Frost Avoidance: Sodium Alginate + CaCl$_2$ Can Postpone Flowering of ‘Kawanakajima Hakuto’ Peach Trees

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Research Article

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Abstract

The objective of this study was to investigate whether treatment with sodium alginate and CaCl$_2$ treatment in the 2$^{nd}$ (swollen bud) blooming stage could delay peach flowers as a strategy to avoid frost. Regarding the expression rate at the 3$^{rd}$ (calyx green) stage, the control treatment (CT, distilled water) showed a sharp increase from 8 days after treatments (DAT), reaching the maximum at 14 DAT. However, the 5AG group (5% sodium alginate + 100 mM CaCl$_2$) and the 7AG group (7% sodium alginate + 100 mM CaCl$_2$) recorded the maximum expression rate at 17 DAT. After the maximum expression, CT showed a sharply diminishing trend, whereas 5AG and 7AG showed gentle decreases. At the 4$^{th}$ (calyx red) stage, CT, 5AG, and 7AG reached the peak expression at 20 DAT (43.5%, 31.9%, and 40.2%, respectively). However, maximum expression levels of all treatments were below ~50%. The 5th (first pink) stage was first expressed in all treatments when the expression rate at the 4$^{th}$ stage peaked in all groups at 20 DAT. Maximum expression was 50.7% in CT, 33.7% in 5AG, and 49.2% in 7AG. This tendency was similar to that at the 4$^{th}$ stage. Particularly, expression in 5AG diminished more slowly than that in CT and 7AG at 3$^{rd}$, 4$^{th}$, and 5$^{th}$ stages. CT group bloomed first at 20 DAT, reaching full bloom at 81.4%, ahead of AG treatments at 28 DAT, while 7AG and 5AG groups bloomed fully at 34 and 37 DAT, respectively. Our results suggest that 5AG treatment is suitable to delay peach flowering when phenological development of the flower buds reaches the 2$^{nd}$ stage.

Introduction

Average temperatures in February and March (early spring) in Southern region of South Korea have increased more than 2 °C and 4°C, respectively, during the last 10 years due to recent abnormal climate. For this reason, peach blooming period has been advanced by about 13 days (https://data.kma.go.kr; https://fruit.nihhs.go.kr). Sugiura et al. (2007) have reported that blooming and maturation periods of Japanese pears, peaches, and Japanese apricots are accelerated due to global warming. Earlier blooming can increase frost damage of peach flower buds and flowers due to low temperatures in late spring. It is an unfavorable factor in peach production. Therefore, avoidance of low temperature at blooming period of peach could be a strategy to prevent frost injury.

Many studies have been conducted on blooming delay of temperate fruit trees using various materials and treatment time. Although treatment with soybean oil in February or March can delay blooming of almond and peach (two days of delay for almond and four to six days of delay for peach), the density of viable flower buds is decreased with increasing concentration of soybean oil (Myers et al., 1996; Samani et al., 2005). Treatment with ethephon can delay blooming of ‘Italian’ prune and ‘Redhaven’ peach by 15 and 9.4 days, respectively. However, fruit yield is decreased by this treatment (Crisosto et al., 1990). In case of ABA treatment, it cannot delay blooming of ‘Nectar’ peaches. In addition, combined treatment of ABA and soybean oil increases flower bud mortality (Deyton et al., 2009).
In this study, we selected sodium alginate and CaCl₂ as materials for delaying peach blooming. Sodium alginate and CaCl₂ have been used for the production of artificial seeds, which encapsulate artificially somatic cell embryos (Rihan et al., 2017). Alginate is a natural polysaccharide that composes cell walls with structural functions similar to agar or carrageenan in brown algae. It has been used in a wide range of industries, such as food additives and pharmaceuticals (Qin et al., 2018). Rihan et al. (2017) have reported that alginate is suitable for producing artificial seeds due to its sensible thickness, weak spinnability of solution, low toxicity of micro-organism, low expense, and fast gelation. The major principle involved in the alginate encapsulation process is that when sodium alginate is dropped into CaCl₂, round and firm beads are formed due to ion exchange between Na⁺ in sodium alginate and Ca²⁺ in CaCl₂ (Saiprasad, 2001).

Therefore, the objective of this study was to determine the encapsulation effect of alginate mentioned above could delay peach blooming. We coated the surface of peach flower buds with a combination of sodium alginate and CaCl₂ and investigated phenological development and blooming time of these coated peach flower buds.

Results

Weather condition at experimental orchard. Figure 1 shows changes of maximum, average, and minimum temperatures and the number of rainy days from March 8, 2021 (the day of treatment) to April 14, 2021 [37 days after treatment (DAT)] at the peach orchard. The average daily temperature range during the experimental period was 14.7 ℃. Below zero temperatures occurred twice during 37 days. The lowest temperature was −2.3 ℃ on March 23, 2021. The average temperature of the experimental period rose about 3.5 ℃ compared to that 10 years ago. The number of rainy days was 12, which corresponded to about 1/3 of the experimental period.

Change of phenological stage in flower buds. Figure 2 shows phenological changes of flower buds from the 3rd stage to the 6th stage. Classification of each stage were as follows: 3rd stage = calyx green, 4th stage = calyx red, 5th stage = first pink, and 6th stage = first bloom. Regarding the expression rate at the 3rd stage, CT showed a sharp increase from 8 DAT, reaching the maximum of 84.6% at 14 DAT, whereas 5AG and 7AG reached the maximum of 70.6% and 71.1%, respectively, at 17 DAT (Fig. 2A). After the maximum expression, the expression in CT decreased drastically, whereas the expression in 5AG and 7AG declined gradually, with 5AG showing a gentler diminishing trend than 7AG.

The fourth stage was first expressed in CT and 5AG on 11 DAT (Fig. 2B). CT, 5AG, and 7AG reached the peak expression of 43.5%, 31.9%, and 40.2% at 20 DAT, respectively. However, maximum expression levels of all treatments were below 50%. After the maximum expression, diminution pattern at the 4th stage was similar to that at the 3rd stage. The expression in 5AG was maintained at 20.3% while that in CT was reduced to 0% at 25 DAT.
The fifth stage was first expressed in all treatments when the expression rate of the 4th stage attained the peak in all treatments at 20 DAT (Fig. 2C). CT showed an expression rate of 33.9%, while 5AG and 7AG showed lower expression levels by 8-fold and 3-fold, respectively, compared to CT. The maximum expression rate was 50.7% in CT, 33.7% in 5AG, and 49.2% in 7AG. This tendency was similar to that at the 4th stage where maximum expression levels in all treatments were below ~ 50%. Particularly, the expression in 5AG diminished more slowly compared to CT and 7AG.

The sixth stage means the blooming phenological stage (Fig. 2D). CT showed the first blooming at 20 DAT. Expression rates in CT, 5AG, and 7AG at 23 DAT were 31.6%, 2.0%, and 6.0%, respectively. The expression rate in 5AG was about 15-fold and 5-fold lower compared to that in CT and 7AG, respectively. CT reached full bloom of 81.4% ahead of AG treatments at 28 DAT. At this time, 5AG and 7AG showed full bloom rates of 34.6% and 53.6%, respectively. Full bloom of 7AG was earlier than that of 5AG at 34 DAT, but was delayed by 6 days compared to that of CT. Full blooming date of 5AG was 37 DAT, which was delayed by 9 days in comparison with CT. Thus, 5% sodium alginate treatment delayed blooming of ‘Kawanakajima Hakuto’ peach trees the most.

Levels of blooming and fruit set. Blooming and fruit set of AG-treated shoots were compared to those of CT (Fig. 3). When more than 80% flowers in CT bloomed, blooming levels of 5AG and 7AG showed significant differences in comparison with CT (Fig. 3A, B, C). Notably, 5AG showed the slowest blooming, corresponding to mostly 4th and 5th stages. In common with blooming, fruit set of AG-treated shoots also exhibited slower development than CT (Fig. 3D, E, F). Fruit sizes of 5AG and 7AG were obviously smaller compared to those of CT. Consequently, sodium alginate + CaCl$_2$ treatment delayed fruit development by delaying blooming.

Discussion

We investigated average temperatures from February to March, 2021 and full blooming periods (Southern region of South Korea). The average temperature was 7.37 °C during the study period. It increased about 3 °C compared to that 10 years ago (https://data.kma.go.kr). The full blooming date of ‘Kawanakajima Hakuto’ in 2011 was April 17, whereas the full blooming date in 2021 was April 4. The full blooming date of ‘Fuji’ apple which belongs to the same family Rosaceae has been advanced by about 8 days compared to that 10 years ago (https://fruit.nihhs.go.kr). This means that blooming period of fruit trees has been advanced with temperature increase in early spring.

Our results showed that 5AG treatment was the most effective by delaying blooming to a maximum of 9 days compared to CT. Sodium alginate and CaCl$_2$ selected as experimental materials in this study are being used mainly for the manufacture of artificial seeds. Redenbaugh et al. (1991) have explained that fundamental requirement of coating for artificial seed production must be durable enough and the concentration capable of germination is required. Alginate is a true block copolymer composed of homopolymeric regions of M and G, termed M- and G-blocks, respectively (Draget, 2009). The capsulation process of sodium alginate and CaCl$_2$ is described as follows. Gelation occurs when divalent ion (Ca$^{2+}$)
takes part in the interchain ionic binding between G-blocks in the polymer chain, giving rise to a three-dimensional network (Rezende et al., 2007). The capsule membrane is made by forming an ordered structure through interactions between sodium alginate and CaCl₂. The gelation process finishes when calcium ions inside the capsule membrane are depleted (Blandino et al., 1999). It has been reported that sodium alginate at a range of 0.5-5.0% and calcium at 30–100 mM are useful for encapsulation of somatic embryos while keeping the viability of embryos (Redenbaugh et al., 1987). Our study revealed that 5AG was more efficient for agricultural use than 7AG. Although 7% sodium alginate also potentiated blooming delay of flower buds, it was difficult to spray it due to its dense concentration. In addition, it was close to a gel type (data not shown). Thus, 7AG was less effective than 5AG.

Coating using sodium alginate + CaCl₂ has been employed for quality maintenance of fruits and flowers. Pansies coated with alginate can maintain fresh appearance for 14 days (Fernandes et al., 2018). Alginate coating can help maintain fruit quality during storage period of peaches, apples, and cherry by keeping hardness and reducing respiration and transpiration rates (Olivas et al., 2007; Maftoonazad et al., 2008; Díaz-Mula et al., 2012). Olivas and Barbosa-Ca´novas (2005) have described that alginate coating with selective permeability to gases is capable of slowing down the metabolism by decreasing internal O₂ concentration and increasing CO₂ concentration. This result supports the flowering delay effect shown by coating sodium alginate + CaCl₂ in our study.

In previous studies related to flowering delay, detailed data about observed blooming process of flower buds were not presented. Here, we investigated concrete expression of phenological stage on every observation date (Fig. 2). We confirmed that the maximum expression at 4th and 5th stages occurred at low levels (below ~ 50%) in the control. Maximum expression at the 3rd stage in the control took 12 days, whereas 4th and 5th stages took 9 and 3 days, respectively. Therefore, 4th and 5th stages of peach blooming generally have shorter expression periods. Furthermore, we found that there were more than 6 days differences in phenological development levels between CT and AG-treated shoots. Blooming and fruit set levels of AG-treated shoots displayed tardier development than CT (Fig. 3). Particularly, 5AG was the most laggard in fruit development with 9 days of blooming delay compared to the other treatments. Noticeably, previous studies of soybean oil treatment reported delay for about 6 days (Myers et al., 1996), while sodium alginate + CaCl₂ treatment delayed 3 more days than soybean oil. However, soybean oil induced bud damage at higher concentration, whereas sodium alginate + CaCl₂ treatment did not trigger any chemical injury.

Although there were 12 rainy days during the experimental period, coating using sodium alginate + CaCl₂ was effective. However, we considered that if it did not rain so frequently during the experiment period, blooming could have been delayed longer than results of this study by sodium alginate + CaCl₂ treatment. On the other hand, we performed another treatment at the same method as described above to seek the optimal treatment time when flower buds development were 4th stage on March 29. However, overall necrosis of flower petals began at the end of 5th stage in 5AG and 7AG, reaching death in severe cases.
Taken together, our study suggests that sodium alginate + CaCl$_2$ treatment is more desirable to delay peach flowering when phenological development of the flower buds is at the 2nd stage. The 5AG treatment is more suitable for actual application to peach orchards.

Methods

Plant materials and treatment with sodium alginate + CaCl$_2$. Before starting this experiment, we rent some parts of a peach orchard (35°07'50.8"N, 128°09'46.7"E). Six-year-old ‘Kawanakajima Hakuto’ peach trees cultivated conventionally at the peach orchard were used in this study. Thus we have no conflict about permissions of this experiment. Treatments were as follows: (1) control (distilled water, CT), (2) 5% sodium alginate + 100 mM CaCl$_2$ (5AG), and (3) 7% sodium alginate + 100 mM CaCl$_2$ (7AG). On March 8, 2021, when most peach flower buds reached the 2nd stage of phenological development in the experimental orchard, 8 shoots on 6 peach trees were randomly selected and labelled for each treatment. We sprayed each treatment solution onto labelled shoots using a compressed sprayer until running off to coat flower buds entirely. In case of 5AG and 7AG treatments, 100 mM CaCl$_2$ was treated before treatment with each sodium alginate concentration to form better gels based on results of our preliminary experiment. In addition, among 2.5, 5, and 7% sodium alginate treatments, 2.5% sodium alginate was difficult to form a gel due to its low density. Thus, it was not used in this study. After all treatments, all flower buds of shoots were checked to make sure they had been coated uniformly. Total numbers of flower buds were counted for each treated shoot.

Observation of flower bud development. Developmental stage and blooming delay of the flower buds of all the treatments were periodically observed at intervals of 2 to 4 days from March 10, 2021 (2 DAT) to April 14, 2021 (80% full bloom in all treatments). Developmental stage of flower buds was evaluated based on the scale of Deyton et al. (2009) and divided into six stages. The number of expressed flower buds on labelled shoots was counted for each stage and presented as a percentage: phenological stage (%) = number of expressed flower buds/number of all flower buds × 100. After full bloom in all treatments, fruit set was compared on April 30 for control and treatment shoots.

Statistical analysis. All data were analyzed using SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA, USA).

Declarations

Data availability

All data generated or analyzed during this study are included in this published article.

Research involving plants

Plant experiments were performed in accordance with relevant guidelines and regulations.
Author contributions

Y. Park, Conceptualization, Methodology, Formal analysis, Writing - Original draft; H. Shin, Conceptualization, Methodology, Writing - Original draft, Visualization, Supervision.

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Competing interests

The authors declare no competing interests.

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**Figures**
Figure 1

Maximum, average, and minimum temperatures and the number of rainy days at the ‘Kawanakajima Hakuto’ peach orchard (35°07'50.8"N,128°09'46.7"E) throughout the experimental period (37 days).
Figure 2

Phenological changes of ‘Kawanakajima Hakuto’ peach flower buds during 37 days after treatments. CT, control (distilled water); 5AG, 5% sodium alginate + 100 mM CaCl2; 7AG, 7% sodium alginate + 100 mM CaCl2; 3rd, calyx green; 4th, calyx red; 5th, first pink; 6th, first bloom.
Figure 3

Comparison of bloom (when more than 80% flowers in the control were bloomed) and fruit set (53rd day after treatments) levels of treated shoots on 6-year-old ‘Kawanakajima Hakuto’ peach trees. A and D, control (distilled water); B and E, 5% sodium alginate + 100 mM CaCl₂; C and F, 7% sodium alginate + 100 mM CaCl₂.