was shortest after treatment with 200 mg·L^{-1} Pro–Ca as a drench. The runner length has a great influence on the determination of bed height in strawberry high bench type culture, and furthermore, the runner length tends to be inversely proportional to the runner diameter [46]. Therefore, it is considered that reducing runner length helps to produce higher quality runners and runner plants.

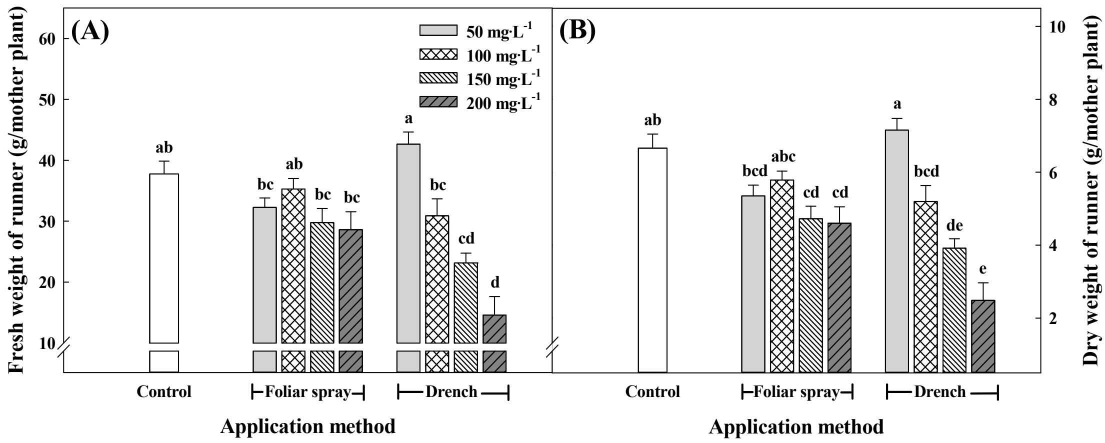

Figure 3. Fresh weight of runners (**A**) and dry weight of runners (**B**) of the strawberry cultivar Maehyang as affected by application method and concentration of Pro–Ca at 15 weeks after treatment. Vertical bars represent the standard deviation of the mean (n = 9). Different letters in the same column indicate significant differences based on Tukey's test ($p \leq 0.05$).

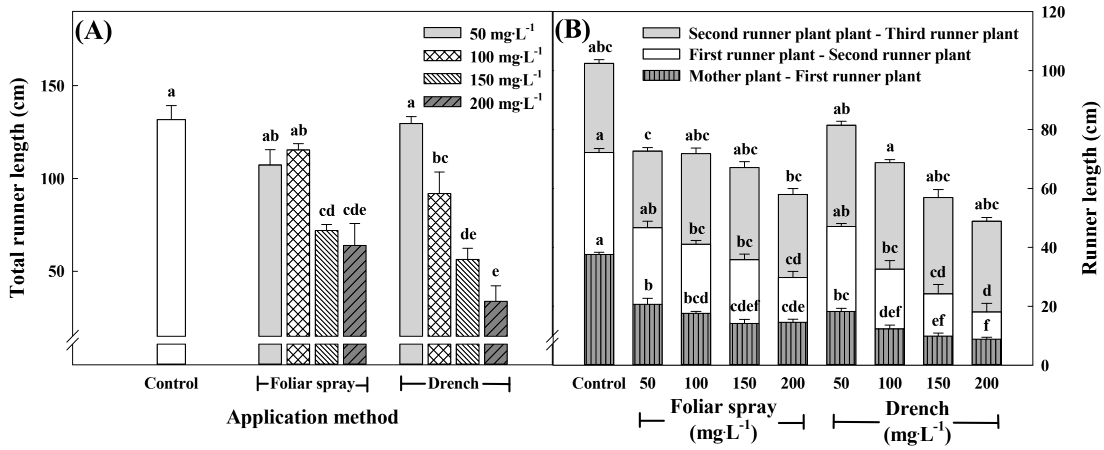

Figure 4. Total runner length (**A**) and comparison of runner length (**B**) of the strawberry cultivar Maehyang as affected by application method and concentration of Pro–Ca at 15 weeks after treatment. Vertical bars represent the standard deviation of the mean (n = 9). Different letters in the same column indicate significant differences based on Tukey's test ($p \leq 0.05$).

3.4. Growth Characteristics of the First, Second, and Third Runner Plants at 15 Weeks after Treatment with Pro–Ca

The growth characteristics of runner plants as affected by application method and concentration of Pro–Ca at 15 weeks after treatment are shown in Table 2. The FW and DW of the first runner plant showed no significant difference under all treatments. The FW of the second and third runner plants showed a tendency to be heavier in the foliar spray than the drench application, and the 50 mg·L^{-1} Pro–Ca concentration resulted in higher FW of the second and third runner plant than the control. In addition, DW of the second and third runner plants were heavier after the 50 mg·L^{-1} Pro–Ca application than the other concentrations of treatments. According to Reekie et al. [17], the net photosynthetic rate of mother and runner plants of the strawberry cultivars Sweet Charlie and Camarosa was increased by application of 62.5 mg·L^{-1} Pro–Ca as a foliar spray. Similarly, Sabatini et al. [47] reported that Pro–Ca positively affected leaf mass area and chlorophyll content in apple and pear trees because net photosynthesis was increased after Pro–Ca application. Also, strawberry plants treated with Pro–Ca exhibited increased total DW, relative growth rate, and unit leaf rate during the nursery period [18]. Moreover, strawberry cultivar Camarosa runner plants treated with 100 mg·L^{-1} Pro–Ca as a foliar spray exhibited a photosynthetic rate increase of 23% compared to non-treated controls [48]. This physiological phenomenon caused by Pro–Ca application occurs as a result of light energy being converted into chemical energy (ATP and NADPH) that is used to reduce atmospheric CO_2 to carbohydrates through the Calvin cycle during photosynthesis [49]. Thus, in the present study, we propose that the net photosynthetic rate was increased by Pro–Ca treatment. In addition, it is considered that the photosynthetic products of the mother plant were more effectively translocated to the runner plant due to the formation of the high quality runner after application of 50 mg·L^{-1} Pro–Ca (Figure 3). In previous studies, Pro–Ca treatment of strawberries focused mainly on the concentration and number of foliar spray application [16–18,43]. Foliar spray applications are most commonly used in PGRs, but can result in non-uniform plant size if careful attention to technique is not used [50]. On the other hand, soil or growth medium drench applications give more uniform results and increased product efficiency at lower concentrations compared to foliar spray application [51]. In general, PGRs are applied directly on the soil or growth medium as a drench, or as a foliar spray, however, it is known that variation in responses occurs among species and cultivars [52]. Here, we determined the best concentration and method of application of Pro–Ca to the strawberry cultivar Maehyang for optimum propagation of runners and runner plants with higher biomass is 50 mg·L^{-1} Pro–Ca applied as a soil or growth medium drench.

Table 2. Growth characteristics of strawberry cultivar Maehyang runner plants as affected by application method and concentration of Pro–Ca at 15 weeks after treatment.

Experiment Factor	Fresh Weight (g/plant)			Dry Weight (g/plant)		
	First Runner Plant	**Second Runner Plant**	**Third Runner Plant**	**First Runner Plant**	**Second Runner Plant**	**Third Runner Plant**
Application method						
Foliar spray	19.2	13.4	4.5	3.9	2.7	0.78
Drench	17.2	11.2	3.3	3.7	2.4	0.67
	n.s.	*	*	n.s.	n.s.	n.s.
Concentration (mg·L^{-1})						
Control (0)	17.5	8.7 ^{b}	3.5 ^{b}	4.0	1.9 ^{b}	0.72 ^{abc}
50	18.5	13.3 ^{a}	5.3 ^{a}	3.9	2.9 ^{a}	1.02 ^{a}
100	19.3	13.7 ^{a}	4.4 ^{ab}	4.1	2.8 ^{a}	0.82 ^{ab}
150	17.3	10.4 ^{ab}	2.9 ^{b}	3.5	2.1 ^{b}	0.50 ^{c}
200	17.7	11.7 ^{ab}	3.1 ^{b}	3.6	2.3 ^{ab}	0.57 ^{bc}
	n.s.			n.s.		

Within each column, * significant difference at $p \leq 0.05$; n.s. no statistically significant difference; means followed by different letters are significantly different according to the Tukey's test at $p \leq 0.05$.

4. Conclusions

The present study revealed that inhibiting the growth of mother plants using drench application of Pro–Ca caused more sensitive responses compared to a foliar spray. Runners had heavier biomass under low concentrations of Pro–Ca (50 mg·L^{-1}) applied as a drench. In addition, application of Pro–Ca (50 mg·L^{-1}) applied as a drench increased the initiation of runner plants and stimulated higher biomass, as measured by DW, of the second runner plant, and the FW and DW of the third runner plant. Overall, the results suggest that 50 mg·L^{-1} Pro–Ca applied as a soil drench is the most suitable way to promote the quality and quantity of strawberry cultivar Maehyang. This knowledge is expected to be beneficial for the practical management of mother plants, runners, and the propagation of runner plants during the nursery period.

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