A DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Assessment of Artificial Pollination Factors Related to Fruit Set, Fruit Quality, and Seed Formation in Yellow-fleshed Kiwifruits

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(Supervised by Professor **Kwan Jeong Song**, Ph.D)

Submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Agriculture

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ABSTRACT

Kiwifruit is a dioecious plant, and artificial pollination is very important for commercial cultivation. Since the effective pollination period of kiwifruits is short, cultivation techniques that allow artificial pollination in a short period of time are needed for stable fruit set. Also, there have been few studies on the correlation between the ploidy of the pollen donor and the quality characteristics of fruit for Korean kiwifruit cultivars. This study was conducted to suggest the effective artificial pollination period and time for yellow-flesh type ‘Halla Gold’ and ‘Sweet Gold’ widely cultivated in the Jeju region, and to investigate the effects of different artificial pollination methods and pollen donors with different polyploidy on fruit quality and seed formation.

This study was conducted to evaluate the effect of artificial pollination period and pollination time on fruit quality and seed formation in 'Halla Gold' and 'Sweet Gold' kiwifruits grown in a non-heated plastic film house in Jeju, Korea. When both ‘Halla Gold’ and ‘Sweet Gold’ cultivars were pollinated on the third day after

blooming, the fruit set rates, fresh weight and dry matter percentage started to decrease. On the other hand, the dry matter percentage, soluble solids content (SSC), acidity and *h* value did not show any significant difference. It could be seen that the seed number and 100-seed weight decreased on the third day after blooming, and the effective pollination period affected the fruit weight and seed development. The fruit set rates of both ‘Halla Gold’ and ‘Sweet Gold’ cultivars with the different artificial pollination time were over 90%. The fruit weight and dry matter showed the tendency of similar level in pollination at 7 and 10 AM and 1 PM and of low level at 4 PM. In both 'Halla Gold' and 'Sweet Gold' cultivars, there was no statistical significance in the SSC, acidity, firmness, *h* value, seed number and 100-seed weight. The results indicates that the efficient pollination period of 'Halla Gold' and 'Sweet Gold' cultivars grown in the non-heated plastic film house in Jeju is within 3 days after blooming, and the pollination time of the day has a minor effect on fruit quality and seed formation.

This study was conducted to investigate the effect of pollen application methods

for artificial pollination on such as pretreatment before pollination, repeated pollination and application types on fruit quality and seed formation. As for repeated pollination, it was observed that the pollen tube in pistil reached and penetrated into the ovule at 3 days after artificial pollination although the patterns varied depending on the number of dry pollen application. In 'Halla Gold' and 'Sweet Gold' cultivars, the number of pollen tubes was clearly higher in repeated pollination than when in single pollination. Furthermore, when pollen application was repeated, the fruit weight, dry matter percentage, number of seeds and 100-seed weight were higher. The firmness was low and the *h* value was high in pollination repeated three times. The soluble solids content and acidity showed no significant difference in all treatments. When pistillate flowers were pollinated with dry pollen immediately after water sprinkle, both 'Halla Gold' and 'Sweet Gold' cultivars showed the lowest fruit weight, dry matter percentage, firmness, number of seeds, and 100-seed weight, whereas dry pollen application 1 h after water sprinkle, immediate or 1 h after suspension medium sprinkle didn't show

significant differences on the fruit quality and seed formation. In wet application using pollen suspension, the fruit weight was lower in both 'Halla Gold' and 'Sweet Gold' cultivars than in conventional dry pollen application, but there was no significant difference in 'Halla Gold' cultivar. Application types with dry pollen and pollen suspension did not show a significant difference in fruit quality and seed formation except the fruit weight of ‘Halla Gold’ cultivar. The results indicate that raindrops or dewdrops on stigma might reduce an efficiency of artificial pollination with dry pollen, but repeated pollination and application types with dry pollen and pollen suspension have a minor influence.

This investigated how the ploidy level of the kiwifruit pollinizer cultivars ‘CK3’ (diploid), ‘T’ line (tetraploid), ‘Bohwa’ (hexaploid) and ‘Chieftain’ (hexaploid) affected fruit set, fruit quality, and seed formation in the tetraploid kiwifruit cultivars ‘Halla Gold’ and ‘Sweet Gold’ cultivated. Pollen tubes growing in the pistil reached and combined with the ovule 3 days after artificial pollination, and their patterns differed depending on the ploidy level of the pollen parent. The

number of pollen tubes observed in ‘Halla Gold’ and ‘Sweet Gold’ pistils was significantly lower following pollination by ‘CK3’ than with the other pollen donors. In all pollen treatments, the fruit set rates were >90%. The fruit weight of both ‘Halla Gold’ and ‘Sweet Gold’ were high following pollination with ‘Chieftain’ and ‘Bohwa’. The dry matter content, soluble solids, and acidity were not significantly different among all pollination treatments. Fruit firmness was higher following pollination with ‘Chieftain’ and ‘Bowha.’ Colorimeter h° values for flesh of ‘Halla Gold’ was low following pollination with ‘CK3’, but there were no differences for ‘Sweet Gold’ among all pollinations. The number of seeds showed a similar trend to fruit weight, but the 100-seed weight was highest with ‘T’ line as the pollinizer. The results indicate that the ploidy level of the pollen donor affects fruit quality more than fruit set. Also, the pollen most suitable for cultivation of ‘Halla Gold’ and ‘Sweet Gold’ is considered to be tetraploid ‘T’ line and hexaploid ‘Bohwa’ and ‘Chieftain’, which showed favorable effects on the weight and firmness of fruits, and the number and weight of seeds without adversely influencing fruit set and dry matter content.

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INTRODUCTION

Kiwifruits are perennial deciduous fruit bearing woody vines, which belong to the genus of *Actinidia* in the family of Acinidiaceae. The original habitat of kiwifruits is China, and two species, *A. chinensis* var. *deliciosa* with green flesh and *A. chinensis* var. *chinensis* with red or yellow flesh, are commercially cultivated (Huang et al.,2004). The base chromosome number of kiwifruits is X = 29, and their ploidy is diverse from diploid to octoploid depending on species or variety (Watanabe et al,1990; Yan et al. 1997; Seal et al., 2013a; Huang 2014). *A. chinensis* var. *chinensis* is either diploid or tetraploid, and *A. chinensis* var. *deliciosa* hexaploid. The fruit size, flesh color and the fruit skin hairiness vary depending on the ploidy (Li et al., 2010).

The main cultivar in Korea is green fleshed 'Hayward', which occupies most of the cultivation area (Park,2009), and besides, yellow or red fleshed kwifruits are also cultivated in small scales, which is gradually increasing (Moon et al.,2012). As consumers in Korea have demanded flavorful kiwifruits with high sugar contents since the 2000s, domestic cultivars such as Jecy Gold, Halla Gold and Sweet Gold

have been bred and distributed (Kim et al., 2007a, 2007b, 2009, 2012,2018; KSVC, 2011). Recently, the cultivation area of yellow fleshed kiwifruits is rapidly expanding in Korea, and 'Halla Gold' and 'Sweet Gold' are the most commonly distributed kiwifruits in the Jeju region.

Kiwifruits are dioecious plants, and artificial pollination is essential for commercial cultivation. Pollen used for artificial pollination has a great effect on seed formation by various factors such as affinity, viability, germination and pollen tube growth (Seal et al., 2013b). Since the size of kiwifruits is associated with the seed number (Hopping, 1976; Vasilakakis et al., 1997), pollination is the most influential factor on the fruit size and harvest yield (Ferguson, 1991). However, as the relationship between the fruit size and the seed number is highly complex, the degree of correlation even varies depending on the cultivar (Lawea et al., 1990), and may also be different due to the pollen donor, tree and the number of fruit set (Hopping, 1990; Seal et al., 2017).

The effective pollination period (EPP) is the number of days required for

pollination that leads to effective production of fruits (Sanzol and Herrero, 2001). The EPP may be affected by temperature, bloom quality and chemical treatment (Sanzol and Herrero, 2001). Generally, artificial pollination for kiwifruits is performed in a fine day and stigma receptivity is associated with the amount of secretion exuded from stigma. The amount of secretion may vary with the time of the day depending on air temperature and humidity, which affects pollen germination and pollen tube penetration. However, as of now, studies on the EPP or pollination time of kiwifruit cultivars bred in Korea have been lacking.

Although high quality pollen is essential in artificial pollination, the other factors such as physiological condition of receptive stigma, application times and methods, and environmental conditions affect ovule fertilization and pollination efficiency. Generally, artificial pollination for kiwifruits is performed in a fine day and exudates on stigma tends to be desiccated around noon due to having high air temperature and low humidity, which hight cause a decline of pollen germination and pollen tube penetration. During artificial pollination, the uniformity of pollen

load on stigma depends on labor proficiency and insufficient or uneven pollen load by unskillful labor requires repeated pollination occasionally. However, up to date, few studies were reported on repeated pollination in kiwifruits, which is not fully understood (Hoping, 1990). Recently, application of suspension pollination has been reported, but studies on it were few, specially in yellow-fleshed kiwifruits until now. While studies on the effective pollination methods for 'Hayward (A. *deliciosa*)' have been reported (Lim and Lee, 2013; Razeto et al., 2005; Gonzalez et al., 1998), those on artificial pollination techniques for other cultivars have been lacking.

Pollination of kiwifruit is performed using the pollen collected donors different polyploidty pollen. Kiwifruit growers in Korea typically use hexaploid A. c*hinensis* var. *deliciosa* cultivars such as 'Machua,' 'Bohwa,' and 'Chieftain' as pollen donors. In addition, most of the commercial pollen used in Korea is imported from China and, while it is inexpensive and easy to obtain, its ploidy level and cultivar source are difficult to determine. Recently, it was reported that the ploidy level of the pollen donor affects the characteristics of fruits including fruit weight, dry matter,

flesh coloration, and nutritional components (Seal et al., 2013a, 2013b; Seal et al, 2016; Stasiak et al., 2019). However, there have been few studies on the correlation between the ploidy of the pollen donor and the quality characteristics of fruit for Korean kiwifruit cultivars (Jeong et al., 2018).

Thus, this study has been conducted to evalvate the effective pollination period, time, pollination methods and pollen donors with different ploidy on fruit quality and seed formation in 'Halla Gold' and 'Sweet Gold', yellow fleshed domestic kiwifruits cultivated commonly in the Jeju region.

LITERATURE REVIEW

General characteristics of kiwifruits

Kiwifruit is a perennial dioecious and deciduous fruit bearing woody vine, which belongs to the genus of *Actinidia* in the family of Acinidiaceae, and it is known to originate from China. Approximately 54 species and 75 taxa exist including commercially important *A. chinensis var. deliciosas* and *A. chinensis var. chinensis* (Huang et al., 2004). Four varieties such as *A. arguta Planch., A. polygama Maxim.*, *A. kolomikta Maxim.* and A. *rufa* Planch. are distributed in Korea (Watanabe et al. 1990; Yan et al. 1997; Cui et al. 2002; Huang 2014).

The polyploidy of kiwifruits varies depending on the species or variety, including diploid, tetraploid, hexaploid and even octoploid (Watanabe et al,1990; Yan et al. 1997; Seal et al., 2013a; Huang 2014). Commonly cultivated *A. chinensis* var. *deliciosas* is hexaploid and *A. chinensis* var. *chinensis* is either diploid or tetraploid (Warrington and Weston, 1990). It is known that diploid species are found in the habitat at 800 m above sea level or lower, tetraploid species in the habitat at between

800 m and 1,400 m, and hexaploid species in the habitat at 1,400 m above sea level or higher (Huang and Liu, 2014). Therefore, polyploidy appears to be associated with adaptation to the environment and the growth of trees.

Basically, kiwifruits are dioecious plants, and their male bloom and female bloom have prominently different characteristics (Schmid 1978; Ferguson 1984). Although female bloom has an external form of a perfect flower, it produces hollow pollen. While bloom develops in the leaf axil, it sometimes develops from small inflorescence. Kiwifruit bloom consists of thin five or more petals in white, yellow or pink. Male bloom typically has short filaments and small pollen in the stamen, and the ovary is retained in a degenerated form.

As for the characteristics of fruits, the fruits of *A. chinensis* var. *deliciosas* are hairy on the surface and have green flesh while those of *A. chinensis* var. *chinensis* are less hairy, which thus makes the surface smooth, and have yellow flesh (Warrington and Weston, 1990). In addition, the fruit size of *A. chinensis* var. *chinensis* is smaller than *A. chinensis* var. *deliciosas*, but its harvest yield is larger. Furthermore, the former has

characteristics of the fast maturation, high sugar content and low acidity.

Kiwifruits have characteristics that the organs such as buds and petals are growing rapidly for 10 weeks from March until anthesis (Polito and Grant, 1984). Although the anthesis of kiwifruit female bloom is 7 - 9 days, fruit set can be induced for the formation of normal fruits only when pollen is applied to the stigma within 2 - 3 days after anthesis. Fruits are growing slowly until 30 - 40 days after anthesis as the cell division stage, and the period of 50 - 60 days after anthesis is when the weight and size of fruits are rapidly increasing. Cell division of the epicarp and the endocarp ends in approximately 20 days and 30 days after anthesis, respectively while the core continues to divides until 110 days after anthesis. In 70 - 80 days after anthesis, hypertrophy of the endocarp and the core slows down drastically, and thus this is the period when the growth of fruits becomes steady.

Seeds start to grow within 4 days after fertilization, and grow constantly for 80 days until they stop to grow in 110 days post-fertilization (Harvey and Fraser, 1988). As the testa is hardened, its color starts to assume pale yellow, and the flesh changes

to pale green. The next is the maturation stage when assimilates are accumulated in fruit cells, and during this period, the fruit weight increases and the flesh changes from pale green to dark green (Hopping, 1976; Woollley et al.,1992).

In general, since the fruit size and the seed number have a positive correlation with each other, the higher number of seeds needs to be formed in order to obtain larger fruits. Normal fruits contain approximately 700 - 1,400 seeds, and the cultivar 'Hayward (A. chinensis var. deliciosas)' reportedly contains up to 1,800 seeds (Hopping, 1976; Pyke and Alspach, 1986).

The most common index to indicate the growth stages of kiwifruits includes days after anthesis, or the sugar content. Recently, Biologische Bundesantalt, Bundessortenamt, Chemische industrie (BBCH) is being used (Richardson et al. 2011). Kiwifruits require post-harvest ripening, in which flavor and taste are increasing only when a certain period of time elapses after harvest. If kiwifruits are harvested before sufficient maturation, their shelf life may be prolonged, but the flavor decreases, lowering the quality. On the contrary, if the harvest is late after

maturation, the flavor is good but the shelf life is short. Predictive studies on setting the suitable harvest time and the quality have been reported (Crisosto, 1992; Richardson et al., 1997; Burdon et al.,2004). In kiwifruits, the sugar content at the harvesting season is most commonly used as an index to evaluate the fruit quality. However, since kiwifruits contain starch even after maturation, it is difficult to measure the accurate sugar content after post-harvest ripening. Recently, the sugar content after post-harvest ripening is estimated using the dry matter percentage of kiwifruits. However, the dry matter percentage may vary depending on the effect of various factors such as kiwifruit cultivar, cultivation factors and the harvesting season (Burdon et al., 2004; Max, 2015).

Pollination and fruit development

In most of deciduous fruit trees, floral buds divide morphologically in July and August, and the reproductive organs develop during winter, blooming occurs in the next spring. The number and size of the reproductive organs increase during winter, but the division of floral buds can be recognized only in the middle of March. Afterwards, until the middle of May, the reproductive organs such as petals form rapidly, which is similar to the process of floral bud division in tangerines, an evergreen fruit tree. Female bloom of kiwifruits contain over 1,400 eggs (Harver and Fraser, 1988), but cannot produce pollen in the stamen (Goodwin, 1986). On the other hand, male bloom is able to produce pollen in the stamen, but eggs and styles exist degenerated. In the production of kiwifruits, the transfer of sufficient pollen to female bloom is important (Ashman et al., 2004; Aizen and Harder, 2007). The amount of pollen adequately transferred to female bloom determines the fruit production and quality, and typically the production of high quality fruits requires 1000 egg fertilizations per bloom (Testolin et al., 1991; Costa et al. , 1993).

Kiwifruit is pollinated by entomophily (Craig et al., 1988), and rarely pollinated by anemophily. The penod of female bloom is typically 7 to 9 days, but the efficient fertilization can be made 4 - 5 days after anthesis. Compared to female bloom, the penod of male bloom is longer because the beginning of anthesis is 2 - 3 days earlier while the end of anthesis is 2 - 3 days later. However, pollen suitable for pollination should be harvested within 2 to 3 days after anthesis, and pollen harvested afterwards has lower activity, unsuitable for pollination.

Although honeybees are reported as an effective pollinator for natural pollination of kiwifruits (Read et al., 1989; Vaissiere et al., 1996; Goodwin, 2012), kiwifruit bloom is unattractive to honeybees since basically it does not have nectar. The direction of honeybees' movement in the orchard is slightly different depending on the type of shelf facilities, but generally honeybees show a long movement in the row direction. Therefore, a certain amount of pollenizers should be planted between the female trees at each row. Honeybees are very sensitive to climate conditions, particularly to temperature and sunlight. Therefore, in order to use honeybees

properly for pollination, the temperature during anthesis should be high, and only when sunlight is sufficient to expose flowers under the kiwifruit vine, honeybees will visit there (Testolin et al., 1990).

Artificial pollination of kiwifruits was first reported in New Zealand and Italy in the 1970s (Hopping and Jerram, 1979; Hopping and Hacking, 1983; King and Ferguson, 1991). Currently, in most farms in Korea, people artificially put pollen of male bloom on the stigma of female bloom. Without such artificial pollination, it is difficult to achieve normal fruiting. A single kiwifruit normally contains 700 to 1,400 seeds (Hopping, 1976; Pyke and Alspach, 1986), and the number of pollen equivalent to that of seeds formed in a fruit should be evenly applied on 25 to 40 stigma.

Male bloom needs to be collected for pollen harvesting at least before 8 AM, and they should be just in a state immediately before opening, not in full bloom. In Korea, a domestic cultivar of ‘Bohwa’ and a New Zealand cultivar of 'Chieftain’ are widely used as the pollenizer of kiwifruits. Bohwa, a domestic cultivar, has been

reported to have more pollen than Chieftain(Oh et al., 2020), and the germination rate and shelf life of pollen are superior to 'Matua', a New Zealand cultivar.

The ideal method for artificial pollination is to use 100% pure pollen, however, the cost of pure pollen is too high for pollination. A pollen diluent containing pigments that allow visual check with the naked eye is used to reduce the waste of pollen caused by repeated pollination. In general, lycopodium powder is used as a pollen diluent in a mixture with pollen at the ratio of 10:1 (Jeong et al., 2018). It is best to perform artificial pollination within 2 days from the day of female bloom, or it should be performed within 4 days at the latest for normal fruit set.

Pollen donors and fruit development

As kiwifruits are dioecious plants, artificial pollination is essential and very important for commercial cultivation. In many plants, pollen donors can affect many important aspects of fruit quality and seed characteristics such as fruit set, fruit size, shape, flesh quality, seed size and seed number (Denney 1992; Seal et al., 2013b). A basis to understand the effects on seeds is that pollen donors make direct genetic contribution to embryo, which has been confirmed through double fertilization in angiosperms. However, the reports on the effects of pollen donors on maternal fruits are still controversial (de Putter et al, 1996; Ehlenfeldt 2003). These effects are associated with the difference in the seed number. In general, the fruit size varies depending on the degree of seed formation in fruits (Hopping, 1976; Vasilakakis et al., 1997). However, the relationship between the seed number and the fruit size is so complex that the degree of such a relationship may even differ depending on the variety (Lawes et al., 1990) as well as the pollen donor, plant age and the number of set fruits (Hopping, 1990; Seal et al.,2018). Pollen donors may

have a different ability to set seeds due to changes in various factors including pollen viability, pollen germination, pollen tube growth and fertilization rate. Typically, since non-crossbreeds or conversely wide crosses have a reduced seed number or abnormal seed development, the fruit size may become smaller (Lim and Luders 1998). In addition, in interploidy crosses, genetic imbalance in the embryo often leads to abnormal seed development (Johnston et al. 1980).

The number of chromosomes in Kiwifruit is X=29 and their ploidy varies from diploid to octoploid depending on the species or variety (Seal et al., 2013a). The fruit size, flesh color, skin hair and environmental resistance vary depending on ploidy (Li et al, 2010). Commonly cultivated *A. chinensis* var. *deliciosas* is known to be hexaploid, and *A. chinensis* var. *chinensis* to be diploid or tetraploid (Warrington and Weston.,1990). Since flowers of diploid *A. chinensis* are normally smaller than those of *A. deliciosa*, it is not economically feasible to produce pollen of diploid *A. chinensis* (Seal et al.,2013b). Hence, kiwifruit growers in Korea typically use hexaploid pollen donors such as 'Machua', 'Bohwa', and 'Chieftain' . In addition, most of commercial

pollen used in Korea is imported from China due to low price and easy accessability. It is not easy to identify the information of the ploidy level and cultivar source of pollen imported from China.

Pollen donors from different varieties affect the characteristics of kiwifruits such as fruit set, soluble solids concentration, flesh firmness, final fresh weight, fruit size as well as fruit shape (Qj et al., 2007). Seal et al. (2016) reported that pollination of diploid A. *chinensis* seedlings with pollen from the hexaploid male A. *chinensis* var. *deliciosa* reduced fruit set, fresh weight, dry matter concentration, weight and number of seeds and expression of red pigmentation in the fruit compared with pollination with pollen from diploid A. *chinensis*. In contrast to many other diploid A. *chinensis* genotypes, pollination of the cultivar 'Hort16A' by males of higher ploidy may be beneficial in terms of fresh fruit weight (Seal et al. 2013).

In recent years, it has increasingly been reported that pollinizers with varying ploidy levels have different effects on fruit yield and characteristics (Li et al., 2010; Jeong et al., 2018; Stasiak et al., 2019). In addition, kiwifruit bacterial canker

(*Pseudomonas syringae* pv. *actinidiae*), the most serious and dangerous disease in kiwifruit, may be dispersed via pollen during artificial pollination.

It was reported that the ploidy level of the pollen donor has an effect on the characteristics of fruits including fruit weight, dry matter, flesh coloration, and nutritional components (Seal et al., 2013a, 2013b; Seal et al, 2016; Stasiak et al., 2019). However, there were few studies dealing with a correlation between the ploidy level of pollen donor and the quality characteristics of fruit for Korean cultivars (Jeong et al., 2018).

CHAPTER I

Evaluation of Artificial Pollination Period and Time Related to Fruit Set and Quality and Seed Formation in Yellow-fleshed Kiwifruits

Abstract

This study was conducted to evaluate the effect of artificial pollination period and pollination time on fruit quality and seed formation in 'Halla Gold' and 'Sweet Gold' kiwifruits grown in a non-heated plastic film house in Jeju, Korea. When both 'Halla Gold' and 'Sweet Gold' cultivars were pollinated on the third day after blooming, the fruit set rates, fresh weight and dry matter percentage started to decrease. On the other hand, the dry matter percentage, soluble solids content (SSC), acidity and *h* value did not show any significant difference. It could be seen that the seed number and 100-seed weight decreased on the third day after blooming, and the effective pollination period affected the fruit weight and seed development. The fruit set rates of both 'Halla Gold' and 'Sweet Gold' cultivars with the different artificial pollination time were over 90%. The fruit weight and dry matter showed the tendency of similar level in pollination at 7

and 10 AM and 1 PM and of low level at 4 PM. In both 'Halla Gold' and 'Sweet Gold' cultivars, there was no statistical significance in the SSC, acidity, firmness, *h* value, seed number and 100-seed weight. The results indicates that the efficient pollination period of 'Halla Gold' and 'Sweet Gold' cultivars grown in the non-heated plastic film house in Jeju is within 3 days after blooming, and the pollination time of the day has a minor effect on fruit quality and seed formation.

Introduction

For stable fruit set and high fruit quality in kiwifruits (*Actinidia chinensis*), the pollination and fertilization of pistillate flowers by viable pollen from staminate flowers are required. Since the fruit size in kiwifruits is correlated with the number of seeds, pollination is the most crucial factor influencing the fruit size and harvest yield over the weight range for exportable fruits (Hopping, 1976; Ferguson, 1991; Vasilakakis et al., 1997; Brantley et al., 2019). Therefore, growers pay a great efforts and attentions to ensure successful pollination. Particularly, in order to optimize the pollination of pistillate flowers, it is very important to understand the effective pollination period (EPP) for successful ovule fertilization. The EPP was proposed by Williams (1970) as a tool to assess the flower receptivity in fruit crops, and defined as a pollination period when fruits can be produced successfully (Sanzol and Herrero, 2001). Various factors may affect the EPP, which include pistillate and staminate cultivars, environmental temperature, flower quality and management practices such as crop load and chemical treatment (Sanzol and Herrero, 2001; Abedi Gheshlaghi, 2019).

In Korea, kiwifruits are mostly grown in Jeju Island and the southern coast areas. As Korean consumers have demanded diverse high-sugar cultivars since the 2000s, the planting of yellow- or red-fleshed kiwifruits have been preferred to that of 'Hayward'

green-fleshed kiwifruits and became main ones recently (Jeong et al., 2018). The major cultivars in Jeju consist of ‘Hall Gold’ and ‘Sweet Gold’ for yellow-fleshed kiwifruits which was bred in Korea and ‘Hayward’ for green-fleshed ones except for ‘Sun Gold’ yellow-fleshed one which is allowed to grow out of New Zealand under contract with Zespri International Co., Ltd. In general, while green-fleshed cultivars (*A. chinensis* var. *deliciosa*) having large flowers maintain petals freshly for 5 days, petals of small flowers from the yellow- or red-fleshed cultivars (*A. chinensis* var. *chinensis*) are withered in shorter days. However, the longevity of flowers or duration of stigma receptivity is not constant even in same cultivar, which differs from growing regions (Abedi Gheshlaghi, 2019). The EPP of commercial green-fleshed 'Hayward' kiwifruit is 3 - 4 days (Galimberti et al.,1987; Gonzalez et al.,1995) and a yellow-fleshed cultivar of 'Hort16A’ has the highest stigma receptivity 2 days after blooming (DAB) (Goodwin et al., 2013). The EPP or DAB for pollination ensuring the good production of fruits with marketable size may also vary with kiwifruit cultivars and growing conditions (Thompson et al., 2014; Abedi Gheshlaghi, 2019). Generally, artificial pollination for kiwifruits is performed in a fine day and stigma receptivity is associated with the amount of secretion exuded from stigma. The amount of secretion may vary with the time of the day depending on air temperature and humidity, which affects pollen

germination and pollen tube penetration. However, as of now, studies on the EPP or pollination time of kiwifruit cultivars bred in Korea have been lacking.

Recently, global warming is getting worse and may provoke a frequent occurrence of detrimental weather to the growth and development of fruit and tree (Ahn et al., 2016; Kumarihami & Song, 2018). It is not easy to maintain the favorable conditions for pollination and fruit set even under protected growing conditions such as plastic film house. Therefore, it is very important for growers to recognize the duration of days and time of the day for artificial pollination securing a satisfied fruit set and quality for each cultivar grown in their orchards. Thus, this study was conducted to evaluate the DAB and time of the day that flowers can be successfully pollinated based on fruit quality and seed formation of two cultivars of yellow-fleshed kiwifruits widely grown in Jeju, Korea.

Materials and Methods

Plant material

The experiment was carried out from 2018 to 2019, on mature vines of the pistillate cultivars (tetraploid), including 8 year-old 'Halla Gold' and 5 year-old 'Sweet Gold' kiwifruit (*A. chinensis* var. *chinensis*), which were trained to a pergola system and grown under the general management practices in an unheated plastic film house at a commercially growing orchard located in Jeju-si, Jeju Special Self-governing Province, Korea.

Artificial pollination

Pollen for artificial pollination was obtained from a hexaploid and staminate cultivar, 'Bohwa' kiwifruit (*A. chinensis* var. *deliciosa*) bred in Korea. The pollen viability for artificial pollination was measured using the FDA and 1% I_2KI staining methods (Pok et al., 2015) before use, and the viability of over 85% was confirmed. Dry pollen mixed with lycopodium power at 1:10 (w/w) was sprayed on pistillate flowers with an artificial pollinator (PS-100, Jeju Bio Tech Co., Korea). Three vines of each pistillate cultivar were used for these experiments. Five fruit

bearing branches from canes with similar length and vigor per each vine were selected and the flowers were pollinated on the morning of blooming day and 1, 2 and 3 days after blooming, respectively for the experiment evaluating pollination period. Also, artificial pollination was performed on five fruit bearing branches per each vine of three vines at 7 AM, 10 AM, 1 PM and 4 PM at 1 day after blooming for the experiment evaluating the pollination time of a day.

Histological analysis of pollen tube growth

In order to observe the pollen tube growth in the pistil with the different DAB of artificial pollination, pistils were collected on the first, second and third day after pollination and were fixed with FAA solution (formalin: acetic acid: 70% ethanol, 1: 1: 18, v/v/v). Fixed ones were kept at 4 ℃ before analyzing pollen tube growth. The fixed ones were softened in 2N NaOH solution at 60 ℃ for 60~90 minutes and washed 4 times with an immersion of distilled water for 15 minutes. The softened ones were stained with 0.1% aniline blue for 24 hours in the dark at room temperature (Yang et al., 2008; Jeong et al., 2018). After being located into a center of block using 4% agarose (Agarose LE, Biomedic Co., Korea), the stained ones were sliced into the 7 μm thickness with Vibratome (Series 1000, The

Vibratome Co., Us). The slices were mounted on the slide glass and then observed under a fluorescence microscope (Leica DMRBE, Leica Co., Germany).

Fruit set rate and fruit quality analysis

To analyze the fruit quality, 30 fruits for each treatment were harvested at 180 days after artificial pollination in both cultivars. The quality characteristics of fruits including the weight of fruits, dry-matter percentage, soluble solids content (SSC), acidity, firmness and color were measured. The weight of fruits was measured immediately after harvest using an electronic scale (EL-2000S, Setra Inc., US). The dry matter percentage was measured using a 2-3 mm thick section cut from the equatorial part of the fruit after drying at 60 ℃ for 24 h (Burden et al., 2016). The SSC and acidity were measured from squeezed fruit juice with a digital sugar and acid analyzer (GMK-707R, G-won Co., Korea). The firmness was measured using a firmness meter (FHM-5, Takemura Co., Japan) after the skin of fruit was peeled off in a thickness of 1 mm (Burden et al., 2017). The color was measured using a colorimeter (CR-400 Chroma Meter, Minolta Co., Japan) after the skin of the fruit was peeled off to a thickness of 2-3 mm.

Assessment of the number and weight of seeds

At 180 days after artificial pollination of 'Halla Gold' and 'Sweet Gold', 20 fruits for each treatment were collected for counting seed number and measuring seed weight. Seeds separated from fruit flesh were washed, dried at 60 ℃ for 24 h and counted using a seed counter (Contador 2, Pfeuffer GmbH Co., Germany). The seed weight based on a 100-seed dry weight was measured using an electronic scale (EL-2000S, Setra Inc., US).

Statistical analysis

Statistical analysis was performed as a completely randomized design at the 95% level using the SPSS program (SPSS version 18, IBM SPSS Software Inc., US) and the significance between the means was analyzed with the Duncan's multiple range test.

Results and Discussion

Pollination period associated with fruit quality and seed formation

The characteristics of fruit quality and seed formation at harvest in 'Halla Gold' and 'Sweet Gold' kiwifruit pollinated at different DAB are shown in Table 1 and Table 2, respectively. The fruit set did not show any difference in both cultivars until 2 DAB, but decreased at 3 DAB. The fresh weight, dry matter percentage, seed number and 100-seeds weight of both cultivars showed almost same tendency which were not different among treatments pollinated at 0 - 2 DAB, however decreased significantly in the pollination at 3 DAB. The SSC and firmness showed a small variation by years with a fact that one year had a significant difference and the other year had no significant difference in both cultivars. The acidity was not significantly different among the treatments pollinated at different DAB in both cultivars for two years. The flesh color development showed no significant difference in 'Hall Gold' for two years, however a significant difference in 'Sweet Gold' for two years, in which the flesh color development was delayed in the pollination at 3 DAB.

It was assumed that the decrease of fruit set, fruit weight, dry matter, seed number, seed weight and flesh color development was affected by flower aging because the

change of petal color from white into light brown started at 2 DAB and the dehiscence of petal started at 3 DAB (Fig. 1). This flower aging might be connected with a deterioration of pollen receptivity or a retardation of pollen tube growth, which reduced the seed number and seed weight due to an inhibition of ovule fertilization (Table 1 and Table 2). According to Gonzalez et al. (1995), an aged kiwifruit flower has a low level of pollen receptivity and subsequently reduces the crop yield along with an increased occurrence of deformed fruits. It has been reported that the EPP of 'Hayward' was 3 days (Galimberti et al., 1987) or 4 days (Gonzalez et al., 1995) and that of ‘AU Fitzgerald’ and ‘AU Golden Sunshine’ was 4 and 5 days, respectively (Thompson, 2014; Brantley, 2016), which indicates that the EPP will be different from cultivars and growing regions. In this study, ‘Halla Hold’ showed an early flower aging in external appearance including petal color change and petal dehiscence than ‘Sweet Gold’, which may be related to a little lower fruit set at 3 DAB of ‘Hall Gold’ compared to that of ‘Sweet Gold’ for two years (Table 1, Table 2 and Fig. 1). This result suggests that flower aging is regulated under genetic constitution might influence EPP and depends on cultivars. Pears had a wide range of the effective pollination period (EPP) with 3 - 13 days and their EPP was prolonged at low temperature and was reduced at high temperature (Vasilakakis and Poringis, 1985). The EPP could be

changed with growing environment conditions such as temperature and humidity which are not constant by years. This represents the reason of that SSC and firmness had a variation of significance by years in this study.

Generally, fruit weight is correlated to seed number and seed weight (Ferguson, 1984; Pyke and Alspach, 1986; Galimberti et al., 1988; Lawes et al., 1990), which was in accord with the results of this study. Also, kiwifruit industry has recognized that some factors including SSC, dry matter, firmness and flesh coloration are key indices determining the maturity and harvesting time of yellow-fleshed cultivars (Feng et al., 2011; Lim and Eom, 2018). This study showed that the pollination period might affect the maturity based on dry matter, SSC, firmness, and flesh chromaticity despite the fact that the SSC didn't have a significant difference in the year of 2018 in both cultivars and the flesh hue value just in 'Halla Gold', too.

The EPP of kiwifruits has been determined based on just one factor of fruit set (Abedi Gheshlaghi, 2019; Gonzalez et al., 1995) or two factors of fruit set and fruit weight (Brantley et al., 2019), which reported the EPP ranged with 3-5 DAB. However, our study showed that all of fruit set, the fruit weight, dry matter, seed number and seed weight were consistently reduced from 3 DAB in both cultivars for two years and the EPP should be determined based on a combination of these factors. Consequently,

the study indicates that the period for pollination of these kiwifruits grown in the non-heated plastic film house in Jeju region might be 3 or 4 DAB at most although it could vary depending on the growing and weather conditions for 'Halla Gold' and 'Sweet Gold'. Also, this result suggests that further studies are inevitably necessary for improving pollination efficiency to allow growers to save a cost and secure a stable fruit set and a high fruit quality since the EPP of kiwifruits is short.

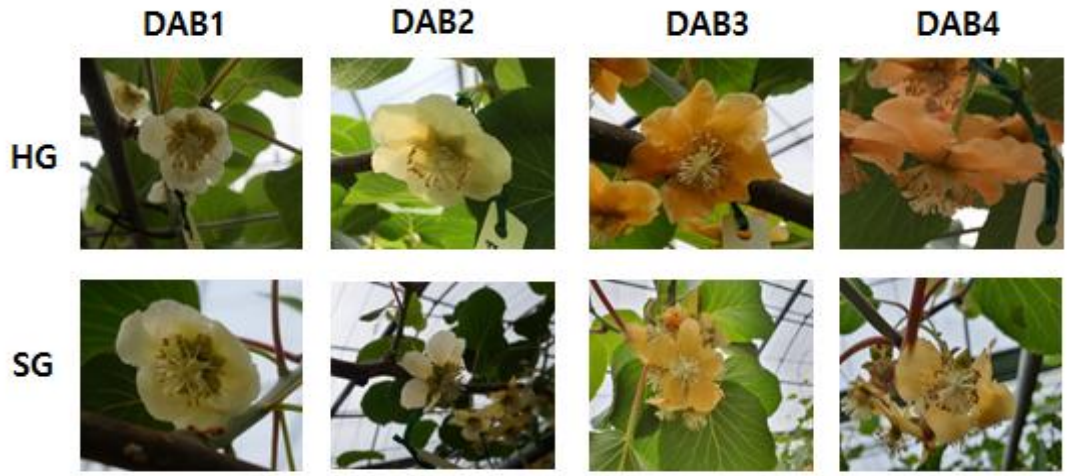

Fig. 1. Morphology of flowers at different days after blooming (DAB) in 'Halla Gold' (HG) and 'Sweet Gold' (SG).

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Table 1. Fruit set rate, fruit quality, and seed formation according to the pollination period in 'Halla Gold' kiwifruit.

Year	DABD[x]	Fruit set rate (%)	Fresh weight (g)	Dry matter (%)	Soluble solids content (°Brix)	Acidity (%)	Firmness (kg/5mmø)	Flesh chromaticity h°	Seed number	100-seeds weight (mg)
2018	1	92.0±0.6[y] a [z]	77.8±1.1a	13.9±0.2a	11.5±0.2	0.9±0.1	5.6±0.2	99.5±0.5	411.0±9.3a	11.8ab
	2	93.3±1.3a	78.4±1.2a	13.6±0.5a	11.9±0.2	0.9±0.1	5.1±0.2	99.9±0.7	407.8±8.3a	12.3ab
	3	92.0±1.0a	77.1±0.8a	13.1±0.3ab	11.9±0.2	1.0±0.1	5.2±0.1	100.9±0.8	414.7±9.9a	12.9a
	4	80.5±0.6b	67.1±0.9b	11.9±0.3b	11.6±0.2	0.9±0.1	5.5±0.2	102.2±0.8	380.0±5.9b	10.1b
	Significance	*	*	*	ns	ns	ns	ns	*	*
2019	1	93.7±0.7a	93.0±3.2a	13.0±0.3a	11.5±0.2ab	1.2±0.1	5.4±0.1	104.4±0.6	514.3±9.3a	12.7ab
	2	92.7±1.2a	94.0±3.2a	12.8±0.2a	12.2±0.2a	1.2±0.1	5.5±0.1	105.0±0.7	509.5±9.0a	13.2a
	3	92.3±0.7a	93.2±0.7a	12.5±0.2a	12.1±0.2a	1.2±0.1	5.3±0.1	104.8±0.7	511.3±7.6a	12.9a
	4	81.8±0.6b	84.8±2.9b	11.6±0.1b	11.0±0.2b	1.1±0.1	5.5±0.1	106.1±0.6	481.0±7.4b	10.8b
	Significance	*	*	*	*	ns	ns	ns	*	*

[z]Mean separation within columns by Duncan"s multiple range test at 5% level (n = 10).

[x]Days after blooming day

[y]Mean standard error

Table 2. Fruit set rate, fruit quality, and seed formation according to the pollination period in 'Sweet Gold' kiwifruit.

Year	DABD[x]	Fruit set rate (%)	Fresh weight (g)	Dry matter (%)	Soluble solids content (°Brix)	Acidity (%)	Firmness (kg/5mmø)	Flesh chromaticity h°	Seed number	100-seeds weight (mg)
2018	1	91.7±1.7[y]a[z]	92.2±0.5a	18.2±0.3a	12.4±0.1	0.9±0.1	5.4±0.1	106.0±1.0b	818.6±7.7a	12.2ab
	2	92.3±.3a	93.3±0.4a	18.2±0.3a	12.1±0.2	1.1±0.1	5.6±0.2	106.8±1.2ab	810.5±8.2a	13.4a
	3	93.3±0.8a	93.4±0.8a	17.7±0.3a	12.4±0.2	1.0±0.1	5.3±0.2	108.6±0.9ab	813.9±9.5a	12.7ab
	4	83.7±1.9b	82.9±2.5b	15.7±.2b	12.3±0.1	0.9±0.1	5.5±0.1	110.3±0.7a	756.1±7.7b	11.2b
	Significance	*	*	*	ns	ns	ns	*	*	*
2019	1	92.7±1.2a	84.0±0.8a	17.2±.0.3a	13.1±0.1a	0.9±0.1	5.0±0.1b	106.2±1.1b	762.1±7.7a	11.4ab
	2	93.7±0.3a	85.2±0.5a	17.6±0.2a	13.7±0.2a	1.0±0.1	5.3±0.1ab	106.1±0.9b	771.9±9.1a	12.6a
	3	92.7±0.5a	84.3±0.8a	17.5±0.2a	13.6±0.2a	1.0±0.1	5.4±0.1a	106.3±1.0b	758.5±7.0a	12.3a
	4	82.2±1.2b	76.8±0.6b	15.7±0.1b	12.2±0.1b	1.0±0.1	5.1±0.1ab	110.7±0.4a	688.5±9.2b	10.5b
	Significance	*	*	*	*	ns	*	*	*	*

[z]Mean separation within columns by Duncan"s multiple range test at 5% level (n = 10).

[x]Days after blooming day

[y]Mean standard error

Pollination time of the day associated with fruit quality and seed formation

The pollen tube growth in the pistil was observed after artificial pollination in order to investigate the fertilization process according to the pollination time of the day (Fig. 2). When flowers of ‘Halla Gold’ and ‘Sweet Gold’ were pollinated with pollen from 'Bohwa' at 7 AM, 10 AM, 1 PM and 4 PM, it was observed that the pollen tube was elongated past the stigma along the style on the first day after pollination in all conditions. On the third day after pollination, the pollen tube started to reach the ovule, and the number of pollen tubes showed similar trends in all conditions. Jeong et al. (2018) reported that the pollen tube started reaching the ovule on the third day after pollination in ‘Halla Gold’ and ‘Sweet Gold’, and our study showed similar trends. As our study showed that there was no difference in pollen tube growth according to the pollination time of the day, it is considered that the pollination time of the day does not affect fruit quality and seed formation.

Table 3 and Table 4 showed the characteristics of fruit quality and seed formation at harvest in 'Halla Gold' and 'Sweet Gold' with the different the pollination time of the day. The fruit set of both 'Halla Gold' and 'Sweet Gold' were over 90%. The fruit weight was significantly higher in pollination at 7 AM, 10 AM and 1 PM than at 4 PM except for that of ‘Sweet Gold’ in the year of 2019. The dry matter showed the significant

difference with the different pollination time of the day, just in the year of 2019. In 'Halla Gold', the seed number of fruit was significantly higher in pollination at 7 AM, 10 AM and 1 PM than at 4 PM just in the year of 2018, but there was no significant difference in 'Sweet Gold' for two years. In both 'Halla Gold' and 'Sweet Gold', no statistical significance was observed in the SSC, acidity, firmness, and flesh *h* value. Although it was reported that pollen adhesion increases and pollination efficiency is improved if artificial pollination is conducted when the amount of pistil exudate is high in the morning (Cacioppo et al., 2018). However, in our study, it was found that the pollination time of the day did not have a significant effect on fruit quality and seed formation. Also, most flowers open between 7 and 11 AM in 'Hort16A' kiwifruit (Goodwin et al., 2013) and then it is considered that stigma holds good receptivity all day for the first 3 DAB (Fig 1), but its receptivity might be different depending on weather conditions between early morning and late afternoon from the 4th day of petal opening. Therefore, the result suggests that further studies on the pollination time of the day according to days after petals open are necessary.

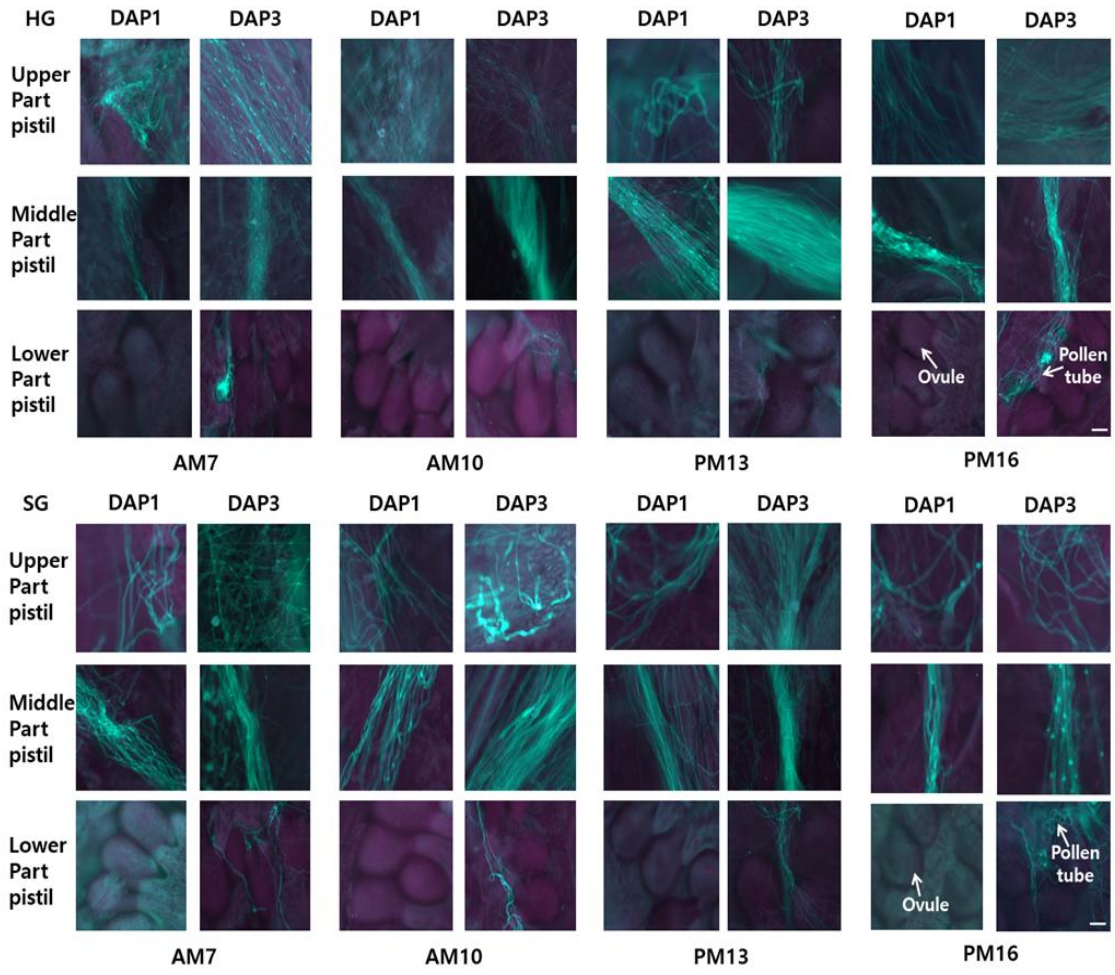

Fig 2. Pollen tube growth in the pistils of 'Halla Gold'(HG) and 'Sweet Gold'(SG) pollinated by repeated pollination with the dry pollen. DAP: days after pollination. Scale bar indicates 100um.

Table 3. Fruit set rate, fruit quality, and seed formation according to the pollination time in 'Halla Gold' kiwifruit.

Year	Pollination time	Fruit set rate (%)	Fresh weight (g)	Dry matter (%)	Soluble solids content (°Brix)	Acidity (%)	Firmness (kg/5mmø)	Flesh chromaticity h°	Seed number	100-seeds weight (mg)
2018	7 AM	92.3±1.2[y]	78.7±1.0a[z]	13.9±0.2	11.5±0.2	0.9±0.1	5.6±0.2	99.*5±0.5	415.0±9.7	12.4
	10 AM	92.0±0.6	76.8±0.9ab	13.6±0.5	11.9±0.2	0.9±0.1	5.1±0.2	100.0±.0.7	411.5±8.9	12.2
	13 PM	91.0±0.6	77.3±.0.8ab	13.1±0.3	11.9±0.2	1.0±0.1	5.2±0.1	101.0±0.8	405.0±9.3	13.3
	16 PM	91.3±0.7	74.6±0.9b	13.0±0.2	11.6±0.2	1.0±0.1	5.5±0.2	101.8±0.9	391.2±7.0	11.8
	Significance	ns	*	ns	ns	ns	ns	ns	ns	ns
2019	7 AM	93.3±0.9	92.5±3.2a	13.1±0.2a	11.8±0.2	1.2±0.1	5.4±0.1	105.3±0.7	510.7±9.8ab	12.7
	10 AM	93.0±0.6	93.1±3.1a	12.9±0.2a	12.2±0.2	1.2±0.1	5.5±0.1	105.4±0.7	514.1±8.9a	12.9
	13 PM	91.7±0.9	92.8±0.7a	12.5±.0.3ab	12.3±0.2	1.1±0.1	5.4±0.1	106.4±0.8	503.8±5.6ab	13.1
	16 PM	90.7±1.5	89.5±3.1b	11.6±0.1b	11.3±0.2	1.1±0.1	5.3±0.1	106.1±0.6	481.0±7.4b	12.1
	Significance	ns	*	*	ns	ns	ns	ns	*	ns

[z]Mean separation within columns by Duncan"s multiple range test at 5% level (n = 10).

[y]Mean standard error

Table 4. Fruit set rate, fruit quality, and seed formation according to the pollination time in 'Sweet Gold' kiwifruit.

Year	Pollination time	Fruit set rate (%)	Fresh weight (g)	Dry matter (%)	Soluble solids content (°Brix)	Acidity (%)	Firmness (kg/5mmø)	Flesh chromaticity h°	Seed number	100-seeds weight (mg)
2018	7 AM	93.3±0.9[y]	93.6±0.6a[z]	17.9±0.2	12.5±0.2	1.0±0.1	5.4±0.1	105.3±1.1	811.6±9.2	12.3
	10 AM	92.3±1.2	94.0±0.8a	17.5±0.3	13.0±0.1	1.0±0.1	5.7±0.1	106.4±1.0	806.8±8.5	13.5
	13 PM	93.3±0.9	93.5±0.4a	17.7±0.3	12.4±.0.2	1.1±0.1	5.6±0.2	106.3±1.1	814.4±9.4	12.7
	16 PM	91.0±1.2	90.3±1.0b	16.8±0.3	12.5±0.2	0.9±0.1	5.5±0.1	106.6±1.0	790.2±8.7	12.3
	Significance	ns	*	ns	ns	ns	ns	ns	ns	ns
2019	7 AM	93.0±0.6	84.5±0.9	17.4±0.2a	13.7±0.2	1.0±0.1	5.5±0.1	106.7±1.0	770.5±7.5	12.1
	10 AM	92.3±1.5	84.3±0.8	17.3±0.2ab	13.2±0.1	0.9±0.1	5.3±0.1	107.3±1.0	763.9±9.9	12.7
	13 PM	93.0±0.6	83.7±.8	17.6±0.2a	13.3±0.2	1.0±0.1	5.3±0.1	106.1±0.9	766.9±6.9	12.2
	16 PM	92.0±1.2	81.1±0.9	17.1±0.2b	12.5±.0.2	1.0±0.1	5.1±0.1	107.2±0.8	743.7±8.6	12.2
	Significance	ns	ns	*	ns	ns	ns	ns	ns	ns

[z]Mean separation within columns by Duncan"s multiple range test at 5% level (n = 10).

[y]Mean standard error

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CHAPTER II

Pollen Application Methods Affecting Fruit Quality and Seed Formation in Artificial Pollination of the Yellow-fleshed Kiwifruits

Abstract

This study was conducted to investigate the effect of pollen application methods for artificial pollination on such as pretreatment before pollination, repeated pollination and application types on fruit quality and seed formation in tetraploid 'Halla Gold' and 'Sweet Gold' kiwifruits grown in a non-heated plastic film house in Jeju, Korea. As for repeated pollination, it was observed that the pollen tube in pistil reached and penetrated into the ovule at 3 days after artificial pollination although the patterns varied depending on the number of dry pollen application. In 'Halla Gold' and 'Sweet Gold' cultivars, the number of pollen tubes was clearly higher in repeated pollination than when in single pollination. Furthermore, when pollen application was repeated, the fruit weight, dry matter percentage, number of seeds and 100-seed weight were higher. The firmness was low and the *h* value was high in pollination repeated three times. The soluble solids content and acidity showed no significant difference in all treatments. When pistillate flowers were

pollinated with dry pollen immediately after water sprinkle, both ‘Halla Gold’ and ‘Sweet Gold’ cultivars showed the lowest fruit weight, dry matter percentage, firmness, number of seeds, and 100-seed weight, whereas dry pollen application 1 h after water sprinkle, immediate or 1 h after suspension medium sprinkle didn’t show significant differences on the fruit quality and seed formation. In wet application using pollen suspension, the fruit weight was lower in both 'Halla Gold' and 'Sweet Gold' cultivars than in conventional dry pollen application, but there was no significant difference in 'Halla Gold' cultivar. Application types with dry pollen and pollen suspension did not show a significant difference in fruit quality and seed formation except the fruit weight of ‘Halla Gold’ cultivar. The results indicate that raindrops or dewdrops on stigma might reduce an efficiency of artificial pollination with dry pollen, but repeated pollination and application types with dry pollen and pollen suspension have a minor influence.

Introduction

Pollination of crops is a key requirement to achieve a sufficient set of crops (Calderone, 2012). Inadequate pollination has been found to be one of the important causative factors of low yields and low quality in many fruit tree species such as olive (Ayerza and Coates, 2004), kiwifruits (Hopping and Hacking, 1983), and pistachio (Vaknin et al., 2002). Kiwifruits (*Actinidia chinensis*) are dioecious plants, which necessitate either natural pollination through flower visiting insects or artificial pollination carried out directly by humans. In natural pollination, the transfer of pollen from staminate flowers to pistillate flowers requires an appropriate climate condition during the flowering season (Testolin et al., 1990). Due to the existence of a short effective pollination time (Gonzalez et., 1995), growers tend to prefer natural pollination by honeybees to artificial pollination in order to ensure a good harvest yield (Costa et al., 1993). The methods of artificial pollination include hand-pollination, blowing or spray of pollen dusting, and spray of pollen suspension (Gonzalez et al., 1998; Testolin et al., 1990; Sale, 1985; Hopping and Hacking, 1983; Hopping and Jerram, 1980). In Korea, for the artificial pollination of kiwifruits, routinely dry pollen and lycopodium powders are mixed and applied to pistillate flowers in order to reduce the management cost.

Although high quality pollen is essential in artificial pollination, the other factors such as physiological condition of receptive stigma, application times and methods, and environmental

conditions affect ovule fertilization and pollination efficiency. Generally, artificial pollination for kiwifruits is performed in a fine day and exudates on stigma tends to be desiccated around noon due to having high air temperature and low humidity, which cause a decline of pollen germination and pollen tube penetration. During artificial pollination, the uniformity of pollen load on stigma depends on labor proficiency and insufficient or uneven pollen load by unskillful labor requires repeated pollination occasionally. However, up to date, few studies were reported on repeated pollination in kiwifruits, which is not fully understood (Hoping, 1990). Therefore, it becomes a main issue how to increase an efficiency of artificial pollination in kiwifruit industry. Recently, application of suspension pollination has been reported, but studies on it were few, specially in yellow-fleshed kiwifruits until now. While studies on the effective pollination methods for 'Hayward (*A. deliciosa*)' have been reported (Gonzalez et al., 1998; Razeto et al., 2005; Lim and Lee, 2013), those on artificial pollination techniques for other cultivars have been lacking.

Thus, this study was conducted to investigate the effect of pollen application methods on fruit set and quality and seed formation in two yellow-fleshed kiwifruits cultivars in Jeju, Korea.

Materials and Methods

Plant material

The experiment was carried out from 2018 to 2019, on mature vines of the pistillate cultivars including 'Halla Gold' (8 years old) and 'Sweet Gold' (5 years old), which are both tetraploidy *A. chinensis* var. *chinensis*. The kiwifruit vines were trained to the pergola system and grown with the general management practices in an unheated plastic film house at commercial fields located in Jeju, Korea.

Pollen preparation, viability testing and pollination

Pollen of hexaploid 'Bohwa' (*A. chinensis* var. *deliciosa*) bred in Korea was used for artificial pollination. The pollen viability test was performed with two staining methods (Pok et al., 2015), consisting of fluorescein diacetate (FDA) and 1% iodine potassium iodide (I_2KI) staining, confirming that the viability of the fresh pollen was ≥90% before use in both years. For preparation of pollen dusting, pollen was carefully mixed with lycopodium powder in a ratio of 1:10. The pollen suspension was prepared with dissolving 4 g of pollen and 0.2 g of Food Red No. 2 (Oh Jung Commercial Co., Ltd., Seoul, Korea) into 1 L of suspension medium. Pollen dusting was sprayed with a spray applicator (PS-100, Jeju Bio Tech Co., Ltd, Jeju, Korea) and pollen suspension was sprayed with a hand sprayer (Apollo Industrial Co., Ltd, Siheung, Korea).

Experiments of pollen application methods were designed with the three kinds of different treatments including the pretreatment of different wetting materials before a spray of pollen dusting, the differently repeated sprays of pollen dusting, and dry and wet pollination. For artificial pollination, 5 fruit bearing branches were selected from each one of three vines with similar vine size and vigor in both pistillate cultivars. The first experiment for the repeated pollination, pollen dusting was sprayed just once at 10 o'clock on the morning of the first day of flowering, twice on the morning and afternoon (16 o'clock) of the first day of flowering, twice on the morning of the first day and second day of flowering, triple on the morning and afternoon of the first day and again on the morning of the second day of flowering. The second experiment for the pretreatment of different wetting materials consisted of non-pretreatment just with pollen dusting spray, water sprinkling (WS) and immediately pollen dusting spray (0), WS and pollen dusting spray one hour later (1), suspension medium spray (SS) and immediately pollen dusting spray (0), suspension medium spray (SS) and pollen dusting spray one hour later (1) on the morning of the second day of flowering. The third experiment was composed of two application types with pollen dusting and pollen suspension, which was sprayed on the morning of the second day of flowering.

Observation of pollen tube growth

In order to observe the pollen tube growth in the pistil from repeated pollination, pistils were

collected on the first and third day after final pollination in 2018 and 2019 and were fixed in FAA solution and kept at 4 ℃ until getting into next processes (Distefano et al., 2009). The fixed pistils were softened in 2N NaOH solution at 60 ℃ for 60~90 minutes and stained with 0.1% aniline blue for 24 h in the dark at room temperature (Yang et al., 2008). The stained pistils were located inside a block using 4% agarose (Agarose LE, Biomedic Co., Korea) and then sectioned in the thickness of 7 μm using Vibratome (Series 1000, The Vibratome Co., USA). The thin sections on the slide glass were observed under a fluorescence microscope (Leica DMRBE, Leica Co., Germany).

Fruit quality analysis

The characteristics of fruit quality were evaluated at harvest with 30 kiwifruits for each treatment. The fruit weight, dry matter percentage, soluble solids content (SSC), acidity, firmness and flesh color were measured. The fruit weight was measured immediately after harvest and the dry matter percentage was measured after drying the equatorial part of the fruit in a thickness of 2-3 mm at 60 ℃ for 24 h (Burdon et al., 2016). The SSC and acidity were measured with the juice squeezed from fruits using a digital sugar and acid analyzer (GMK-707R, G-won Co., Korea). The firmness of the fruit was measured using a firmness meter (FHM-5, Takemura Co., Japan) after removing the peel and external flesh of fruits in a thickness of 1 mm. The flesh color was measured using a colorimeter (CR-400 Chroma Meter, Minolta Co., Japan) after removing the skin and external flesh in a thickness of 2-3 mm.

Assessment of the number and weight of seeds

At 180 days after anthesis, 20 kiwifruits from each of three vines for each treatment were harvested and seeds were counted using a seed counter (Countador, Pfeuffer GmbH Co., Germany) after separating from the flesh carefully. The weight of seeds was represented as a 100-seed weight, measured using an electronic scale (EL-2000S, Setra Inc., USA).

Statistical analysis

Statistical analysis was performed using the SPSS program (SPSS version 18, IBM SPSS Software Inc., US). Significance at the 95% level was tested, and then the significance between the means was analyzed with Duncan's multiple range test.

Results and Discussion

Repeated pollination with dry pollen

In order to investigate the fertilization process upon repeated artificial pollination, the pollen tube growth in the pistil after pollination was observed (Fig. 1). When pollination of 'Halla Gold' and 'Sweet Gold' cultivars was performed in the morning only, performed in the morning and repeated in the afternoon on the same day, performed in the morning and repeated in the morning on the following day, and performed in the morning and repeated in the afternoon on the same day and the morning on the following day, it could be seen that the pollen tube was extended past the stigma along the style on the first day in all treatment conditions. On third day after pollination, the number of pollen tubes in the style increased, and the pollen tubes reached the ovule in all treatment conditions. Jeong et al. (2018) reported that the pollen tube started reaching the ovule on third after pollination in 'Sweet Gold' and 'Halla Gold', which is in agreement with our observations. However, the number of pollen tubes in the ovule was clearly higher in the treatment group where pollination was repeated than in the treatment group where pollination was performed only once. Such a difference is considered to affect the weight of fruits and the number of seeds (Table 1 and Table 2).

When 'Halla Gold' and 'Sweet Gold' cultivars were pollinated repeatedly with dry pollen, the characteristics of fruit quality and seed formation were shown in Table 3 and Table 4. The fresh

weight was shown to be the highest in the treatment where pollination was performed in the morning and repeated in the morning on the following day while it was low when pollination was performed only once in the morning. The dry matter percentage was high when artificial pollination was performed in the morning and repeated in the afternoon on the same day or repeated in the morning on the following day while it was typically low when pollination was performed in the morning and repeated twice in the afternoon on the same day and in the morning on the following day. The SSC, acidity and *h* value did not show any statistically significant difference. Firmness was shown to be low in the case of triple repeated pollination where pollination was performed in the morning and repeated twice in the afternoon on the same day and the morning on the following day. The number of seeds and 100-seed weight were higher in repeated pollination than in a single pollination in the morning.

Pistillate flowers in most of pollination-dependent plants should receive sufficient pollen to maximize ovule fertilization (Aizen and Harder, 2007; Ashman et al., 2004). However, it was reported that repeated hand-pollination of the same flower with dry pollen over one or more days might bear smaller fruits with fewer seeds than a single pollination in 'Hayward' kiwifruits, which was related to the arrest or inhibition of pollen tube growth caused by following pollen tubes (Hoping, 1990). The previous report was not accord with our study showing that the fruit weight, dry matter percentage, firmness, seed weight, and 100-seed weight appeared to be high if a sufficient amount of pollen was repeatedly infiltrated into the pistil. This conflict might be caused

by some factors such as different cultivars or polyploidy levels and pollen application types. Therefore, it is considered that further studies on repeated artificial pollination are needed to interpret where these conflicts result from or what types of factors influence.

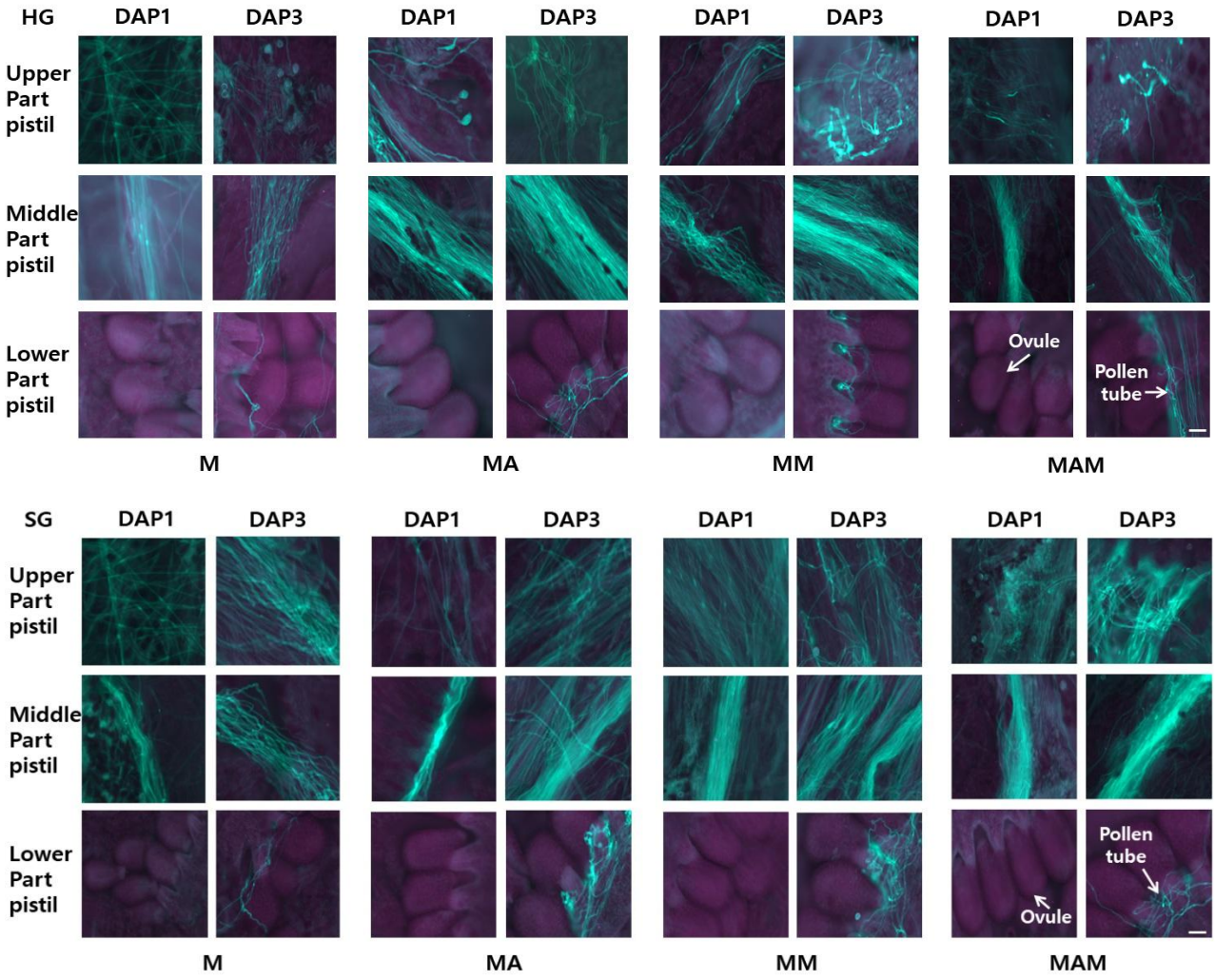

Fig. 3. Pollen tube growth in the pistils of 'Halla Gold'(HG) and 'Sweet Gold'(SG) pollinated with by repeated pollination with the dry pollen. DAP: days after pollination, M: pollination only in morning on the day of full bloom, MA: pollination in morning and in afternoon on the day of full bloom, MM: pollination in morning on the day of full bloom and in morning on the following day, MAM: pollination in morning and in afternoon on the day of full bloom and in morning on the following day. Scale bar indicates 100um.

Table 5. Fruit quality and seed formation of kiwifruits resulted from repeated pollination with the dry pollen in 'Halla Gold' kiwifruit.

Year	Repeated pollination	Fruit weight (g)	Dry matter (%)	Soluble solids content (°Brix)	Acidity (%)	Firmness (kg/5mmø)	Flesh chromaticity h°	Seed number	100-seeds weight (mg)
2018	M[a](once)	77.8±0.9[y] b[z]	13.0±0.2bc	11.8±0.2	0.9±0.1	5.0±0.1bc	98.3±0.7	408.0±9.5b	12.0b
	MA(twice)	82.6±1.1a	13.7±0.2ab	11.8±0.2	0.9±0.1	5.6±0.2ab	99.9±0.5	439.3±11.0a	14.1a
	MM(twice)	84.3±0.4a	14.5±0.3a	11.6±0.2	0.9±0.1	5.5±0.2a	99.9±0.5	441.6±12.7a	14.3a
	MAM(triple)	81.0±1.2ab	12.3±0.3c	11.9±0.2	0.9±0.1	4.8±0.1c	100.5±0.7	428.4±7.3ab	12.8b
	Significance	*	*	ns	ns	*	ns	*	*
2019	M[a](once)	91.6±3.3b	12.7±0.2	11.4±0.2	1.2±0.1	5.4±0.1	108.3±0.4	509.7±9.0b	12.5b
	MA(twice)	95.4±3.2ab	13.1±0.2	11.3±0.2	1.2±0.1	5.5±0.1	107.3±0.5	547.1±5.8a	14.9a
	MM(twice)	96.9±3.5a	12.6±0.3	13.5±0.1	1.1±0.1	5.4±0.1	107.9±0.5	549.7±8.1a	14.4a
	MAM(triple)	93.5±3.2ab	12.5±0.3	11.3±0.2	1.2±0.1	4.7±0.1	107.4±0.5	526.1±8.6ab	13.1ab
	Significance	*	ns	ns	ns	ns	ns	*	*

[z]Mean separation within columns by Duncan's multiple range teat at 5% level.

[y]M: pollination only in morning on the day of full bloom, MA: pollination in morning and in afternoon on the day of full bloom, MM: pollination in morning on the day of full bloom and in morning on the following day, MAM: pollination in morning and in afternoon on the day of full bloom and in morning on the following day.

[y]Mean standard error

Table 6. Fruit quality and seed formation of kiwifruits resulted from repeated pollination with the dry pollen in 'Sweet Gold' kiwifruit.

Year	Duplicate pollination	Fruit weight (g)	Dry matter (%)	Soluble solids content (°Brix)	Acidity (%)	Firmness (kg/5mmø)	Flesh chromaticity h°	Seed number	100-seeds weight (mg)
2018	M[a](once)	92.7±1.2[y] c[z]	17.1±0.1bc	12.4±0.1	1.0±0.1	5.6±0.1a	110.6±0.7a	808.2±8.5b	11.8b
	MA(twice)	98.0±0.7a	18.3±0.3a	12.3±0.2	1.0±0.1	5.4±0.1a	110.0±0.7a	845.3±5.5a	13.2a
	MM(twice)	97.5±0.4ab	18.1±0.3ab	12.3±0.1	1.0±0.1	5.5±0.1a	106.9±1.1b	843.6±7.4ab	13.6a
	MAM(Triple)	94.1±0.9bc	16.7±0.3c	12.7±0.1	1.0±0.1	4.5±0.1b	111.5±0.2a	826.4±8.6ab	12.5a
	Significance	*	*	ns	ns	*	*	*	*
2019	M[a](once)	80.4±0.6c	17.4±0.2ab	13.1±0.1	0.9±0.1	5.1±0.1a	108.5±0.9	760.4±7.6b	10.9b
	MA(twice)	86.4±0.5a	17.7±0.2a	13.5±0.2	1.0±0.1	5.4±0.1a	107.1±1.1	780.4±9.1a	13.0a
	MM(twice)	87.2±0.5a	17.6±0.2a	13.3±0.2	0.9±0.1	5.1±0.1a	107.9±1.0	790.8±8.2a	13.0a
	MAM(Triple)	83.8±0.8b	16.7±0.2b	13.2±0.1	0.9±0.1	4.8±0.1b	108.9±0.9	785.6±9.7a	12.3ab
	Significance	*	*	ns	ns	*	ns	*	*

[z]Mean separation within columns by Duncan's multiple range teat at 5% level.

[a]M: pollination only in morning on the day of full bloom, MA: pollination in morning and in afternoon on the day of full bloom, MM: pollination in morning on the day of full bloom and in morning on the following day, MAM: pollination in morning and in afternoon on the day of full bloom and in morning on the following day.

[y]Mean standard error

Pretreatment of wetting materials on stigma

Fruit quality and seed formation upon artificial pollination with the pretreatment of water and pollen suspension were represented in Table 3 for 'Halla Gold' and Table 4 for 'Sweet Gold'. The fruit weight, dry matter percentage, SSC and firmness of 'Halla Gold' and 'Sweet Gold' kiwifruits were shown to be the lowest for both of 2 years in the case of pollination with pollen dusting performed immediately after water sprinkle on pistillate flowers. Likewise, the number of seeds and 100-seed weight were the lowest in the pollination with dry pollen sprayed immediately after water sprinkle. However, fruit acidity did not show a statistically significant difference. It could be seen that the *h* value from the colorimeter was typically higher except the year of 2019 in 'Sweet Gold' cultivar when artificial pollination was performed immediately after the pretreatment of water. In kiwifruits, Cacioppa et al. (2018) reported that pollen adhesion was lowered when it was delivered with water. Furthermore, it was reported that pollen might lose viability due to osmotic shock when it is suspended directly in water and pollen might maintain viability about for 3 h when it is suspended in suspension medium (Hoping, 1990). Also, high temperature and high humidity should be avoided in order to maintain the viability of pollen on stigma. From the results of this study, it appeared that the immediate pollination after water sprinkle might lead to lower viability of pollen due to the effect on pollen adhesion to the pistil and pollen tube penetration and burst. The result was in accord with that in orchards, artificial pollination on a sunny day normally results in superior fruit quality and seed formation rather than on a rainy day. Thus, it is

recommended that artificial pollination with pollen dusting should be avoided until raindrops or dewdrops on stigma disappear. However, it is considered that further studies on such artificial pollination conditions and proper timing are needed.

Table 7. Fruit quality and seed formation of kiwifruits resulted from pollination with the dry pollen of combined with the pretreatment of water or pollen suspension in 'Halla Gold' kiwifruit.

Year	Modes of pollination	Fruit weight (g)	Dry matter (%)	Soluble solids content (°Brix)	Acidity (%)	Firmness (kg/5mmø)	Flesh chromaticity h°	Seed number	100-seeds weight (mg)
2018	Non-pretreatment	80.6±1.1[y]a[z]	13.5±0.2ab	11.4±0.2	1.0±0.1	5.2±0.1ab	100.5±0.7ab	413.8±8.3a	12.0a
	WS(0)[x]	71.7±2.4b	13.0±0.3b	11.2±0.2	1.0±0.1	5.0±0.1b	100.8±0.7a	321.1±10.9b	6.9b
	WS(1)	82.5±0.9a	13.7±0.2ab	11.8±0.1	0.9±0.1	5.2±0.1ab	99.9±0.5ab	404.7±10.2a	11.9a
	SS(0)	83.0±0.7a	14.5±0.3a	11.6±0.2	0.9±0.1	5.5±0.2ab	98.1±0.6b	403.5±9.9a	11.1a
	SS(1)	84.7±0.8a	13.7±0.3ab	11.9±0.2	0.9±0.1	5.7±0.1a	98.8±0.5ab	414.5±12.3a	12.1a
	Significance	*	*	ns	ns	*	*	*	*
2019	Non-pretreatment	95.6±3.4a	13.0±0.3bc	11.5±0.2ab	1.0±0.1	5.4±0.1ab	105.1±0.6ab	541.8±9.3a	13.6a
	WS(0)[x]	82.7±1.8b	12.4±0.2c	10.8±0.1c	1.0±0.1	4.9±0.1c	107.1±0.5a	472.3±9.9b	10.7b
	WS(1)	95.3±0.8a	13.2±0.2bc	11.2±0.1bc	0.9±0.1	5.2±0.1bc	100.3±0.6c	544.0±9.8a	13.8a
	SS(0)	95.5±0.7a	14.4±0.3a	11.7±0.1ab	1.0±0.1	5.8±0.1a	103.5±0.4b	528.8±9.0a	13.2ab
	SS(1)	96.7±0.4a	13.8±0.2ab	12.0±0.1a	0.9±0.1	5.6±0.1ab	103.3±0.4b	553.0±8.5a	14.0a
	Significance	*	*	*	ns	*	*	*	*

[z]Mean separation within columns by Duncan's multiple range teat at 5% level.

[x]WS: water sprinkling , SS: pollen suspension medium spray, o: immediately pollen dusting spray, 1: pollen dusting spray one hour later.

[y]Mean standard error

Table 8. Fruit quality and seed formation of kiwifruits resulted from pollination with the dry pollen of combined with the pretreatment of water or pollen suspension in 'Sweet Gold' kiwifruit.

Year	Modes of pollination	Fruit weight (g)	Dry matter (%)	Soluble solids content (°Brix)	Acidity (%)	Firmness (kg/5mmø)	Flesh chromaticity h°	Seed number	100-seeds weight (mg)
2018	Non-pretreatment	94.3±1.2a[z]	18.7±0.3a	12.6±0.1a	0.9±0.1	5.5±0.1a	109.5±0.2b	832.9±11.6a	12.0a
	WS(0)[x]	88.4±0.8b	16.8±0.2b	11.9±0.2b	1.1±0.1	4.8±0.1b	111.5±0.2a	537.9±13.8b	6.5b
	WS(1)	95.2±0.8a	18.1±0.2a	12.3±0.2a	1.0±0.1	5.4±0.1a	111.1±0.2a	814.4±11.4a	11.1a
	SS(0)	96.4±0.5a	18.6±0.3a	12.5±0.2a	1.0±0.1	5.3±0.1a	110.2±0.1a	807.5±12.1a	11.7a
	SS(1)	95.8±0.7a	18.7±0.3a	12.7±0.1a	1.0±0.1	5.3±0.2a	109.8±0.7b	811.9±10.4a	11.1a
	Significance	*	*	*	ns	*	*	*	*
2019	Non-pretreatment	80.2±0.9a	17.4±0.2b	13.5±0.2a	1.0±0.1	5.4±0.1	109.8±0.9	743.1±7.5a	11.6a
	WS(0)[x]	73.4±0.8c	16.7±0.2ab	11.7±0.1b	0.9±0.1	5.0±0.2	107.1±2.4	536.2±17.6b	6.9b
	WS(1)	78.4±1.1ab	19.0±0.2a	12.3±0.1ab	1.0±0.1	5.2±0.1	110.7±0.2	751.4±14.2a	11.6a
	SS(0)	76.3±0.5b	18.5±0.2ab	12.3±0.1ab	1.0±0.1	5.5±0.1	110.7±0.2	763.1±9.4a	11.3a
	SS(1)	78.0±0.5b	18.6±0.2ab	12.3±0.4ab	1.0±0.1	5.1±0.1	109.9±0.1	752.4±8.2a	11.1a
	Significance	*	*	*	ns	ns	ns	*	*

[z]Mean separation within columns by Duncan's multiple range teat at 5% level.

[x]WS: water sprinkling , SS: pollen suspension medium spray, o: immediately pollen dusting spray, 1: pollen dusting spray one hour later.

[y]Mean standard error

Application types with dry pollen and wet pollen

Table 5 shows the characteristics of the fruit quality and seed formation of 'Halla Gold' and 'Sweet Gold' kiwifruits at harvest upon the different application types with dry and wet pollen. The fruit weight, the number of seeds and the 100-seed weight of 'Halla Gold' and 'Sweet Gold' cultivars were higher in dry pollen pollination than in liquid pollination, although a significant difference was observed only in fruit weight of 'Sweet Gold' cultivar. The dry matter percentage, SSC, acidity, firmness and *h* value did not show any statistically significant difference.

Lim et al. (2014) reported that wet pollen application using pollen suspension and dry pollen application using lycopodium powders did not show a significant difference in the number of seeds, SSC, acidity and firmness in 'Hayward' cultivar, and out study also showed the similar results in 'Halla Gold' and 'Sweet Gold' cultivars. Therefore, it was found that the use of pollen suspension instead of lycopodium powders as the pollen diluent in 'Halla Gold' and 'Sweet Gold' cultivars did not have the effects on fruit quality and seed formation. Also, it is considered that wet application using pollen suspension will help reducing the operating expenses in orchards since its labor and pollen requirements are small. However, further studies on the appropriate dilution ratio of pollen in the suspension according to the cultivar are needed.

Table 9. Fruit quality and seed formation of kiwifruits resulted from the different application types of pollen for articial.

Cultivar	Year	modes of pollination	Fresh weight (g)	Dry matter (%)	Soluble solids content (°Brix)	Acidity (%)	Firmness (kg/5mmø)	Flesh chromaticity h°	Seed number	100-seeds weight (mg)
Hall Gold	2018	Dry pollen	80.6±1.1[y]	13.5±0.2	11.4±0.2	1.0±0.1	5.2±0.1	98.8±0.5	413.8±8.3	12.0
		Wet pollen	76.6±1.9	13.7±0.2	12.9±0.2	0.9±0.1	5.2±0.2	99.1±0.6	397.9±9.1	10.1
		Significance	ns	ns	ns	ns	ns	ns	ns	ns
	2019	Dry pollen	95.6±3.4	13.0±0.3	11.5±0.2	1.2±0.1	5.4±0.1	107.1±0.5	541.8±9.3	12.6
		Wet Pollen	92.1±3.3	13.3±0.3	12.4±0.2	1.2±0.1	5.6±0.1	108.6±0.5	529.1±8.4	12.2
		Significance	ns	ns	ns	ns	ns	ns	ns	ns
Sweet Gold	2018	Dry pollen	94.3±1.2	17.7±0.3	12.6±0.1	0.9±0.1	5.5±0.1	111.1±0.2	831.9±11.6	12.0
		Wet Pollen	89.1±1.8	18.3±0.3	12.7±0.2	1.0±0.1	5.3±0.2	111.5±0.2	810.1±9.7	11.1
		Significance	*	ns	ns	ns	ns	ns	ns	ns
	2019	Dry pollen pollination	82.1±0.9	17.5±0.2	13.6±0.2	1.0±0.1	5.4±0.1	109.7±0.9	744.1±7.5	11.6
		Liquid pollination	77.4±1.0	18.3±0.2	13.0±0.2	1.0±0.1	5.1±0.1	107.9±1.0	733.7±12.7	11.7
		Significance	*	ns	ns	ns	ns	ns	ns	ns

[z]Mean separation within columns by T-teat at 5% level.

[y]Mean standard error

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CHAPTER III

Response of Fruit Set and Quality and Seed Formation to Ploidy Levels of Pollen Donor in Yellow-fleshed Kiwifruits

Abstract

This study investigated how the ploidy level of the kiwifruit pollinizer cultivars 'CK3' (diploid), 'T' line (tetraploid), 'Bohwa' (hexaploid) and 'Chieftain' (hexaploid) affected fruit set, fruit quality, and seed formation in the tetraploid kiwifruit cultivars 'Halla Gold' and 'Sweet Gold' cultivated in an unheated greenhouse in Jeju, Korea. Pollen tubes growing in the pistil reached and combined with the ovule 3 days after artificial pollination, and their patterns differed depending on the ploidy level of the pollen parent. The number of pollen tubes observed in 'Halla Gold' and 'Sweet Gold' pistils was significantly lower following pollination by 'CK3' than with the other pollen donors. In all pollen treatments, the fruit set rates were >90%. The fruit weight of both 'Halla Gold' and 'Sweet Gold' were high following pollination with 'Chieftain' and 'Bohwa'. The dry matter content, soluble solids, and acidity were not significantly different among all pollination treatments. Fruit firmness was higher following pollination with 'Chieftain' and

'Bowha.' Colorimeter h° values for flesh of 'Halla Gold' was low following pollination with 'CK3', but there were no differences for 'Sweet Gold' among all pollinations. The number of seeds showed a similar trend to fruit weight, but the 100-seed weight was highest with 'T' line as the pollinizer. The results indicate that the ploidy level of the pollen donor affects fruit quality more than fruit set. Also, the pollen most suitable for cultivation of 'Halla Gold' and 'Sweet Gold' is considered to be tetraploid 'T' line and hexaploid 'Bohwa' and 'Chieftain', which showed favorable effects on the weight and firmness of fruits, and the number and weight of seeds without adversely influencing fruit set and dry matter content.

Introduction

Kiwifruit (*Actinidia* spp.) is a dioecious plant that, if it is to be grown economically and profitability, requires a sufficient proportion of male plants to be present during culture to provide the necessary pollen for the female plants to bear enough fruit. Staminate and pistillate flowers in kiwifruit have considerably different characteristics (Schmid, 1978; Ferguson, 1984). Although pistillate flowers have the external morphology of a perfect flower, they produce hollow pollen. In contrast, staminate flowers have short filaments and small viable pollen in their stamen while their ovary remains degenerate. Therefore, artificial pollination is essential to cultivate kiwifruit commercially. Currently in Korea, most of kiwifruit growers perform artificial pollination by manually transferring pollen from staminate flowers to the stigmata of pistillate flowers (Oh et al., 2020). Without this farm practice, adequate fruit set would not be achieved. Viability, compatibility, pollen tube growth, and fertilization rates of pollen used in artificial pollination become crucial factors determining seed formation and fruit set (Seal et al., 2013a, 2013b).

Pollination of kiwifruit is performed using the pollen of *Actinidia chinensis* var. *chinensis*, *A. chinensis* var. *deliciosa*, and hybrids of these two varieties. Since flowers of typically diploid *A. chinensis* var. *chinensis* are smaller than those of hexaploid *A. chinensis* var. *deliciosa*, it may be uneconomical to produce pollen from the former due to it lower pollen production (Seal et al., 2016). Hence, kiwifruit growers in Korea typically use hexaploid *A. chinensis* var. *deliciosa* cultivars

such as ‘Machua’, ‘Bohwa’ and ‘Chieftain’ as pollen donors. Since the flowering time of these three hexaploid cultivars is later than the pollination time of cultivated yellow-fleshed kiwifruit, it is difficult to use their pollen collected in the same year. In addition, most of the commercial pollen used in Korea is imported from China and, while it is inexpensive and easy to obtain, its ploidy level and cultivar source are difficult to determine.

Recently, it was reported that the ploidy level of the pollen donor affects the characteristics of fruits including fruit weight, dry matter, flesh coloration, and nutritional components (Seal et al., 2013a, 2013b ; Seal et al, 2016; Stasiak et al., 2019). However, there have been few studies on the correlation between the ploidy of the pollen donor and the quality characteristics of fruit for Korean kiwifruit cultivars (Jeong et al., 2018). Thus, this study was conducted to investigate the effect of pollen donor ploidy level on the fruit set, fruit quality, and seed formation in two cultivars of yellow-fleshed kiwifruit cultivated in Jeju, Korea.

Materials and methods

Plant material

The experiment was carried out from 2018 to 2019 on mature vines of the pistillate cultivars of *A. chinensis* var. *chinensis* (tetraploid) 'Halla Gold' (8 years old) and 'Sweet Gold' (5 years old) grown under standard management practices in an unheated greenhouse at a commercial orchard located in Jeju-si, Korea.

Pollen preparation, viability testing and pollination

Diploid 'CK3' (*A. chinensis* var. *chinensis*), tetraploid 'T' line (*A. chinensis* var. *chinensis*), and hexaploid 'Chieftain' (*A. chinensis* var. *deliciosa*), kiwifruit breeds from New Zealand, and hexaploid 'Bohwa' (*A. chinensis* var. *deliciosa*), a kiwifruit breed from Korea, were used as pollen donors for artificial pollination. They were also grown under standard management practices in unheated greenhouses at the grower fields in Jeju-si ('Bohwa' and 'Chieftain') and Seogwipo-si ('CK3' and 'T 'line), Korea.

Flowers at the popcorn stage just prior to opening were collected from the pollen donor kiwifruit vines, then the anthers were separated and dried at room temperature to release pollen. The collected pollen was stored at −4 °C in tubular glass vials with air-tight caps. Pollen viability was determined with two staining tests (Fig. 4) (the fluorescein diacetate test, and 1% iodine

potassium iodide (I_2KI) test; Pok et al., 2015), confirming that the viability of the fresh pollen was ≥90% in both years (Tables 10).

The pollen was carefully mixed with lycopodium powder in the ratio 1:10, after which artificial pollination of the 'Halla Gold' and 'Sweet Gold' kiwifruit flowers was performed on the second day after full bloom using a spray applicator (PS-100, Jeju Bio Tech Co., Korea). Eight pollination treatments were designed and conducted (Fig. 5), with pollen from each of the four pollinators used for each of the two recipients.

Histological analysis of pollen tube growth

To observe pollen tube growth after artificial pollination, pistils were collected on the first, second, and third day after pollination in 2018 and 2019, fixed in FAA solution (formalin:acetic acid:70% ethanol, 1:1:18 v/v/v) and kept at 4 ℃ until the next treatment (Distefano et al., 2009). The fixed pistils were softened at 60 ℃ for 60–90 min in 2N NaOH solution and stained with 0.1% aniline blue for 24 h in the dark at room temperature (Yang et al., 2008). The stained tissues were then sectioned into slices 7 μm thick on a slide glass using a Vibratome (Series 1000, The Vibratome Co., Us) and then observed under a fluorescence microscope (Leica DMRBE, Leica Co., Germany).

Fruit set rate and fruit quality analysis

The percentage of fruit set was recorded for each pollination treatment ("Halla Gold," Table 11; "Sweet Gold," Table 12). At 180 days after full bloom 30 kiwifruits were selected from each pollination treatment for fruit quality assessment. The fruit weight at harvest, dry matter content, soluble solids content (SSC), acidity, firmness, and flesh color were measured. The fruit weight was measured immediately after harvest, and the dry matter content of an equatorial section of fruit with a thickness of 2–3 mm was measured after drying at 60 °C for 24 h (Burdon et al., 2016). The SSC and acidity were measured using a digital sugar and acid analyzer (GMK-707R, G-won Co., Korea). The firmness of fruits peeled to a thickness of 1 mm was measured at right angles using a firmness meter (FHM-5, Takemura Co., Japan) (Burdon et al., 2017). The flesh color of fruits peeled to a thickness of 2–3 mm was measured using a colorimeter (CR-400 Chroma Meter, Minolta Co., Japan).

Assessment of the number and weight of seeds

To assess the seed number and weight, the seeds were collected from 12 'Sweet Gold' kiwifruit and 30 'Halla Gold' kiwifruit for each pollination treatment at 180 days after full bloom. The collected kiwifruit seeds were properly washed, dried and counted using a seed counter (Countador, Pfeuffer GmbH Co., Germany). The weight of seeds was expressed as a 100-seed weight, measured using an electronic scale (EL-2000S, Setra Inc., US).

Statistical analysis

Statistical analysis was performed using the SPSS program (SPSS version 18, IBM SPSS Software Inc., US). Significance at the 95% level was tested, and subsequently, Duncan's multiple range test was performed to compare significance between means.

Table 10. Percentages of pollen viability at the popcorn phenophase stage of flower buds of four different pollen donor genotypes.

Year	Pollen donor	I_2KI (%)	FDA (%)
2018	CK3 (2x)	93.6±0.6[y]a[z]	91.3±1.5a
	T line (4x)	93.4±1.1a	90.9±1.1a
	Bohwa (6x)	91.5±1.5b	88.0±1.2b
	Chieftain (6x)	91.7±1.6b	87.4±1.6b
	Significance	*	*
2019	CK3 (2x)	92.8±1.1a	90.3±1.4a
	T line (4x)	93.1±1.1a	90.2±1.2a
	Bohwa (6x)	90.8±1.6b	87.5±1.3b
	Chieftain (6x)	91.4±1.7ab	88.1±0.8b
	Significance	*	*

[z]Mean separation within columns by Duncan"s multiple range test at 5% level (n = 10).

[y]Mean standard error

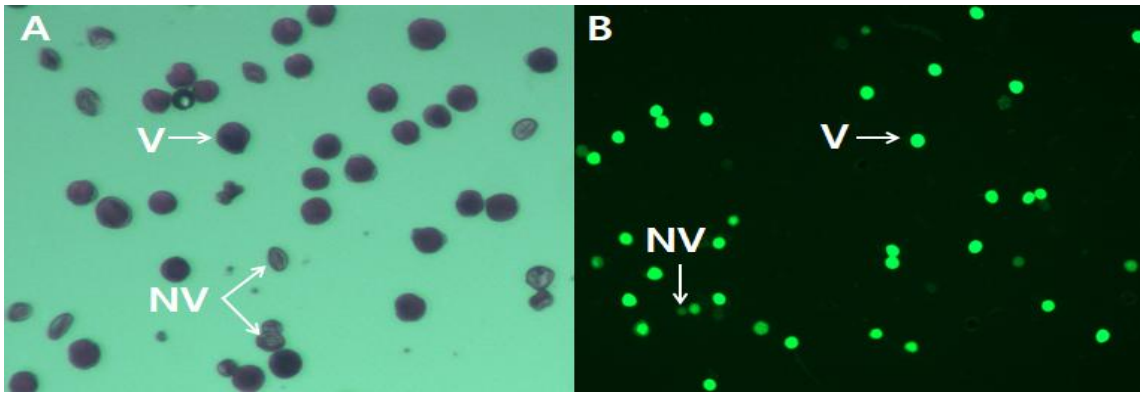

Fig. 4 Viable and nonviable pollen grains in I_2KI (A) and FDA (B) staining tests. V, viable; NV, nonviable.

Results and discussion

To examine fertilization in kiwifruit using dry pollen of different ploidy levels, pollen tube growth in the pistil was observed after artificial pollination (Fig. 5). Elongation of the pollen tubes was observed beyond the stigma along the style from the first day of pollination. Previous studies on pollen tube growth have reported variable findings. In *A. chinensis* var. *deliciosa* "Hayward," the pollen tube started entering the style at 7 h after pollination, reached the base of the style after 31 h, and after ~40 h entered the ovule, initiating fertilization (Jerram, 1979). In contrast, Gonzàlez and Coque (1995) reported that the pollen tube reached the base of the style 2 d after pollination, and entered the ovule at 3 d after pollination. It is considered that these differences were caused by the ploidy level, viability, and compatibility of the pollen. Jeong et al. (2018) reported that the pollen tube started entering the ovule at 3 d after pollination in 'Sweet Gold' and 'Halla Gold', and the trends in our study were similar.

It was reported that the pollen tube growth rate in the style decreased and that the fertilization rate dropped substantially when a diploid cultivar of *A. chinensis* var. *chinensis* was pollinated with pollen from a hexaploid cultivar of *A. chinensis* var. *deliciosa* (Harvey et al., 1991). In our study, as in this previous report, the number of pollen tubes observed was noticeably reduced in the tetraploid

pistillate cultivars ‘Halla Gold’ and ‘Sweet Gold’ when pollinated with the pollen of the diploid staminate cultivar ‘CK3’ (Fig. 5). Such a difference is considered to have an effect on the weight of fruits and the formation of seeds (Tables 11 and 12).

The results for fruit quality parameters and seed formation at harvest time of ‘Halla Gold’ and ‘Sweet Gold’ pollinated with the pollen of different ploidy levels are shown in Tables 11 and 12. The fruit set rate was >90% with the pollen of all pollinizers, with no statistically significant difference among the treatments. The fruit weight of ‘Halla Gold’ and ‘Sweet Gold’ was lowest with the pollen of ‘CK3’ (diploid), and was high but not different significantly with the tetraploid and hexaploid pollen. The dry matter content, SSC, and acidity of fruits were not significantly different among all the pollinizers in both ‘Halla Gold’ and ‘Sweet Gold’ except for just SSC in the year 2018. Fruit firmness was high in ‘Halla Gold’ and ‘Sweet Gold’ pollinated with hexaploid ‘Bohwa’ and ‘Chieftain’. The h° values from the colorimeter in ‘Sweet Gold’ were lower with ‘CK3’ than with ‘T’ line, ‘Bohwa’ and ‘Chieftain’ in both years, and in ‘Halla Gold’ in 2019.

Table 11. 'Halla Gold' kiwifruit data: fruit set rate, fruit quality, and seed formation following pollination with the dry pollen of four different pollen donor genotypes.

Year	Pollen donor	Fruit set rate (%)	Fresh weight (g)	Dry matter (%)	Soluble solids (°Brix)	Acidity (%)	Firmness (kg/5mm ø)	Flesh chromaticity (h°)	Seed number	100-seeds weight (mg)
2018	CK3 (2x)	92.7±1.8[y]	67.6±1.8b[z]	14.4±0.4	11.6±0.3	1.0±0.1	4.7±0.1b	98.8±0.5	354.6±9.1c	9.0b
	T line (4x)	95.3±0.7	82.9±2.1a	15.4±0.4	11.9±0.3	0.8±0.1	4.9±0.1b	97.8±0.5	384.7±7.4ab	16.0a
	Bohwa (6x)	93.3±0.7	77.5±3.5a	14.8±0.5	11.5±0.3	1.1±0.1	5.3±0.2a	98.0±0.9	437.4±8.4a	13.0ab
	Chieftain (6x)	94.7±0.7	80.7±5.4a	15.4±0.3	11.4±0.3	1.2±0.1	5.6±0.2a	99.3±0.7	406.3±12.9a	12.0ab
Significance		ns	*	ns	ns	ns	*	ns	*	*
2019	CK3 (2x)	92.7±0.9	88.4±3.5b	12.7±0.3	11.1±0.2	1.2±0.1	4.9±0.1b	99.2±0.2b	472.1±9.9b	12.0c
	T line (4x)	94.3±0.9	91.5±3.4ab	12.5±0.3	11.5±0.2	1.1±0.1	4.8±0.1b	108.3±0.4a	500.7±9.1ab	19.0a
	Bohwa (6x)	95.0±0.6	95.8±3.7a	12.7±0.2	10.9±0.2	1.2±0.1	5.4±0.1a	108.3±0.4a	536.1±7.3a	15.0ab
	Chieftain (6x)	95.3±0.9	95.6±3.4a	12.6±0.2	11.3±0.2	1.2±0.1	5.5±0.1a	107.3±0.5a	502.6±9.2ab	14.0b
Significance		ns	*	ns	ns	ns	*	*	*	*

[z]Mean separation within columns by Duncan"s multiple range test at 5% level (n = 10).

[y]Mean standard error

Table 12. 'Sweet Gold' kiwifruit data: fruit set rate, fruit quality, and seed formation following pollination with the dry pollen of four different pollen donor genotypes.

Year	Pollen donor	Fruit set rate (%)	Fresh weight (g)	Dry matter (%)	Soluble solids (°Brix)	Acidity (%)	Firmness (kg/5mm ø)	Flesh chromaticity (h°)	Seed number	100-seeds weight (mg)
2018	CK3 (2x)	93.3±1.8[y]	86.6±3.2b[z]	18.2±0.3	11.6±0.3c	1.0±0.2	4.5±0.3b	98.8±0.5c	799.8±14.7b	9.0c
	T line (4x)	92.0±1.2	90.8±2.9ab	18.0±0.5	13.7±0.5a	1.0±0.1	4.7±0.2b	106.8±0.4b	823.1±10.7ab	14a
	Bohwa (6x)	96.0±1.2	95.0±2.3a	17.2±0.4	12.9±0.4ab	1.0±0.2	5.6±0.3a	106.2±0.5b	855.2±10.6a	11ab
	Chieftain (6x)	94.7±0.7	95.8±2.5a	17.4±0.5	13.7±0.2a	0.9±0.1	5.8±0.2a	109.0±0.3a	824.4±11.2a	11b
Significance		ns	*	ns	*	ns	*	*	*	*
2019	CK3 (2x)	93.0±0.6	70.8±2.8b	18.0±0.4	13.5±0.2	0.9±0.1	4.6±0.1b	110.2±0.3c	642.1±12.3c	8.0c
	T line (4x)	95.0±0.6	77.2±1.9a	18.0±0.4	13.6±0.3	0.8±0.1	4.6±0.2b	110.6±0.3bc	716.8±8.2b	13a
	Bohwa (6x)	94.3±1.8	79.0±0.8a	17.9±0.5	13.2±0.2	0.9±0.1	5.8±0.1a	111.6±0.3ab	792.4±9.2a	11ab
	Chieftain (6x)	95.0±1.0	80.0±1.4a	18.2±0.3	14.1±0.4	1.1±0.1	5.2±0.2ab	112.0±0.2a	754.5±7.2ab	10ab
Significance		ns	*	ns	ns	ns	*	*	*	*

[z]Mean separation within columns by Duncan"s multiple range test at 5% level (n = 10).

[y]Mean standard error

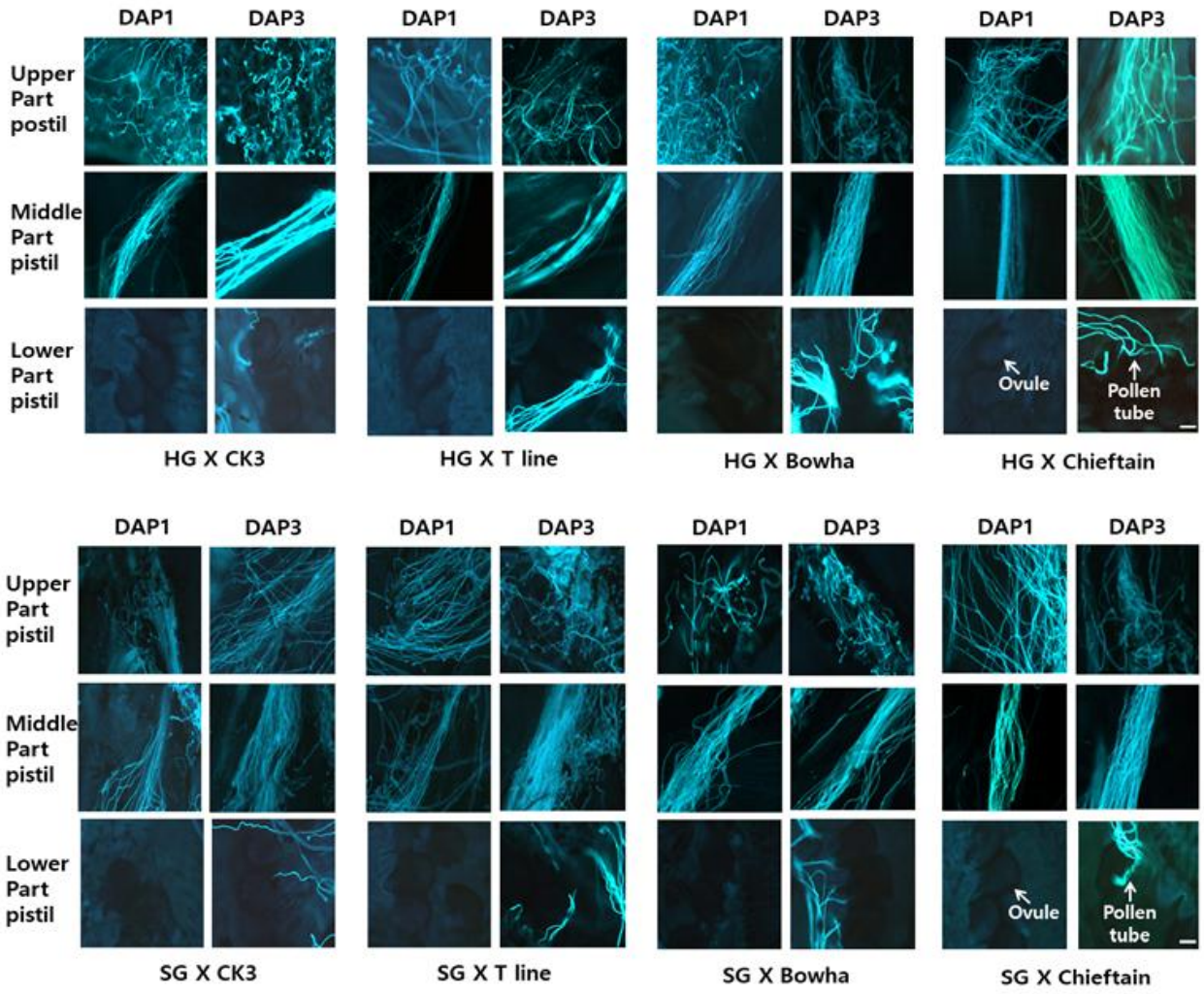

Fig 5. Pollen tube growth in the pistils of 'Halla Gold' (HG) and 'Sweet Gold' (SG) pollinated with the dry pollen of four different pollen donor genotypes ('CK3', 'T' line, 'Bowha', and 'Chieftain'). DAP: days after pollination. Scale bar indicates 100 μm.

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CONCLUSIONS

Kiwifruit is a dioecious plant, and artificial pollination is very important for commercial cultivation. Since the effective pollination period of kiwifruits is short, cultivation techniques that allow artificial pollination in a short period of time are needed for stable fruit set. Also, there have been few studies on the correlation between the ploidy of the pollen donor and the quality characteristics of fruit for Korean kiwifruit cultivars. This study was conducted to suggest the effective artificial pollination period and time and to investigate the effects of different artificial pollination methods and pollen donors with different polyploidy on fruit quality and seed formation in yellow-flesh type 'Halla Gold' and 'Sweet Gold' widely cultivated in the Jeju region.

When both 'Halla Gold' and 'Sweet Gold' cultivars were pollinated on the third day after blooming, the fruit set rates, fresh weight and dry matter percentage started to decrease. On the other hand, the dry matter percentage, soluble solids content (SSC), acidity and h value did not show any significant difference. It could be seen that the seed number and 100-seed weight decreased on the third day after blooming, and the effective pollination period affected the fruit weight and seed development. It was assumed that the decrease of fruit set, fruit weight, dry matter, seed number, seed weight and flesh color development was affected by flower aging because the change of petal color from white into light brown started at 2 DAB and the dehiscence of petal started at 3 DAB. This flower aging might be connected with a deterioration of pollen

receptivity or a retardation of pollen tube growth, which reduced the seed number and seed weight due to an inhibition of ovule fertilization.

According to Gonzalez et al. (1995), an aged kiwifruit flower has a low level of pollen receptivity and subsequently reduces the crop yield along with an increased occurrence of deformed fruits. It has been reported that the EPP will be different from cultivars and growing regions. In this study, 'Halla Hold' showed an early flower aging in external appearance including petal color change and petal dehiscence than 'Sweet Gold', which may be related to a little lower fruit set at 3 DAB of 'Hall Gold' compared to that of 'Sweet Gold' for two years. This result suggests that flower aging is regulated under genetic constitution might influence EPP and depends on cultivars. The EPP could be changed with growing environment conditions such as temperature and humidity which are not constant by years. The EPP of kiwifruits has been determined based on just one factor of fruit set (Gonzalez et al., 1995; Abedi Gheshlaghi, 2019) or two factors of fruit set and fruit weight (Brantley et al., 2019), which reported the EPP ranged with 3-5 DAB. However, our study showed that all of fruit set, the fruit weight, dry matter, seed number and seed weight were consistently reduced from 3 DAB in both cultivars for two years and the EPP should be determined based on a combination of these factors. Consequently, the study indicates that the period for pollination of these kiwifruits grown in the non-heated plastic film house in Jeju region might be 3 or 4 DAB at most although it could vary depending on the growing and weather conditions for 'Halla Gold' and 'Sweet Gold'.

This study showed that there was no difference in pollen tube growth according to the pollination time of the day, it is considered that the pollination time of the day does not affect fruit quality and seed formation. Although it was reported that pollen adhesion increases and pollination efficiency is improved if artificial pollination is conducted when the amount of pistil exudate is high in the morning (Cacioppo et al., 2018). However, in our study, it was found that the pollination time of the day did not have a significant effect on fruit quality and seed formation. Also, most flowers open between 7 and 11 AM in 'Hort16A' kiwifruit (Goodwin et al., 2013) and then it is considered that stigma holds good receptivity all day for the first 3 DAB, but its receptivity might be different depending on weather conditions between early morning and late afternoon from the 4th day of petal opening. Therefore, the result suggests that further studies on the pollination time of the day according to days after petals open are necessary.

As for repeated pollination, it was observed that the pollen tube in pistil reached and penetrated into the ovule at 3 days after artificial pollination although the patterns varied depending on the number of dry pollen application. Pistillate flowers in most of pollination-dependent plants should receive sufficient pollen to maximize ovule fertilization (Ashman et al., 2004; Aizen and Harder, 2007). However, it was reported that repeated hand-pollination of the same flower with dry pollen over one or more days might bear smaller fruits with fewer seeds than a single pollination in 'Hayward' kiwifruits, which was related to the arrest or inhibition of pollen tube growth caused by following pollen tubes (Hoping, 1990). The previous report was not

accord with our study showing that the fruit weight, dry matter percentage, firmness, seed weight, and 100-seed weight appeared to be high if a sufficient amount of pollen was repeatedly infiltrated into the pistil. This conflict might be caused by some factors such as different cultivars or polyploidy levels and pollen application types. Therefore, it is considered that further studies on repeated artificial pollination are needed to interpret where these conflicts result from or what types of factors influence.

When pistillate flowers were pollinated with dry pollen immediately after water sprinkle, both 'Halla Gold' and 'Sweet Gold' cultivars showed the lowest fruit weight, dry matter percentage, firmness, number of seeds, and 100-seed weight, whereas dry pollen application 1 h after water sprinkle, immediate or 1 h after suspension medium sprinkle didn't show significant differences on the fruit quality and seed formation. In kiwifruits, Cacioppa et al. (2018) reported that pollen adhesion was lowered when it was delivered with water. Furthermore, it was reported that pollen might lose viability due to osmotic shock when it is suspended directly in water and pollen might maintain viability about for 3 h when it is suspended in suspension medium (Hoping, 1990). Also, high temperature and high humidity should be avoided in order to maintain the viability of pollen on stigma. From the results of this study, it appeared that the immediate pollination after water sprinkle might lead to lower viability of pollen due to the effect on pollen adhesion to the pistil and pollen tube penetration and burst. The result was in accord with that in orchards, artificial pollination on a sunny day normally results in superior fruit quality and seed formation rather

than on a rainy day. Thus, it is recommended that artificial pollination with pollen dusting should be avoided until raindrops or dewdrops on stigma disappear. However, it is considered that further studies on such artificial pollination conditions and proper timing are needed.

In wet application using pollen suspension, the fruit weight was lower in both 'Halla Gold' and 'Sweet Gold' cultivars than in conventional dry pollen application, but there was no significant difference in 'Halla Gold' cultivar. Application types with dry pollen and pollen suspension did not show a significant difference in fruit quality and seed formation except the fruit weight of 'Halla Gold' cultivar. Lim et al. (2014) reported that wet pollen application using pollen suspension and dry pollen application using lycopodium powders did not show a significant difference in the number of seeds, SSC, acidity and firmness in 'Hayward' cultivar, and out study also showed the similar results in 'Halla Gold' and 'Sweet Gold' cultivars. Therefore, it was found that the use of pollen suspension instead of lycopodium powders as the pollen diluent in 'Halla Gold' and 'Sweet Gold' cultivars did not have the effects on fruit quality and seed formation. Also, it is considered that wet application using pollen suspension will help reducing the operating expenses in orchards since its labor and pollen requirements are small. However, further studies on the appropriate dilution ratio of pollen in the suspension according to the cultivar are needed.

This was investigated how the ploidy level of the kiwifruit pollinizer cultivars 'CK3' (diploid), 'T' line (tetraploid), 'Bohwa' (hexaploid) and 'Chieftain' (hexaploid) affected fruit set, fruit quality, and seed formation in the tetraploid kiwifruit cultivars 'Halla Gold' and 'Sweet Gold' cultivated.

Harvey et al. (1991) reported that the pollen tube growth rate in the style decreased and that the fertilization rate dropped substantially when a diploid cultivar of A. *chinensis* var. *chinensis* was pollinated with pollen from a hexaploid cultivar of A. *chinensis* var. *deliciosa.*

In our study, as in this previous report, the number of pollen tubes observed was noticeably reduced in the tetraploid pistillate cultivars 'Halla Gold' and 'Sweet Gold' when pollinated with the pollen of the diploid staminate cultivar 'CK3'. Such a difference is considered to have an effect on the weight of fruits and the formation of seeds.

In kiwifruit, the h° value (rather than L, a and b values) is used as an index of ripening, and it is generally accepted that a lower h° value indicates increased ripening of fruit (Seal et al., 2013b). Therefore, when 'Halla Gold' and 'Sweet Gold' were pollinated with the pollen of 'Bohwa' and 'Chieftain', the reason that the firmness or h° was higher than when pollinated with other pollen (and despite similar dry matter and SSC values) could be attributed to a delay in ripening. Also, the firmness of kiwifruits, which ripen after harvest, is an important factor affecting postharvest storage and distribution processes (Feng et al., 2003). Therefore, it is considered that studies on the effect of the ploidy level of pollen on postharvest fruit quality are needed in the future. The number of seeds showed a similar trend to fruit weight, but the 100-seed weight was highest with 'T' line as the pollinizer. Several studies have reported the correlation between fruit weight and number of seeds (Galimberti et al., 1988; Pyke and Alspach, 1986; Ferguson, 1984; Hopping, 1976). In particular, fruit weight in kiwifruit has been reported to be more highly correlated with seed

weight than with number of seeds (Lawes et al., 1990). In this study, a correlation between fruit weight and seed formation (such as seed number and seed weight) was observed. When 'Halla Gold' and 'Sweet Gold' were pollinated with the pollen of diploid 'CK3,' the low fruit weight was correlated to low seed number and low seed weight, together. However, when pollinated with the pollen of tetraploid 'T' line, the high seed weight compensated for the low seed number resulting in fruit weights similar to those arising from hexaploid pollen donors. Hence, it is postulated that fruit weight is correlated with seed weight as well as seed number. This result is in accordance with those in previous studies (Seal et al., 2013a, 2013b, 2016). In addition, Buxton (2005) suggested that seed number was correlated with dry matter percentage, but such a correlation was not observed in this study.

Lawes et al. (1990) indicated that fruit set and fruit weight were correlated to seed development but not to fertilization because fertilized zygote fails to develop perfect seeds. This phenomenon is caused by the lack of coordination between embryo and endosperm due to a violation in the 2:3 genomic ratio between embryo and endosperm, called an interploid block. It has been reported not only in kiwifruit (Seal et al., 2013a, 2013b, 2016) but also in other crops such as kiwiberry (Stasiak et al., 2019) and citrus (Cameron and Burnett, 1978). In the case of the interspecific or intraspecific hybridization of kiwifruit reported by Seal et al. (2013a, 2013b, 2016) and Lawes et al. (1990), the ratios showed greater deviation (3:4, 4:5, and 4:7), and normal seed development failed. These results suggest that a threshold of deviation from a ratio of 2:3 might exist, though further studies

would be needed to confirm this hypothesis. The results of this study indicate that suitable pollen donors for the cultivation of 'Halla Gold' and 'Sweet Gold' are the tetraploid 'T' line and hexaploid 'Bohwa' and 'Chieftain' cultivars, since these showed more favorable trends in terms of fruit weight and firmness, and the number and weight of seeds than 'CK3'. While this study showed compatibility differences when two tetraploid A. *chinensis* var. *chinensis* cultivars of kiwifruit were pollinated with the pollen of four cultivars with different ploidy levels, it is likely that a number of kiwifruit growers will continue to use inexpensive imported pollen of unknown cultivar and ploidy level. Since the cultivation area for yellow-fleshed or red-fleshed type (A. *chinensis* var. *chinensis*) kiwifruit is predicted to increase in Korea, further studies to identify pollen parents suitable for a particular cultivar are needed.

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ABSTRACT IN KOREAN

키위는 자웅이주 식물로 상업적 재배를 위해서는 인공수분이 매우 중요하다. 키위는 유효수분기간이 짧기 때문에 안정적인 착과를 위해서는 짧은 기간에 인공수분 할 수 있는 재배기술이 필요하다. 또한 화분친의 배수성이 국내 육성 키위 품종의 과실 품질에 미치는 영향에 대한 연구는 아직 보고된 바가 없다. 따라서 본 연구는 황색 계통으로 제주지역에서 많이 재배하고 있는 'Halla Gold'와 'Sweet Gold' 품종에 대해 효과적인 인공 수분 시기 및 수분 시간을 제시하고, 인공 수분 방법 및 배수성이 다른 화분친이 과실 품질 및 종자 형성에 미치는 영향을 알아보고자 수행되었다.

제주지역 무가온 플라스틱 하우스에서 재배되고 있는 'Halla Gold'와 'Sweet Gold' 품종에 인공 수분 시기와 수분 시간에 따른 과실 품질 및 종자 형성에 미치는 영향을 알아보고자 수행하였다. 'Halla Gold'와 'Sweet Gold' 모두 개화 후 3일차에 수분하였을 때 착과율과 생체중, 건물률이 감소하기 시작하였다. 반면 당도, 산도, 건물율, H값은 유의적 차이가 나타나지 않았다. 종자무게와 백립중은 개화 후 3일차에 감소하는 경향을 확인 할 수 있었고, 효과적인 수분기간은 생체중과 종자 충실도에 영향을 미치는 것을 확인할 수 있었다.

인공 수분 시간에 따른 'Halla Gold'와 'Sweet Gold'의 착과율은 모두 90% 이상으로 나타났

다. 생체중은 오전 7시와 10시에 수분하였을 때 높게 나타났고, 16시 수분할 때에는 낮게 나타났다. 'Halla Gold'에서 건물률은 오전 7시와 10시에 수분했을 때 높게 나타났고 16시 수분했을 때 낮게 나타났지만, 'Sweet Gold'에서는 유의적 차이가 없었다. 'Halla Gold'와 'Sweet Gold' 모두에서 당도, 산도, 경도, H값, 종자수, 백립중의 통계적 유의성은 없었다. 따라서 제주지역의 무가온 플라스틱 하우스에서 재배되는 'Halla Gold'와 'Sweet Gold'의 수분 가능한 기간은 개화 후 3일 이내로 판단되었고, 수분 시간은 과실 품질 및 종자형성에 큰 영향을 주지 않는 것을 확인하였다.

'Halla Gold'와 'Sweet Gold' 품종에 인공 수분 방법에 따른 과실 품질 및 종자 형성에 미치는 영향을 알아보고자 수행하였다. 중복적으로 인공 수분 했을 때가 생체중, 건물율, 종자 수와 백립중이 높게 나타났다. 당도와 산도는 모든 처리에서 유의적 차이가 없었다. 3번 중복 인공 수분한 처리에서 경도는 낮게, H값은 높게 나오는 경향을 나타냈지만, 'Halla Gold'에서는 유의적 차이가 없었다. 또한 암꽃의 물 처리 후 바로 수분하였을 때 'Halla Gold'와 'Sweet Gold' 모두 생체중 및 건물률, 경도, 종자수, 백립중이 가장 낮게 나타났다. 중복 인공 수분에서는 암술 내 화분관 신장은 인공 수분 3일 후에 밑씨에 도달하여 결합하는데, 그 양상은 인공 수분 횟수에 따라 차이가 있었다. 'Halla Gold'와 'Sweet Gold'는 화분관의 수는 한번 인공 수분할 때보다

중복적으로 인공 수분하였을 때 확연하게 많이 관찰되었다. 물을 뿌린 직후에 인공 수분하였을 때 'Halla Gold'와 'Sweet Gold' 은 모두 생체중, 건물중, 경도, 종자수, 백립중이 가장 낮은 반면, 물을 뿌린 1시간 후, 화분현탁액을 뿌린 직후, 화분현탁액을 뿌린 1시간 후에는 과실 품질 및 종자 형성에 유의한 차이가 나타나지 않았다. 화분 현탁액을 이용한 물수분은 'Halla Gold'와 'Sweet Gold'에서 생체중이 기존 dry pollen pollination보다는 낮게 나타났지만 'Halla Gold'에서는 유의적 차이가 없었다. 또한 화분증량제를 수용액 형태의 화분현탁액을 이용한 물수분과 분말 형태의 석송자를 이용한 수분은 생체중을 제외한 과실 품질 및 종자 형성도에서 크게 차이를 나타내지 않았다. 따라서 화분 현탁액을 이용한 물수분은 인공수분 노동력과 꽃가루의 소요량이 작기 때문에 효율적인 수분방법이 될 수 있을 것이다.

4배체 'Halla Gold'와 'Sweet Gold' 품종에 배수성이 다른 'CK3' (diploid), 'T' line (tetraploid), 'Bowha' (hexaploid), and 'Chieftain' (hexaploid) 품종이 과실 품질 및 종자 형성에 미치는 영향을 알아보고자 수행하였다. 암술 내 화분관 신장은 인공수분 3일 후에 밑씨에 도달하여 결합하는데, 그 양상은 배수성이 다른 화분친에 따라 차이가 있었다. 'Halla Gold'와 'Sweet Gold'는 화분관의 수는 'CK3'으로 수분하였을 때 다른 꽃가루에 비해 확연하게 적게 관찰되었다. 'Halla Gold'와 'Sweet Gold'의 모든 화분 처리에서 착과률이 90%을 나타냈다. 'Halla Gold'와 'Sweet

Gold'의 과실 무게에서는 'Chieftain'과 'Bowha'의 수분에서 높게 나타났다. 당도는 'Sweet Gold'에서는 'Chieftain'에서 높게 나타났으나 'Halla Gold'에서는 큰 차이가 없었다. 건물률과 산도는 모든 처리에서 유의적 차이가 없었다. 경도는 'Halla Gold'에서는 'Chieftain'에서 높게 나타났고, 'Sweet Gold'에서는 'Bowha'에서 높게 나타났다. 색차계 h° 은 'Halla Gold'와 'Sweet Gold' 모두 'CK3'에서 낮게 나타났다. 'Halla Gold'와 'Sweet Gold'의 종자 수는 과중과 비슷한 경향을 보였으나, 백립중은 'T' line에서 가장 높게 나타났다. 화분의 배수성 수준이 착과율보다 과일 품질에 더 많은 영향을 준다는 것을 확인할 수 있었다. 또한 'Halla Gold'와 'Sweet Gold' 재배에 적합한 꽃가루는 생체중과 과실 경도, 종자수, 종자무게에서 높은 경향을 나타낸 4배체 'T' line과 6배체 'Bohwa'와 'Chieftain'으로 판단된다.

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