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Effects of honey bee (*Apis mellifera* L.) colony size on the pollination of greenhouse-cultivated watermelon (*Citrullus lanatus* L.) under forcing cultivation

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Abstract

We investigated the effects of honey bee (*Apis mellifera* L.) colony size on the pollination of greenhouse-cultivated watermelon grown under the forcing cultivation system. The highest pollination activity of bees was observed (14.3 ± 5.0 honey bees/day) when the bee colony size was 10,000 followed by 7,500 and 5,000 honey bees. There was a positive correlation between the bee colony size and pollination activity (R = 0.262) but insignificant difference in fruit set with different honey bee colony sizes (88%–91%). Evaluation of physical properties revealed that the weight and shape of watermelon were also not significantly different among different colony sizes. However, larger the bee colony size, higher the number of seeds were fertilized and rate of seed fertilization ($p > 0.05$). Number of seeds and content of sugar were negatively correlated (R = -0.714). Fertilized seeds showed a significant increase in mealy flesh, which has a negative effect on fruit quality, compared with that of the unfertilized seeds. Overall, we found that a colony size of 5,000 honey bees was the most effective for the pollination of watermelon grown under forcing cultivation. A comparison of the effects of bee pollination with those of artificial pollination suggested that artificial pollination can be effectively replaced by bee pollination in the forcing cultivation of watermelon, because fruit set, weight, and shape by bee pollination were similar to those achieved by artificial pollination.

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Introduction

Watermelon (*Citrullus lanatus* L.), which originated in Africa, is an important crop cultivated worldwide as a perennial vine plant (Wehner, 2008; Chomicki and Renner 2015; Paris, 2015). In South Korea, the domestic production of watermelon is 506,471 t and the cultivation area is 12,661 ha, making it an important economic crop. It is the most extensively cultivated crop in Korea predominantly produced by greenhouse cultivation covering an area 3.6 times larger than that of outdoor cultivation (Statistics Korea, 2018).

Watermelon is an entomophilous plant and has both pistillate and staminate flowers that bloom separately (Walters, 2005). However, as greenhouse cultivation isolates the plants from the

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outside environment, it is difficult to achieve natural pollination by insects. Therefore, honey bees as pollinators are commonly used along with artificial pollination (Watanabe *et al.,* 2001; Lee *et al.,* 2013). However, increasing farm management cost due to higher labor cost is increasing the reliance on pollination by honey bees for greenhouse-cultivated watermelon (Yoon *et al.,* 2017). The cropping season of greenhouse-cultivated watermelon comprises periods of forcing cultivation (winter and early spring), semi-forcing cultivation (spring and early summer), and retarding cultivation (fall). Watermelon is cultivated depending on the consumption period of certain variety of watermelon (Chon *et al.,* 1998; Lee *et al.,* 2006). The environmental conditions within a greenhouse can be affected by the solar radiation amount and the average temperature. Furthermore, the foraging behavior of honey bees is influenced by the effects of environmental conditions on a honey bee colony such as temperature, light intensity, ultraviolet radiation amount and wind speed (Esch and Burns 1996; Heinrich, 1996; Kevan *et al.,* 2001).

However, information on the efficient use of honey bees during the flowering and cultivation periods of watermelon is needed. The aim of this study was to investigate the pollination activity and pollination effects of honey bees based on different colony sizes in a forcing cultivation system, which is economically more rewarding than other watermelon cultivation techniques. We also examined how the quality of harvested fruits varied and determined the optimal bee colony size. We investigated the effective pollination distance honey bees in a greenhouse to determine the appropriate placement location of the honey bee colony within a facility. Finally, we compared the fruit set and quality of harvested fruits obtained by artificial pollination with those obtained by bee pollination to determine whether bee pollination can replace artificial pollination.

Materials and Methods

Experimental insects and crops

This study was conducted from February 13 to 23, 2017, in six vinyl greenhouses (each with an area of 660 m^2) in Wolchon-ri, Haman-gun, Gyeongsangnam-do, Korea (35°18’35”N, 128°18’27”E). Watermelon (*Citrullus lanatus* L.) ‘Speed honey’ cultivar (Nongwoo Bio, Suwon, Gyeonggi, Korea) was grown under forcing cultivation. On December 28, 2016, watermelon was planted in two rows of 154 plants, with the rows spaced 40-cm apart, in each greenhouse. The experimental insects were a three-way cross hybrid honey bee (*Apis mellifera* L.) selected by the Rural Development Administration (Lee *et al*., 2014). The queens used in this study, was produced at “Saryangdo” (34º50’53”N, 128º11’37”E) in July 2016, and colony was cultivated until October. Since then, the colony has been overwintering from November 2016 to January 2017.

Pollination effect according to bee colony size and pollination method

On February 13, 2017, to investigate the effect of bee colony size on pollination, six of bee colonies (5,000 workers × 2 colonies, 7,500 workers × 2 colonies and 10,000 workers × 2 colonies) were introduced to six vinyl houses, respectively. The colonies were installed at points 10 m from the entrances of greenhouse, and were removed from the greenhouses 10 days after installation. The pollination activity of honey bees, fruit set, and physical properties of the harvested watermelon were investigated to compare the pollination effects. We confirmed the relationship between bee colony size and pollination activity and fruit set. Artificial pollination was also performed to compare the efficiency of the different pollination methods. On February 23, at 10:00 h, artificial pollination was carried out by randomly selecting 5 staminate flowers and 10 pistillate flowers from the third flower cluster in each greenhouse, and rubbing the staminate flowers on the stigmas for 5 s. The flowers that were subjected to artificial pollination were sealed with a paper cup until February 23 to prevent pollination by honey bees. The control group consisted of 10 pistillate flowers randomly selected just before the bloom of the third flower cluster, and these were maintained sealed with a paper cup and a mesh (1 mm × 1 mm) throughout the study period.

Pollination effect according to distance from the colony box

To determine the effective pollination distance in the greenhouse, pollination activity and fruit set were examined at 20 m intervals for up to 100 m from the location of the colony box. The data were analyzed for significant differences in pollination activity and fruit set according to the distance from the colony box, and the relationship between the distance from the colony box and pollination activity and fruit set was determined.

Analysis of pollination effect

Pollination effects were assessed by evaluating the pollination activity of honey bees, fruit set of the cultivated watermelons, and physical properties of the harvested fruits. Pollination activity was defined as the number of bees sitting on a watermelon flower in the greenhouses for 10 min at 2 h intervals, from 10:00–16:00 h on February 21–23. Fruit set was investigated 15 d after pollination (March 8, 2017). Fruit set was calculated as the ratio of setting fruits to the number of plants in each test plot. The fruit was harvested 48 d after pollination (April 5, 2017). The weight, peduncle property (weight, length, and diameter), and blossom-end diameter of the non-cleaved fruits were measured to determine the physical properties of the fruit. The width (cm), Length/Diameter ratio, soluble solids (Brix), number of fertile seeds, and seed set were investigated after fruit cleavage. The pollination ratio was calculated as the ratio of the number of fertile seeds to the total number of seeds in one fruit. The relationship between the number of seeds and sugar content of fruit was investigated to confirm the difference in the commercial value of fruits according to the number of fertilized seeds. The size of mealy flesh was calculated as the length of the mealy flesh area surrounding the seed minus the length of the seed. The length of the fruits and seeds was measured using a digital calipers (500-181; Mitutoyo, Kawasaki, Japan), weight of fruits was measured using an electronic balance (AND, CB-3000; Seoul, Korea), and sugar content of fruits was measured using a digital sugar meter (PR-32α; Atago, Tokyo, Japan).

Statistical analyses

Differences in pollination effects (pollination activity, fruit set, and physical properties of fruit) according to different colony sizes and pollination effects (pollination activity and fruit set) according to the distance from the colony box were tested by one-way ANOVA, followed by Tukey's HSD as *post-hoc* analysis. Spearman's correlation was used to determine the correlation of pollination activity and fruit set with different colony sizes. Further, the correlation of pollination activity and fruit set with different colony sizes at different distances was analyzed by Pearson's correlation. The correlation between the fertilized seed number and fruit sugar content was analyzed by Pearson's correlation, and the regression equation was derived by the linear regression analysis. Comparisons between the size of mealy flesh of fertilized and unfertilized seeds were performed by a *t*-test. All statistical analyses were performed using SPSS PASW 22.0 for Windows (IBM, Chicago, IL, USA)..

Results

Pollination effect on greenhouse-cultivated watermelon in winter according to the bee colony size

Pollination activity according to the bee colony size

The pollination activity of honey bees during watermelon flowering period was the highest, with 14.3 ± 5.0 bees/day, when the colony size was of 10,000 bees, followed by 7,500 and 5,000 bees, but the difference was not significant (one-way ANOVA test: $F_{2,15}$ = 0.745, p = 0429; Table 1). There was no significant difference in the pollination activity of honey bees based on colony size at different distances from the colony box installed in the test plot (20 m: $F_{2,15}$ = 0.096, p = 0.909; 40 m: $F_{2,15}$ = 0.116, p = 0.891; 60 m: $F_{2,15}$ = 0.615, p = 0.554; 80 m: $F_{2,15}$ = 1.260, p

Table 1. Pollination activity of bees per day in the greenhouse according to the distance from the colony box

Colony size (No. of honey bees)	N	Distance from colony box (m)					Total
		20	40	60	80	100	
5,000	6	2.8 ± 0.9z	2.8 ± 1.7	2.6 ± 2.2	1.9 ± 0.7	1.5 ± 0.6	10.3 ± 5.5
7,500	6	2.9 ± 1.2	3.0 ± 1.3	2.5 ± 1.5	2.6 ± 2.0	1.9 .90.8	10.5 0.6.8
10,000	6	3.1 ± 1.7	3.2 ± 1.1	3.0 ± 1.2	2.7 ± 0.5	2.8 ± 1.8	14.3 ± 5.0
Total	18	2.9 ± 1.2	3.0 ± 1.3	2.7 ± 1.5	2.4 ± 1.1	2.1 ± 1.3	11.7 ± 5.8

[z] Number of bees (mean ± SD)
1) The pollination activity of honey bees did not differ significantly among different colony sizes and distances from the colony box (ANOVA, p > 0.05).

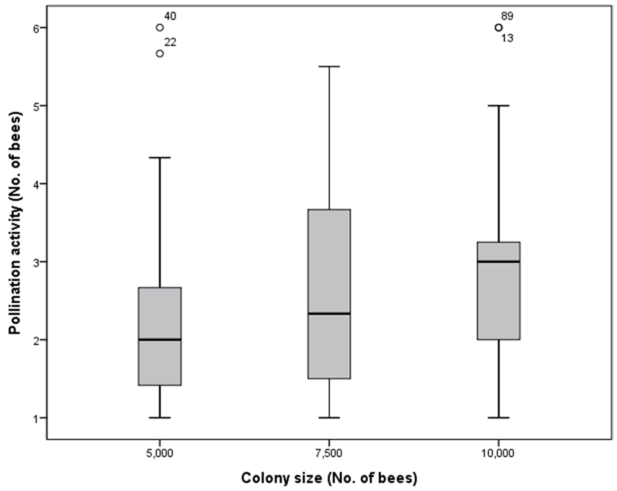

Fig. 1. Relationship between pollination activity and different colony sizes.
1) Spearman's Rho coefficient = 0.262, p = 0.019

= 0.316; 100 m: $F_{2,15}$ = 2.844, p = 0.092; Table 1). However, a weak positive correlation was found between bee colony size and pollination activity; therefore, the larger the bee colony size, the greater the pollination activity (Spearman's Rho coefficient = 0.262, p = 0.019; Fig. 1).

Fruit set and characteristics according to the bee colony size and pollination method

The bee colonies with 5,000, 7,500, and 10,000 bees presented the fruit set of 90.5%, 88.4%, and 91.7%, respectively, which did not vary significantly (one-way ANOVA test: $F_{2,9}$ = 8.561, p = 0.350; Fig. 3). A comparison of the fruit set among different pollination methods revealed that no treatment group showed a fruit set of 0%. Artificial pollination showed a fruit set of 85.6%, which was marginally lower than that by honey bee pollination, with no significant difference (Welch's ANOVA test: $F_{3,6.731}$ = 3.752, p = 0.071). There was no correlation between the bee colony size and fruit set (Pearson's correlation: p > 0.05).

Evaluation of the physical properties of watermelon pollinated by honey bees from different-sized colonies revealed that the average weight of watermelon was approximately 6 kg for all colony sizes, and there was no significant difference among the different colony sizes. Furthermore, the weight, length, and diameter of peduncle, blossom-end diameter and width, and L/D ratio also did not show any significant difference (fruit weight: $F_{3,31}$ = 0.613, p = 0.612; peduncle weight: $F_{3,31}$ = 0.673, p = 0.575; peduncle length: $F_{3,31}$ = 0.114, p = 0.951; peduncle diameter:

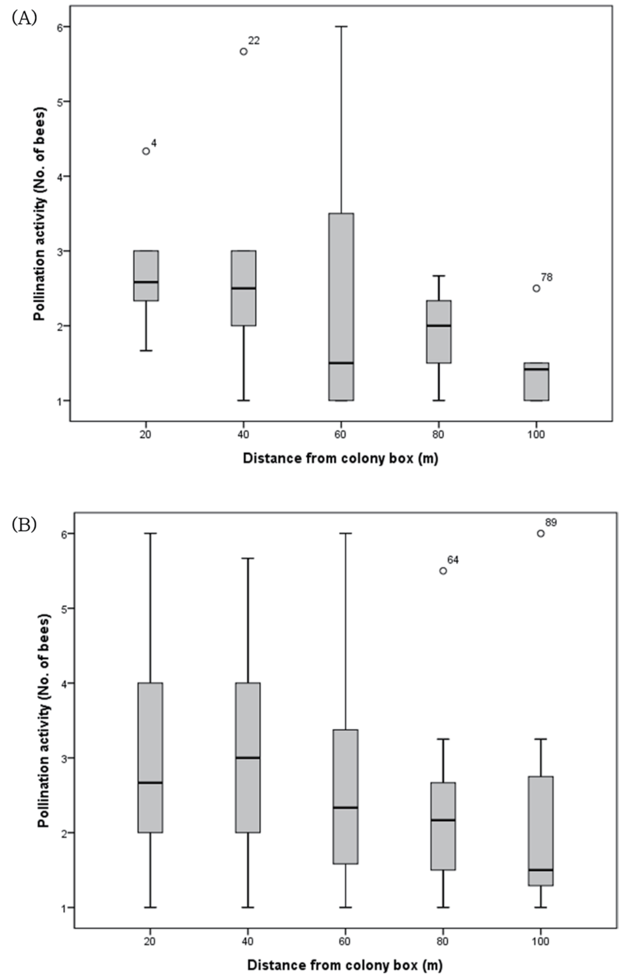

Fig. 2. Relationship between the pollination activity of bees for a colony size of 5,000 bees and distance from the colony box in (A) and between the pollination activity of bees and distance from the colony box in (B).

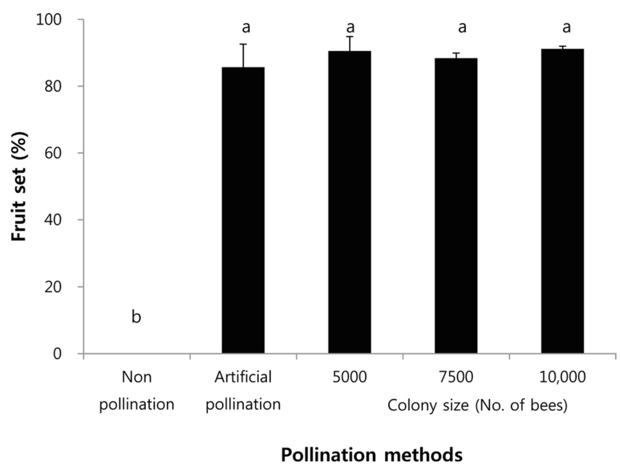

Fig. 3. Fruit set of watermelon in terms of different pollination methods and colony sizes of honey bees. Different letters indicate significant differences among the pollination methods, as determined by one-way ANOVA and Tukey's HSD (p < 0.05).

Table 2. Correlations between pollination activity in the greenhouse according to different colony sizes and distances from the colony box

Pollination activity × Distance from the colony box	Colony sizes (workers)			Total[z]
	5,000	7,500	10,000	
R	-0.386*	-0.253	-0.111	-0.246*
p	0.046	0.233	0.567	0.028

[z] Correlations between the total pollination activity and distance from the colony box
1) Pearson's correlation coefficients were calculated for pollination activity according to different colony sizes and distances from the colony box. As each set of values was used to calculate the two correlations, "*" indicates a significant correlation at $p < 0.05$ (two-tailed).

$F_{3,31} = 0.414$, $p = 0.744$; fruit width: $F_{3,31} = 2.030$, $p = 0.130$; L/D ratio: $F_{3,31} = 0.078$, $p = 0.971$; Tables 4 and 5). The soluble solid content did not show any difference with bee colony size, but artificial pollination showed a 1.3-times higher soluble solid content than that by bee pollination (soluble solid: $F_{3,31} = 30.354$, $p = 0.0001$). By artificial pollination, the number of fertile seeds obtained was 328.8, which was the lowest (only 44% of that obtained by bee pollination). There was no significant difference in the number of seeds based on the bee colony size, but the number of seeds tended to increase with the increase in bee colony size (number of fertile seeds: $F_{3,31} = 12.107$, $p = 0.0001$). Artificial pollination presented a fertilization rate of 60.2%,

Table 3. Fruit set of watermelon in the greenhouse according to the distance from the colony box

Colony size (No. of honey bees)	N	Distance from colony box (m)				
		20	40	60	80	100
5,000	6	91.7 ± 1.7z	92.2 ± 1.3	91.1 ± 2.6	89.2 ± 5.7	83.6 ± 20.7
7,500	6	87.0 ± 4.9	90.2 ± 3.4	87.5 ± 1.7	89.4 ± 4.0	85.9 ± 7.9
10,000	6	88.0 ± 7.9	93.7 ± 2.1	91.7 ± 2.4	92.7 ± 1.4	84.7 ± 12.4
Total	18	88.9 ± 5.4	92.0 ± 2.7	90.1 ± 2.8	90.5 ± 4.1	84.8 ± 13.3

[z] Fruit set ratio (mean ± SD) %
1) Fruit set did not differ significantly among different colony sizes and distances from the colony box (ANOVA, $p > 0.05$).

Table 4. Effects of different colony sizes on the physical properties of non-cleaved watermelon

Colony size (No. of honey bees)	N	Weight (Kg)	Peduncle			Blossom-end Diameter (cm)
			Weight (g)	Length (cm)	Diameter (cm)	
Artificial pollination	5	5.9 ± 0.3z	9.8 ± 1.6	9.7 ± 0.7	3.8 ± 1.6	10.6 ± 5.3
5,000	10	6.2 ± 0.5	9.6 ± 2.5	10.1 ± 0.8	4.1 ± 1.0	10.5 ± 2.2
7,500	10	6.1 ± 0.6	9.6 ± 2.6	9.8 ± 0.7	3.4 ± 1.1	8.0 ± 1.5
10,000	10	6.2 ± 0.5	9.1 ± 2.8	10.0 ± 0.5	3.7 ± 1.0	10.2 ± 2.8

[z] mean ± SD
1) The physical properties of non-cleaved watermelon did not differ significantly among different colony sizes (ANOVA, $p > 0.05$).

Table 5. Effects of different colony sizes on the physical properties of cleaved watermelon

Colony size (No. of honey bees)	N	Width (cm)	L/D Ratio*	Soluble solid (Brix)	Seed set (%)	No. of fertile seeds
Artificial pollination	5	23.9 ± 2.6z	1.07 ± 0.11	13.0 ± 0.8a	60.2 ± 31.6b	328.8 ± 203.9b
5,000	10	23.3 ± 0.9	1.07 ± 0.01	10.2 ± 0.6b	87.8 ± 4.8a	711.6 ± 103.9a
7,500	10	23.4 ± 1.0	1.06 ± 0.03	10.1 ± 0.6b	85.0 ± 7.3a	725.1 ± 134.8a
10,000	10	23.4 ± 1.0	1.06 ± 0.02	9.9 ± 0.5b	91.7 ± 4.9a	749.5 ± 131.1a

[z] mean ± SD
[*] Fruit length to diameter
1) Different letters indicate significant differences among colony sizes, based on the results of one-way ANOVA and Tukey's HSD ($p < 0.05$).

which was 25%–31% lower than that of bee fertilization; thus, the number of fertile seeds was similar (seed set: $F_{3,31}$ = 7.205, p = 0.001).

Pollination effect of honey bees on greenhouse-cultivated watermelon in winter according to the distance from colony box

Pollination activity according to the distance from the colony box

Pollination activity according to the distance from the colony box during the flowering period of greenhouse-cultivated watermelon in winter was the highest (3.0 ±1.3 bees) at the distance of 20 m from the bee colony box, but there was no significant difference among the distances evaluated (one-way ANOVA test: $F_{4,75}$ = 1.364, p = 0.255; Table 1). There was no significant difference in the pollination activity of bees with distance from the colony box for the different bee colony sizes (5,000 bees: $F_{4,22}$ = 1.152, p = 0.359; 7,500 bees: $F_{4,19}$ = 0.410, p = 0.799; 10,000 bees: $F_{4,24}$ = 0.129, p = 0.970). However, as the distance from the colony box increased, the pollination activity of the bees tended to decrease, and a weak negative correlation was observed (R = -0.386, p = 0.046; Table 2). The bee colony size of 5,000 bees was negatively correlated with the distance from the colony box, whereas the other bee colony sizes did not show any significant correlation (R = -0.246, 0 = 0.028; Fig. 2).

Fruit set according to the distance from the colony box

There was no significant difference in the fruit set of watermelon according to the distance from the colony box (one-way ANOVA test: $F_{2,57}$ = 0.482, p = 0.620; Table 3). No significant difference was observed in the fruit set according to the distance from the colony box irrespective of the bee colony size (5,000 bees: $F_{4,15}$ = 0.518, p = 0.724; 7,500 bees: $F_{4,15}$ = 0.534, p = 0.713; 10,000 bees: $F_{4,24}$ = 1.226, p = 0.341). There was no correlation between the distance from the colony box and fruit set (p > 0.05).

Discussion

The honey bee colony size that can be used for induced pollination might vary depending on the flowering characteristics of the crops and the cultivation method adopted (Goodwin *et al*., 2011; Delaplane *et al*., 2013). In this study, the size of bee colony for the effective pollination of greenhouse-cultivated watermelon was investigated. The results revealed that the number of bees performing pollination activity when the colony size was of 10,000 bees was approximately 1.4 times higher than that with the other colony sizes (Table 1). As the colony size increased, the number of bees carrying out pollination activity tended to increase (Fig. 1), suggesting that the larger the size of the bee colony, the more favorable the pollination of watermelon. However, there was no significant difference in fruit set according to the bee colony size, and there was no correlation. There was also no difference in the fruit set between artificial pollination and bee pollination (Fig. 3). This might be because in greenhouse-cultivated watermelon, all the flowers that bloom throughout the flowering period do not undergo fertilization and only the targeted pistillate flowers are pollinated within a short time; thus, requiring less labor from the bees. The number of bees foraging and the distance from the colony box installed in the greenhouse showed a weak negative correlation. However, there was no correlation between the fruit set and the distance from the colony box. It has been found that the farther the distance from the colony box, the more energy is consumed for foraging (Esch and Burns, 1996). Therefore, we expected a negative correlation between the pollination activity of bees and distance from the colony box. However, the effective foraging distance of honey bees is known to be 2 km from the colony box, and honey bees can forage for up to a maximum distance of 10 km (Steffan-Dewenter and Kuhn, 2003; Hagler *et al.,* 2011). Thus, the relatively short distance of the vinyl greenhouse (100 m) might not have had a significant effect on the pollination behavior of bees. Hence, there was no difference in the pollination of watermelon according to the installation position of the honey bee colony box (at the entrance or in the middle of the greenhouse). The evaluation of the physical properties of fruits according to bee colony size and pollination method, including artificial pollination, revealed that the weight, length, and diameter of peduncle and blossom-end diameter of fruits, which are affected by the growth conditions, were the same in all the experimental plots, confirming the same growth environment. Furthermore, the weight and shape of fruits did not exhibit any significant difference with the bee colony size, suggesting that these parameters are affected by the growth environment after fruit set. The content of soluble solids, number of fertile seeds, and seed set did not show significant differences according to

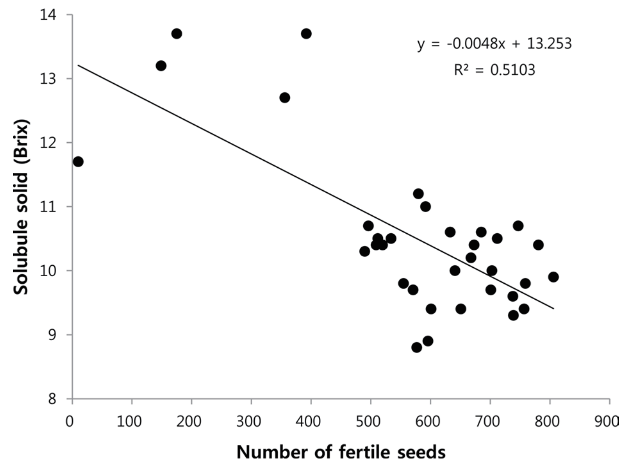

Fig. 4. Regression analysis between the content of soluble solids and number of fertile seeds (black circles).

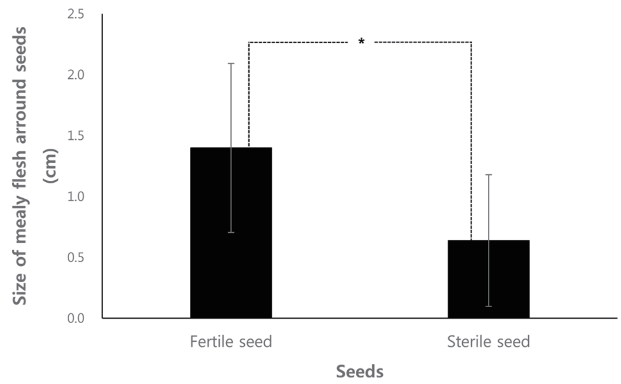

Fig. 5. Comparison of the size of mealy flesh around the seeds between the fertile seeds and sterile seeds. "*" indicates significant difference at $p < 0.05$ (t-test) between the fertile seeds and sterile seeds.

the bee colony size, but the number of fertile seeds tended to increase as the size of the colony size increased. Compared with that of artificial pollination, bee pollination increased the number of seeds two-fold. This might have been due to differences in the frequency of visitation by bees on the pistillate flowers. Artificial pollination of pistillate flowers is only carried out once, whereas honey bees pollinate the pistillate flowers several times during the flowering period, thereby increasing the number of fertilized seeds (RDA, 2014). The number of fertile seeds is expected to increase as the number of bees carrying out pollination increases with the bee colony size. However, in the present study, as there was no significant difference in the pollination activity of bees according to the bee colony size, there was also no significant difference in seed set and number of fertile seeds according to the bee colony size. Interestingly, a significant negative correlation was found between the sugar content and number of fertile seeds ($R = -0.714$, $p = 0.0001$; Fig 4). These results suggest that the production of seeds is related to the consumption of energy (sugar) during crop growth and fruit development (Weber *et al.,* 1996; Hill *et al.,* 2003). Furthermore, fertilized black seeds increased the size of mealy flesh two-fold compared with that of unfertilized yellow seeds, and therefore, are expected to affect the storage of watermelon (*t*-test: $t_{88} = 5.800$, $p = 0.0001$; Fig. 5) (Kuvare, 2005). Our results suggest that the larger the bee colony size, the higher the fruit set. However, the excessive number of bees in the limited space of a greenhouse might have an adverse effect on watermelon quality owing to the increase in the number of seeds resulting from excessive pollination. Therefore, we confirmed that a bee colony size of 5,000 bees is the most effective in the forcing cultivation of watermelons. We also found that pollination by honey bees can effectively replace artificial pollination. However, Lee *et al.* (2008) reported that environmental conditions inside a greenhouse during the flowering period of watermelons are not suitable for bee activity and that artificial pollination is more effective than bee pollination, which is inconsistent with the findings of our study. This difference might be because bee activity and fruit set can vary depending on the environmental conditions inside a greenhouse and the watermelon varieties cultivated. Therefore, a close examination of the experimental conditions (Lee *et al*., 2008) and the environmental conditions (in this study) is necessary.

Artificial pollination or bee pollination is required for the pollination of watermelon in greenhouses during winter, because natural pollination by outdoor insects is difficult (Lee *et al.,* 2006). As the effective flowering period of watermelons is relatively short, approximately 2 weeks, techniques for using honey bees efficiently are required (RDA, 2014). Our findings suggest an ideal bee colony size when using bees for pollinating winter greenhouse-cultivated watermelon with a flowering period in February. However, as the direction of the greenhouse was not considered in this study, there are limitations in terms of applying the results of this study to all watermelon cultivation environments. The temperature inside a greenhouse might change due to convection depending on the direction of the greenhouse, which might cause a difference in the behavior of honey bees. Dag and Eisikowitch (1995) reported a significant difference in fruit set based on the direction of greenhouse and colony box. Therefore, it is necessary to examine temperature change and pollination behavior according to the direction of greenhouses. As there are various watermelon cultivation systems in Korea (Chon *et al.,* 1998), comparative studies to determine the honey bee colony size that is the most effective for pollination according to the flowering time are required.

Acknowledgements

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