A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Cultivar Differences in Floral Structures and Effect of Low Air Temperature on Pollen Germination in *Fragaria* x *ananassa* Duch.

딸기 품종별 화기 구조의 차이 및 야간 기온이 화분 발아에 미치는 영향

BY

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ABSTRACT

This study was conducted to investigate the causes of fruit malformation in the Korean strawberry 'Maehyang' and to develop cultural techniques to reduce the incidence of the symptoms. The first chapter of this thesis analyzes cultivar differences in morphological characteristics of flower organs. In the second chapter, cultivar differences in pollen germination as affected by low temperature during the dark period are discussed. In Chapter 1, three Korean cultivars

‘Maehyang’, ‘Seolhyang’, and ‘Keumhyang’, and a Japanese cultivar ‘Akihime’ were grown in a plastic greenhouse, and their flower organs were observed. The shape of the receptacle in ‘Maehyang’ was longer and narrower in stage 3 than in the other cultivars. ‘Maehyang’ had the more rows of ovules, which might indicate a difference in pistil development between distal and proximal regions of the receptacle. Among the four tested cultivars, there was no significant difference in pistil length in primary or tertiary flower clusters. However, in the secondary flower cluster, where the incidence of fruit malformation was reported to be greatest, ‘Maehyang’ had a shorter pistil than ‘Seolhyang’, ‘Keumhyang’ and ‘Akihime’, with ‘Keumhyang’ having the longest pistil. The Stigma surface of ‘Maehyang’ was smoother than those of the other cultivars and had a larger number of beaks on the stigma. Anther shapes at stage 3 were dependent on cultivar and flower cluster. Among the tested cultivars, ‘Maehyang’ had the narrower and longer anthers throughout the primary to tertiary flower clusters. ‘Maehyang’ had a smallest number of pollen grains in the secondary flower cluster, while ‘Keumhyang’ had the largest number. These morphological differences of flower organs may be related to the higher incidence of fruit malformation in ‘Maehyang’.

In Chapter 2, the two Korean cultivars ‘Maehyang’ and ‘Seolhyang’ were cultivated in a greenhouse. When flower buds became visible in early October,

plants of each cultivar were transferred to a plant factory with artificial lighting. Four different air temperatures (5, 10, 15, and 20°C) were tested during the dark-period (14 h d^{-1}), and photoperiod (10 h d^{-1}) air temperature was maintained at 25°C. For the determination of pollen viability and germination, three fully-opened flowers with dehiscent anthers were obtained from each flower cluster of plants grown at the plant factory. An in vitro pollen viability test was performed using the acetocarmine staining technique and pollen grains germinated in germination medium. 'Maehyang' showed lower pollen viability and pollen germination compared with 'Seolhyang' in all temperature treatments. Both cultivars had relatively low pollen viability at 25°C/5°C and 25°C/10°C. The percentage of viable pollen was significantly lower in 'Maehyang' than in 'Seolhyang' at 25°C/15°C in both primary and secondary flower clusters and also lower at 25°C/5°C and 25°C/10°C in the tertiary flower cluster. The percentage of pollen germination in primary flower cluster was lower at high night temperature (25°C/15°C and 25°C/20°C) than at the other two temperature treatments. In the secondary flower cluster, 'Maehyang' showed a lower pollen germination than 'Seolhyang' at 10°C and 20°C, with the lowest percentage of pollen germination occurring at 25°C/20°C in 'Maehyang' but at 25°C/5°C in 'Seolhyang'. In the Tertiary flower cluster, the pollen germination percentage was significantly higher in 'Seolhyang' than in 'Maehyang' at all tested night temperatures except 10°C,

and the lowest percentage of pollen germination was observed at 25ºC/10ºC in both cultivars. The research results of the present study could be used for developing new cultivation techniques to produce high-quality strawberry fruits in winter cultivation, so called forced cultivation.

Keywords: cultivar, flower cluster, malformation, morphological characteristics, pollen viability, pollen germination

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INTRODUCTION

The total area used for the cultivation of strawberries (*Fragaria* x *ananassa* Duch.) in Korea is about 6,403 ha, with an average yield of 3,311kg/10a (KOSIS, 2015). The major strawberry cultivars popular to Korean growers and consumers, 'Akihime' and 'Red Pearl' were originally bred in Japan and are popular due to their high yield and easy adaptability to forced culture cropping. The cultivation area of these Japanese cultivars in Korea, however, has dramatically decreased since 2005.

Korea joined the International Union for the Protection of New Varieties of Plants (UPOV) in 2002, through which the strawberry became subject to plant variety protection. Starting in 2011, Korean strawberry growers have been required pay a royalty for using foreign cultivars. With these circumstances, breeding of new domestic cultivars has accelerated, and many Korean cultivars have been introduced since 2005. 'Maehyang', 'Seolhyang', and 'Keumhyang' are the major Korea cultivars well adaptive to forced culture cropping, demonstrating excellent fruit quality; their development resulted in a very rapid increase in cultivation area of Korean domestic strawberry cultivars (3.8% in 2005 to 92.4%

in 2015), with the remaining area still occupied by Japanese-bred cultivars (RDA, 2015).

The fruit quality of 'Maehyang' among the Korean-bred strawberry cultivars is very high, showing a high sugar-acid ratio, great aroma values, and high firmness. Because of these advantageous characteristics, 'Maehyang' fruits have been exported to Hong Kong, Singapore, Japan, Malaysia, and other countries (KATI, 2014) at high price and account for almost 100% of the Korean strawberry export. However, 'Maehyang' occasionally shows a high incidence of malformed fruit, which might be a major reason for the dramatic decrease in cultivation area from 9.2% in 2005 to 1.7% in 2014 (RDA, 2015) and for stagnation of its export at around 3,700-ton and 33 million USD (Kim, 2015).

There are multiple causes of malformed berries in strawberry, one of which is insufficient pollination, which reduces the number of fertilized ovules, the source of localized stimuli for receptacle expansion, causing uneven receptacle growth and a malformed fruit (Gilbert, 1985). Malformed fruits are a source of economic loss to growers since they reduce yield and quality. Fruit malformation is mainly caused by genetic and environmental factors (Ariza et al., 2011). The major Korean strawberry cultivar showing high incidence of fruit malformation is 'Maehyang', based mainly on observation of growers. However, there is little detailed scientific information about cultivar differences in floral structure or

pollen development or about the influence of environmental factors on pollen viability, pollen grain germination, fertilization, and fruit development not only in the 'Maehyang' cultivar, but also in other recently bred Korean and Japanese cultivars.

In this study, as one of the early steps of the research to investigate the causes of fruit malformation of 'Maehyang' and to develop cultural techniques to reduce the incidence of the symptoms, two basic objectives were set. They were to clarify the cultivar differences in floral morphology of 'Maehyang' from that of other major strawberry cultivars (in Chapter 1) and to understand effect of night air temperature on pollen viability and germination of pollen grains in 'Maehyang' compared to those in 'Seolhyang' (in Chapter 2).

LITERATURE REVIEW

Fruit of malformation in strawberry

Nitsch (1950) stated that fruit development depended on the number of fertilized achenes on the receptacle. Successful fertilization and adequate numbers of achenes are necessary to achieve marketable shape and size of strawberry fruits. Poor pollen quality in strawberry plants results in poor fertilization, which in turn leads to a reduction in fruit set (Gilbert and Breen, 1987). Achene enlargement usually occurs gradually from the basal to the apical zone of the receptacle, causing the symmetric heart-shape typical of the strawberry fruit (Perkins-Veazie, 2010). However, a loss of symmetry because of an uneven reduction in receptacle size has been commonly described in some strawberry cultivars rendering misshapen fruits (Carew et al., 2003).

The vegetative and reproductive development of strawberry is mainly controlled by genetic factors, although they are also influenced by environmental conditions (Braun and Kender, 1985; Paroussi et al., 2002). Some diploid and polyploidy species were anatomically investigated by Rudloff (1930), who found that development of seed buds and anthers ceased at widely different stages, which corresponded with the male and female sterility series. The quality of stamens and

carpels can also vary with the position of the flowers on the inflorescence, which can lead to the production of malformed fruits (Kronenberg, 1959). Incomplete pollination of a strawberry flower results in malformed fruits, and the extent of fruit malformation is related most strongly to the number of pistils not fertilized (Albregts and Howard, 1982).

Among the environmental conditions, temperature is one of the most important factors that affect fruit and seed set. Temperature can affect it at different stages of the development process, such as pollen viability and germination (Higuchi et al., 1998; Sato and Peet, 2005), stigmatic receptivity (Hedhly et al., 2003), and ovule longevity (Sanzol and Herrero, 2001). High temperature stress can affect the growth and development of pollen, which in turn can impinge on fruit set and seed development (Ledesma and Sugiyama, 2005); it has also been shown that pollen from different strawberry cultivars have differing responses to high temperature stress. In 'Raiho' and 'Tochiotome' strawberry cultivars, misshapen fruit were related to the presence of small achenes resulting from high temperatures during the fruiting season (Fujishige et al., 2001; Pipattanawong et al., 2009). The incidence of fruit malformation in strawberry is related to a localized loss of achene functionality resulting from temperatures below 7°C during pollen and embryo development (Ariza et al., 2011).

Floral morphology of strawberry

The strawberry flower develops centripetally, with the sepals appearing first, followed by the petals, stamens, and carpels, respectively (Taylor et al., 1997). Under unfavorable conditions, as in poor light and low temperature, flower parts are suppressed in a regular pattern, fist the stamens, next petals, then sepals, epicalyx, and finally the pistils. Fruit malformation-causing factors, such as low temperatures, affect the functional integrity of either male or female reproductive structures by acting from pre-anthesis, when sporogenesis, gametogenesis and maturation of the reproductive structures occur until fruit development (Ariza et al., 2011).

The pistil consists of three parts: the stigma, which provides the cells for pollen reception and germination; the style, through which the pollen tubes grow; and the ovary, which contains one or more ovules, each containing an embryo sac, the female gametophyte (Knox, 1984). The stigmas of many genera of flowering plants have been surveyed morphologically (Heslop-Harrison and Shivanna, 1977; Heslop-Harrison, 1981) and are regarded as a gland, which provides the receptive cells for pollination and may comprise a few or many modified epidermal cells. The stigmas are specialized elongate papillae composed of either unicellular or multicellular units; in some cases, the receptive cells may be bullate or flattened in appearance. The surface characteristics of stigmas in the living state are readily

observed by scanning electron microscopy without any pretreatment (Knox, 1984).

Each stamen is made up of paired anthers on a filament or stalk. Pollens in the anthers mature before the flower or the anthers open, but usually the anthers do not crack until after the flower opens and the anthers dry a little (Darrow, 1966). The anthers open at the sides under tension so that pollens are thrown onto pistils and petals. The pollens are at first heavy and sticky but later becomes dry and is carried by air currents and they remain viable for several days under ordinary conditions. The pollens come from the anthers of the stamen is carried by bees to the stigmas of pistils effective pollination. If all pistils are pollinated, a well-shaped berry may develop. If few are pollinated, an irregular-shaped berry develops (Andersson et al., 2012). Pollen quality is measured not only by its ability to germinate, but also by the ability of the pollen tube to elongate down the style for successful fertilization (Ledesma and Sugiyama, 2005).

Pollen viability and germination as affected by air temperature

Adverse environmental conditions such as high or low temperature and water stress induce pollen sterility to different degrees (Shivanna, 2003). Thompson (1969) presented data on the effect of temperature on pollen formation of one English and four Dutch cultivars. He observed a decrease in individual fruit weight

when flowering plants were exposed to 10ºC and short days (12 h). The fruit was relatively small and had fewer viable achenes. Thompson (1969) reasoned that low temperature must have lowered the production of fertile pollen. Heat-induced short-term reductions in cropping occur frequently in extended-season ever-bearing strawberry production in the UK. A 2-year study evaluated the effects of high temperatures on the performance of pollen in two ever-bearing strawberry cultivars (cvs 'Everest' and 'Diamante'), illustrating that pollen from both cultivars lost the ability to germinate after exposure to a period of high temperature (30/20ºC day/night) (Karapatzak et al., 2012).

The combination of cold and wet weather may disrupt pollen germination. Garren (1981) has reported that bloom temperatures in Oregon are frequently below 15.6ºC, which is low enough to impair pollen germination. Hedhly et al (2003) concluded that high temperature reduced stigmatic receptivity in the sweet cherry, while low temperature increased it. Low temperatures may negatively impact the pollen by reducing its germination and growth rate, which could limit fertilization success (Thompson and Liu 1973; Jakobsen and Martens, 1994). A study with *Brassica napus* suggested that the major cause of low pollen fertility under high temperatures was reduced pollen germination (Young et al., 2004).

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CHAPTER 1

Cultivar Differences in Morphological Characteristics of Flower Organs

INTRODUCTION

Flowering responses are often used to make inferences about floral induction, initiation, and differentiation (Durner and Poling, 1987). Flowers also vary in size and number of parts, with variation occurring between cultivars and according to the position or rank of the flower in the inflorescence (Guttridge, 1985).

Within a strawberry flower, the order of primordial induction is sepals, petals, stamens, and pistils (Sattler, 1973). In strawberry, it has been reported that low temperatures during flower development produce physical injury to floral organs at several stages of flower development. Malformed fruit have been observed in strawberry cultivars during winter, suggesting that cold temperatures are also involved in the incidence of malformed fruits by affecting reproductive success.

Differences in floral and fruit characteristics of strawberry were previously investigated, especially with respect to the relationship between the maturity of pistils at anthesis and the final fruit shape. Darrow (1927) stated that 'pistil-sterility' of hermaphrodite varieties was the only important type of sterility in the strawberry. He regarded it as one of the outstanding problems in the development of the strawberry. If all the pistils in a flower are frozen, the receptacle cannot develop and dries out. If only a number of the pistils of a flower become frozen, the other pistils may develop; however, only the part of the receptacle with the fertilized pistil will swell properly, resulting in malformed fruits (Inaba, 2001). Valleau (1918) and Herold (1941) have observed that, in addition to normally developed pollen grains, a larger or smaller number of degenerated grains may occur in strawberry pollen. Generally, stamen number per flower ranges from 20-35. The conical receptacle contains up to 500 pistils (Connor, 1970; Darrow, 1927). These pistils develop first at the base of the receptacle and appear sequentially in a spiral-like fashion up to the tip. Underdeveloped pistils occur most often at the tip of the receptacle (Valleau, 1918).

The quality of stamens and carpels can vary with the position of the flower on the inflorescence, which can lead to the production of malformed fruit (Kronenberg, 1959). Fruit malformation in 'Ai-berry' was mainly related to genetics of floral structure (Yoshida, 1991). Due to the limited detailed scientific

information about the cultivar differences in floral structures and pollen development not only in ‘Maehyang’ cultivar but also other recently bred Korean and Japanese cultivars, we investigated the flower structures of ‘Maehyang’, ‘Seolhyang’, ‘Keumhyang’, and ‘Akihime’ in order to obtain a deeper comprehension of the relationship between flower morphology and fruit malformation.

Materials and Methods

Plant material

On September 18, 2015, transplants of three Korean cultivars (‘Maehyang’, ‘Seolhyang’, and ‘Keumhyang’) and a Japanese cultivar (‘Akihime’) were transplanted and cultivated until April 2016 in a greenhouse of experimental farm of Seoul National University located in Suwon. The plants were fertigated using Yamazaki nutrient solution.

Observation of flower organs using field emission scanning electron microscopy

Sampled flowers were classified into five developmental stages: stamen differentiation (stage 1), pistil differentiation (stage 2), anthesis (stage 3), dehiscence of anther (stage 4), and discoloration of anther (stage 5) (Yoshida, 1991) (Fig. 1-1). Productive organs such as stigma, pistil, anther, pollen, and receptacle were observed using field emission scanning electron microscopy (SEM). The distance between the two lowest ovaries was regarded as the width of the receptacle, and the height of the receptacle was measured from the line connecting the lowest ovaries to the apical ovary.

Pistils, stamens, and receptacles were collected from primary to tertiary flower clusters at each developing stage. These materials were fixed with Karnovsky's solution at 4°C for 3 h, washed with 0.05 M sodium cacodylate buffer, post-fixed with 2% osmium tetroxide and 0.1 M cacodylate buffer at 4°C for 2 h, and washed with distilled water. The plant parts were then dehydrated in a graded ethanol series (30, 50, 70, 80, 90, and 100%) for 10 min in each concentration, and dried in a critical point drier (CPD 030; BAL-TEC Inc., Balzer, Liechtenstein, Germany) with liquid CO_2 as a transitional fluid. The specimens were then mounted onto metal stubs and coated with gold using a sputter-coater (JFC-1100E, JEOL, Tokyo, Japan). Finally, the specimens were examined with a field emission scanning electron microscope (Supra 55 VP, Carl Zeiss, Oberkochen, Germany) operated at an accelerating voltage of 2 kV.

Statistical analysis

Data were analyzed using SAS 9.4 version (SAS Inst. Inc., Cary, NC, USA) for Duncan's multiple range test (DMRT) at $P \leq 0.05$.

Fig. 1-1. Developmental stages flower in *Fragaria* x *ananassa* Duch. (1-5). Stage 1, stamen differentiation and receptacle diameter (Φ) ≈ 5-6 mm; stage 2, pistil differentiation, receptacle Φ ≈ 7-8 mm; stage 3, anthesis; stage 4, anther dehiscence; stage 5, anther discoloration.

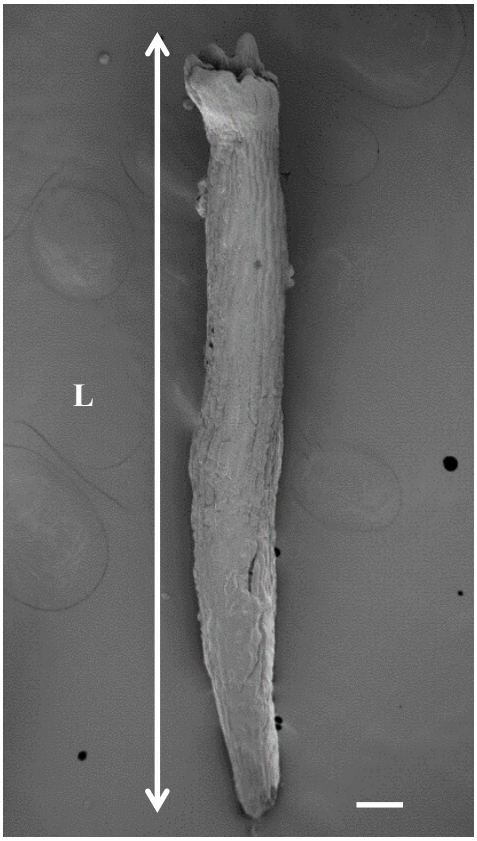

Fig. 1-2. Scanning electron micrograph of a pistil. Scale bar indicate 100μm.

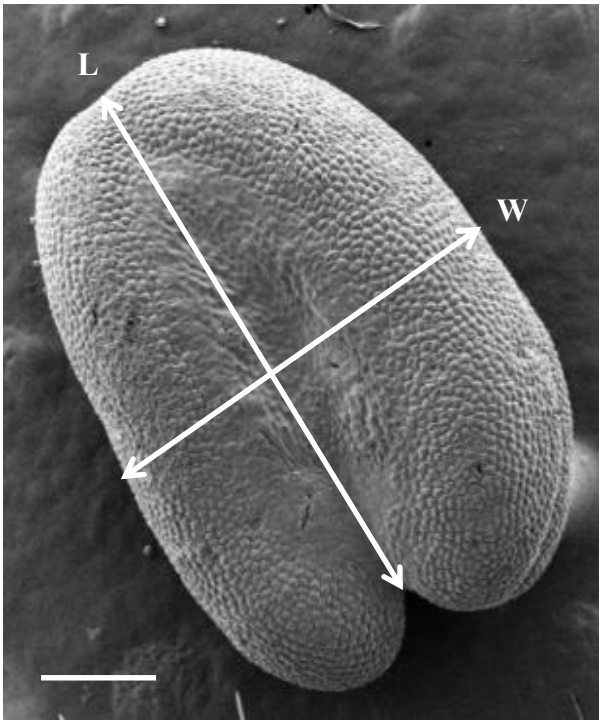

Fig. 1-3. Scanning electron micrograph of an anther. Scale bar indicate 250μm.

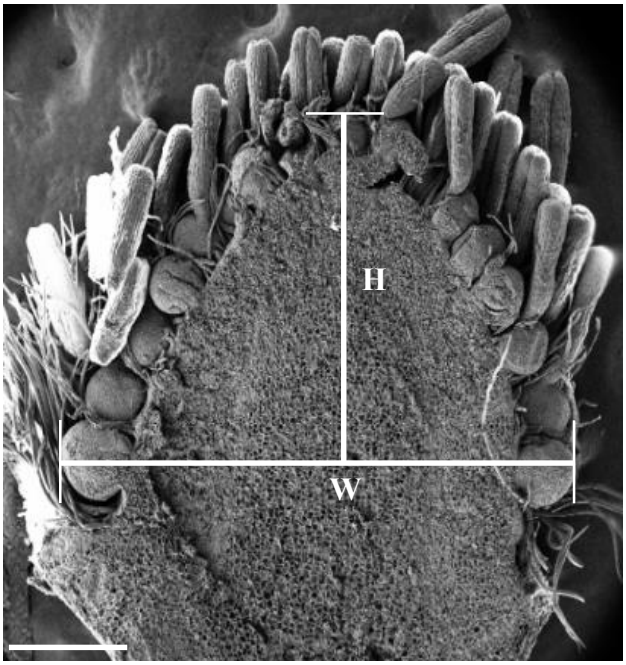

Fig. 1-4. Scanning electron micrograph of a receptacle. Scale bar indicate 500μm.

RESULTS AND DISCUSSION

Receptacle shape

The shape the of receptacle in 'Maehyang' was longer and narrower in stage 3 than in the other cultivars (Table 1-4). 'Maehyang' had the more rows of ovules, which might indicate a difference in pistil development between distal and proximal regions of the receptacle. Yoshida (1992) reported that the flower of 'Ai-berry' had the largest number of rows of pistils from the base to the apex of the receptacle among the tested 6 Japanese cultivars and also showed the highest incidence of malformed fruit. For similar reasons, a higher incidence of fruit malformation might be observed in 'Maehyang'.

Pistil length and stigma surface

Among the four tested cultivars, there was no significant difference in pistil length in primary or tertiary flower clusters (Table 1-1). However, in the secondary flower cluster, where the incidence of fruit malformation was reported to be greatest, 'Maehyang' had a shorter pistil than 'Seolhyang', 'Keumhyang' and 'Akihime', with 'Keumhyang' having the longest pistil. This suggests that there might be a relationship between pistil length and incidence of fruit malformation.

The stigma surface of ‘Maehyang’ was smoother than those of the other cultivars and had a larger number of beaks on the stigma (Fig. 1-5). These morphological differences of stigma and pistil may be related to the incidence of fruit malformation in various strawberry cultivars. More research is needed to clarify the relationships between morphological characteristics of pistil and success rates of pollination and fertilization, as well as incidence of fruit malformation.

Anther shape and number of pollen grains

Anther shapes at stage 3 were dependent on cultivar and flower cluster. Among the tested cultivars, ‘Maehyang’ had the narrower and longer anthers (the greater length to width ratio) throughout the primary to tertiary flower clusters (Table 1-2). ‘Maehyang’ had a smallest number of pollen grains in the secondary flower cluster, while ‘Keumhyang’ had the largest number (Table 1-3). A small number of pollen grains in an anther, especially in the secondary flower cluster, might be related to the higher incidence of fruit malformation in ‘Maehyang’.

Yoshida (1992) reported that the development rate of pistils in the distal region of the receptacle was faster than that in proximal region in ‘Ai-berry’ a

cultivar showing the highest incidence of fruit malformation. To capture pollen grains, stigmas depend on biotic and abiotic pollinators like bees and use rapid and strong adhesive interactions to retain pollen grains (Edlund et al., 2004). Water immediately surrounds grains that land on a wet stigma, while grains that land on dry stigmas mobilize their lipid-rich pollen coat to form an interface between the two cell surfaces. This interface converts to a histochemically distinguishable form thought to promote water flow (Elleman and Dickinson, 1986; Elleman et al., 1992). There have been some studies about the morphological characteristics of flower organs; however, few studies have analyzed the relationships between morphological characteristics and incidence of fruit malformation in the forced culture cropping type such as 'Maehyang'. Further research is necessary to understand the relationships between morphological characteristics and incidence of fruit malformation. This information can be used to suggest a new cultivation technique for strawberries.

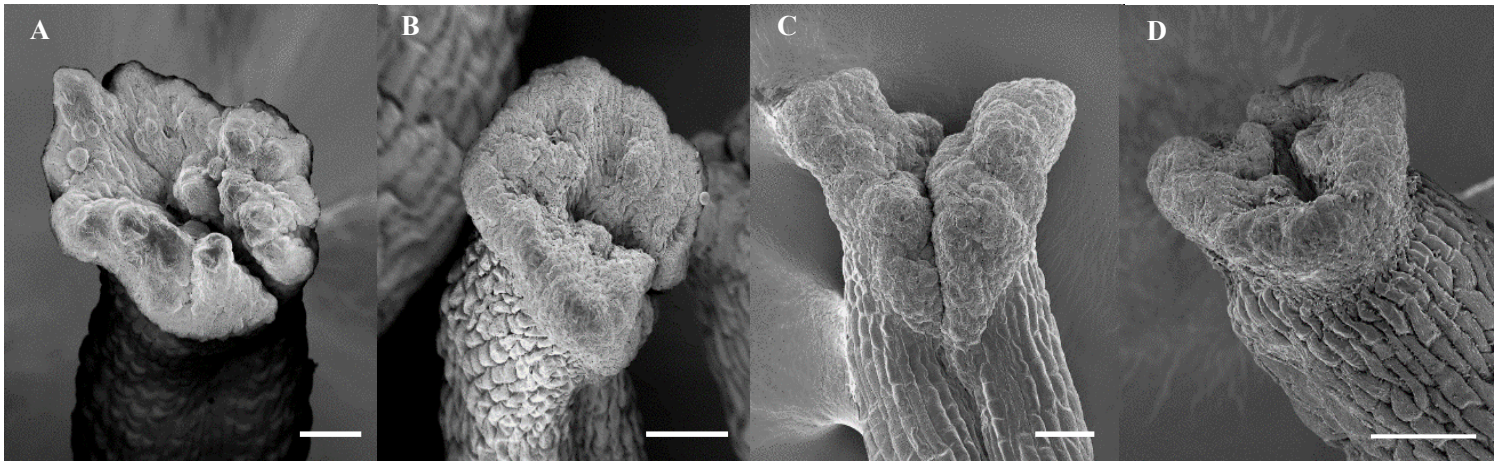

Fig. 1-5. Different shapes of stigma at anthesis (stage 3) in four cultivars. A: 'Maehyang', B: 'Seolhyang', C: 'Keumhyang', and D: 'Akihime'. Scale bar indicate 50μm.

Table 1-1. Length of pistil at anthesis as affected by flower clusters and cultivars.

Cultivar	Primary FC[z]	Secondary FC	Tertiary FC
	Length (mm)	Length (mm)	Length (mm)
Maehyang	1.47[y] a	1.45 c[x]	1.55 a
Seolhyang	1.55 a	1.85 ab	1.89 a
Keumhyang	1.20 a	1.96 a	1.74 a
Akihime	1.43 a	1.62 bc	1.78 a

[z] FC: flower cluster

[y] n=30

[x] Mean separation within a column by Duncan's multiple range test at $P = 0.05$.

Table 1-2. Height and width of anther at anthesis as affected by flower clusters and cultivars.

Cultivar	Primary FC[z]			Secondary FC			Tertiary FC		
	Length (L, mm)	Width (W, mm)	L/W	Length (L, mm)	Width (W, mm)	L/W	Length (L, mm)	Width (W, mm)	L/W
Maehyang	1.10[y] a[x]	0.82 ab	1.35 ab	1.03 a	0.87 a	1.27 a	1.21 a	0.76 b	1.60 a
Seolhyang	1.24 a	0.95 a	1.30 b	1.11 a	0.77 a	1.43 a	1.50 a	0.98 a	1.54 a
Keumhyang	1.15 a	0.73 b	1.57 a	0.95 a	0.79 a	1.21 a	1.37 a	1.12 a	1.22 b
Akihime	1.07 a	0.77 ab	1.38 ab	0.95 a	0.71 a	1.33 a	1.21 a	1.07 a	1.12 b

[z] FC: flower cluster

[y] n=30

[x] Mean separation within a column by Duncan's multiple range test at $P = 0.05$.

Table 1-3. Cultivar difference in the number of pollen grains of flowers in the secondary flower cluster.

Cultivar	Number of pollen grains ($x10^3$/flower)
Maehyang	75[z] b[y]
Seolhyang	086 b
Keumhyang	199 a
Akihime	115 b

[z] n=30

[y] Mean separation within a column by Duncan's multiple range test at $P = 0.05$.

Table 1-4. Cultivar differences in height and width of receptacle of flowers in tertiary flower cluster at anthesis.

Cultivar	Height (H, mm)	Width (W, mm)	H/W
Maehyang	6.31[z] ± 1.47[y]	5.60 ± 1.31	1.13 ± 0.02
Seolhyang	5.87 ± 0.76	6.28 ± 0.92	0.93 ± 0.02
Keumhyang	6.34 ± 0.64	6.89 ± 0.11	0.92 ± 0.09
Akihime	6.43 ± 2.01	6.20 ± 0.79	1.02 ± 0.24

[z] n=30

[y] Mean ± SD

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CHAPTER 2

Cultivar Differences in Pollen Germination as Affected by Low Temperature during Dark Period

INTRODUCTION

Both mature pollen and receptive pistils are present in strawberry flowers a few days before anthesis. After contact with the receptive stigma, a pollen grain generates a pollen tube that grows down through the style. The generative cell fertilizes the egg cell located in the ovary at the base of the style. The fertilized ovule is called an achene (Pipattanawong et al., 2009) and is attached to the receptacle by vascular bundles. In one cultivar a well-shaped berry resulted when only 40% of the achenes were viable (Guttridge and Turnbull, 1975).

Malformed fruits have been observed in strawberry cultivars during winter, suggesting that cold temperatures are also involved in the incidence of malformed fruit by affecting reproductive success. According to Darrow (1966), environmental conditions during autumn, when strawberry flowers are generally

initiated, determine both the number of pistils on the receptacle and the number of cells per fruit. Environmental conditions such as relative humidity and air temperature affect pollen function and bee activity. It has been reported that low temperatures during flower development induced a reduction in pollen quality (Voyiatzis and Paraskevopoulou-Paroussi, 2002) and pistil fertility (Inaba, 2001). Poor pollen quality in strawberry plants results in low fertilization rates, which in turn lead to reduction in fruit set (Gilbert and Breen, 1987) and an increase in the formation of malformed fruits called 'nubbins' (Kronenberg, 1959). Flowers of 'Ever-bearing' strawberry exposed to cool clear nights are most susceptible to frost. Spring frosts are typical of western Oregon (Garren, 1981). Many primary flowers at anthesis are above the leaf canopy and are most often damaged by spring frost. In some cultivars, the number of malformed fruit increases at high temperature because of decreased pollen activity (Ledesma and Sugiyama, 2005).

Anther and pollen degeneration are caused by several factors, including inadequate chilling of plants (Risser, 1997), low temperatures (<17°C) (Braak, 1968), and low light intensity shortly before and during flowering (Smeets, 1980). Extended periods of low temperature may cause poor fruit set in the strawberry (Garren, 1981; Kronenberg, 1959). 'Jucunda' fruit developing in a 17-26°C regime reached maximum size, but those at 14°C were severely damaged and did not experience receptacle enlargement. The fruit set in 'Jucunda' appears to be

especially sensitive to temperatures of 14°C and below (Kronenberg et al., 1959). Braak (1968) mentions that, in all his experiments with ‘Jucunda’ a decrease in fruit set paralleled a progressive abortion of stamens. ‘Jucunda’ plants fertilized with foreign pollen (that from cv. Deutsch Evern) produce well-formed fruit. It is possible that low temperature reduces the viability of 'Jucunda' pollen. Thompson (1969) presented data on the effects of temperature on pollen formation of one English (Redgauntlet) and four Dutch cultivars. Thompson (1969) reasoned that low temperature must have lowered the production of fertile pollen.

Major strawberry cultivars are forced culture cropping type in Korea, which bloom from October to February. The temperature must be controlled at blooming, because pollen viability and germination are known to be affected by temperature. The risk of reduced pollen fertility is high at low temperature, and this can cause damage in the form of misshapen and malformed fruit. In this chapter, we investigated the effect of night air temperature on pollen viability and germination of pollen grains in ‘Maehyang’ compared to those in ‘Seolhyang’.

MATERIALS AND METHODS

Plant materials

On September 18, 2015, two Korean cultivars (‘Maehyang’ and ‘Seolhyang’) were transplanted into an experimental farm of Seoul National University in Suwon. When flower buds became visible in early October, plants of each cultivar were transferred to a plant factory with artificial lighting. Four different air temperatures (5, 10, 15, and 20°C) were used during the dark-period (14 h d^{-1}), and photoperiod (10 h d^{-1}) air temperature was maintained at 25°C. Plants were irrigated with Yamazaki nutrient solution. For the determination of pollen viability and pollen germination, three fully-opened flowers (Fig. 1-1, stage 4) with dehiscent anthers were obtained from each flower cluster of plants grown in the plant factory.

Pollen viability

The flowers were gently tapped with a pen to collect pollen into a petri dish. The pollen was then mixed and spread with a paintbrush onto drops of 0.1% acetocarmine solution on microslides. After 15 min, viability was examined under a light microscope (x50). Pollen that stained red was considered viable, and the

percentage of viable pollen from 152 to 2974 was determined for flower samples in each flower cluster.

Pollen germination

The flowers were gently tapped with a pen to collect pollen into a petri dish. The pollen was then mixed and spread with a paintbrush onto drop of germinating medium and placed on several microscope slides. The germination medium consisted of 8% sucrose, 0.6% agar, and 50 $mg \cdot L^{-1}$ boric acid. The slides were put in separate petri dishes, where the relative humidity was kept high by lining the petri dish cover with moist filter paper and sealing with adhesive tape. The petri dish was incubated for 2 h in a growth chamber of 25°C under dark condition.

Statistical analysis

Data were analyzed using SAS 9.4 version (SAS Inst. Inc., Cary, NC, USA). Duncan's multiple range test was used to analyze the differences among night temperatures and Fisher's least significant difference test was used to analyze the differences between cultivars. Significant differences were determined at $P \leq 0.05$.

RESULTS AND DISCUSSION

Pollen viability

'Maehyang' showed lower pollen viability compared with 'Seolhyang' in all temperature treatments. Both cultivars had relatively low pollen viability at 25°C/5°C and 25°C/10°C. The percentage of viable pollen was significantly lower in 'Maehyang' than in 'Seolhyang' at 25°C/15°C in both primary and secondary flower clusters and also lower at 25°C/5 °C and 25°C/10°C in the tertiary flower cluster (Table 2-1). Shivanna (2003) reported that adverse environmental conditions such as high or low temperature and water stress induce pollen sterility to different degrees. Also, Thompson (1969) mentioned that low temperature reduced the production of fertile pollen. Night air temperature equal to or lower than 15°C reduced the percentage of pollen viability in both cultivars, especially in the secondary flower cluster of 'Maehyang', which might be related to the higher incidence of fruit malformation in this cultivar.

Pollen germination

'Maehyang' and 'Seolhyang' showed different behaviors above and below the optimal temperatures, indicating that two cultivars have slightly different

temperature tolerances. In general, the pollen germination percentage of 'Seolhyang' was higher than that of 'Maehyang' (Table 2-2). The percentage of pollen germination in primary flower cluster was lower at high night temperature (25°C/20°C and 25°C/15°C) than at the other two temperature treatments. In the secondary flower cluster, 'Maehyang' showed a lower percentage of pollen germination compared with 'Seolhyang' at 10°C and 20°C, and the lowest percentage of pollen germination occurring at 25°C/20°C in 'Maehyang' and at 25°C/5°C in 'Seolhyang'. In the tertiary flower cluster, the percentage of pollen germination was significantly higher in 'Seolhyang' than in 'Maehyang' at all tested night temperatures except 10°C, and the lowest percentage of pollen germination was observed at 25°C/10°C in both cultivars. Guttridge et al. (1980) suggested that anther failure in 'Redgauntlet' is a physiological disorder due to varying sensitivity to the environment and to a male infertility problem. The variation in fruit malformation among some strawberry cultivars may be related to their differences in anther quality and pollen production (Gilbert and Breen, 1987). Risser (1997) observed that low temperature negatively affected pollen germination of 'Chandler'. Poor pollen performance and stamen sterility may significantly decrease the fruit setting and cause unfavorable fruit formation in strawberries (Voyiatzis and Paraskevopoulou-Paroussi, 2002a). Night air temperature lower than 15°C reduced the percentage of pollen germination in both

strawberries, especially in the secondary flower cluster of ‘Maehyang’, which showed a low percentage of pollen germination at temperatures lower than 20°C. This finding might be related to the higher incidence of fruit malformation in ‘Maehyang’ in this flower cluster.

Table 2-1. In vitro pollen viability of ‘Maehyang’ and ‘Seolhyang’ strawberries cultivated at different night temperature.

Cultivar	Night air temperature	The percentage of pollen viability (%)		
		Primary FC[z]	Secondary FC	Tertiary FC
Maehyang	5°C	51.2[y] a[ns x]	39.4 ab[ns]	33.3 b*
	10°C	33.9 a[ns]	37.0 ab[ns]	35.6 b*
	15°C	46.1 a*	27.6 b*	59.5 a[ns]
	20°C	37.2 a[ns]	55.9 a[ns]	70.4 a[ns]
Seolhyang	5°C	47.1 a	52.4 a	59.7 b
	10°C	47.5 a	52.6 a	84.8 a
	15°C	60.0 a	70.4 a	59.6 b
	20°C	51.5 a	63.0 a	63.8 b

[z] FC: flower cluster

[y] Number of pollen grains counted varies by flower samples from 152 to 2974.

[x] Value followed by the same letter in each column is not significantly different from each other within the same cultivar according to Duncan’s multiple range test at a 5% significance level.

[ns,*] Nonsignificant or significant at $P \leq 0.05$ between 2 cultivars according to Fisher’s least significant difference test.

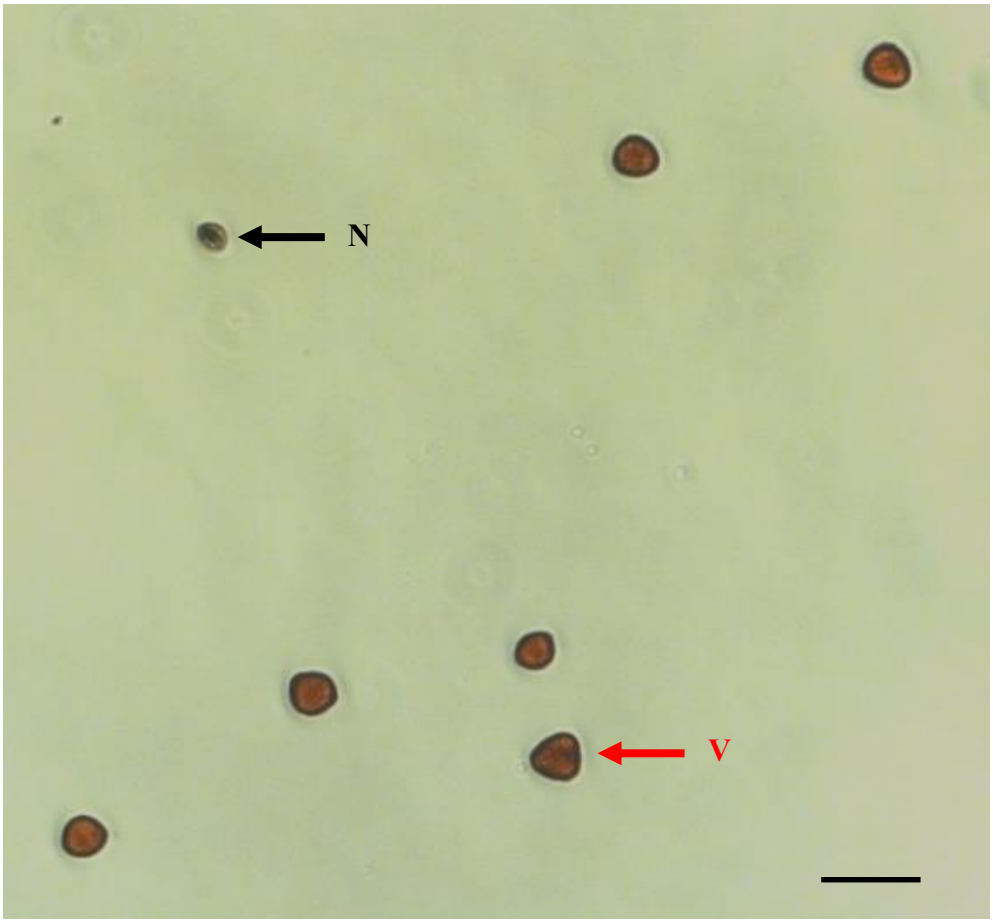

Fig. 2-1. In *vitro* pollen viability test using acetocarmine staining technique. N: nonviable; V: viable. Scale bar indicate 5μm.

Table 2-2. In vitro pollen germination of 'Maehyang' and 'Seolhyang' strawberries cultivated at different night temperature.

Cultivar	Night air temperature	The percentage of pollen germination (%)		
		Primary FC[z]	Secondary FC	Tertiary FC
Maehyang	5°C	15.9[y] a[ns x]	11.3 a[ns]	17.8 b*
	10°C	21.8 a[ns]	7.8 b*	8.8 a[ns]
	15°C	15.7 a[ns]	10.0 a[ns]	13.9 b*
	20°C	4.8 a[ns]	6.4 b*	12.5 b*
Seolhyang	5°C	30.3 a	21.3 a	33.2 a
	10°C	27.6 a	29.9 a	28.7 a
	15°C	8.4 a	22.9 a	45.9 a
	20°C	10.9 a	26.2 a	34.0 a

[z] FC: flower cluster

[y] Number of pollen grains counted varies by flower samples from 165 to 2039.

[x] Value followed by the same letter in each column is not significantly different from each other within the same cultivar according to Duncan's multiple range test at a 5% significance level.

[ns,*] Nonsignificant or significant at $P \leq 0.05$ between 2 cultivars according to Fisher's least significant difference test.

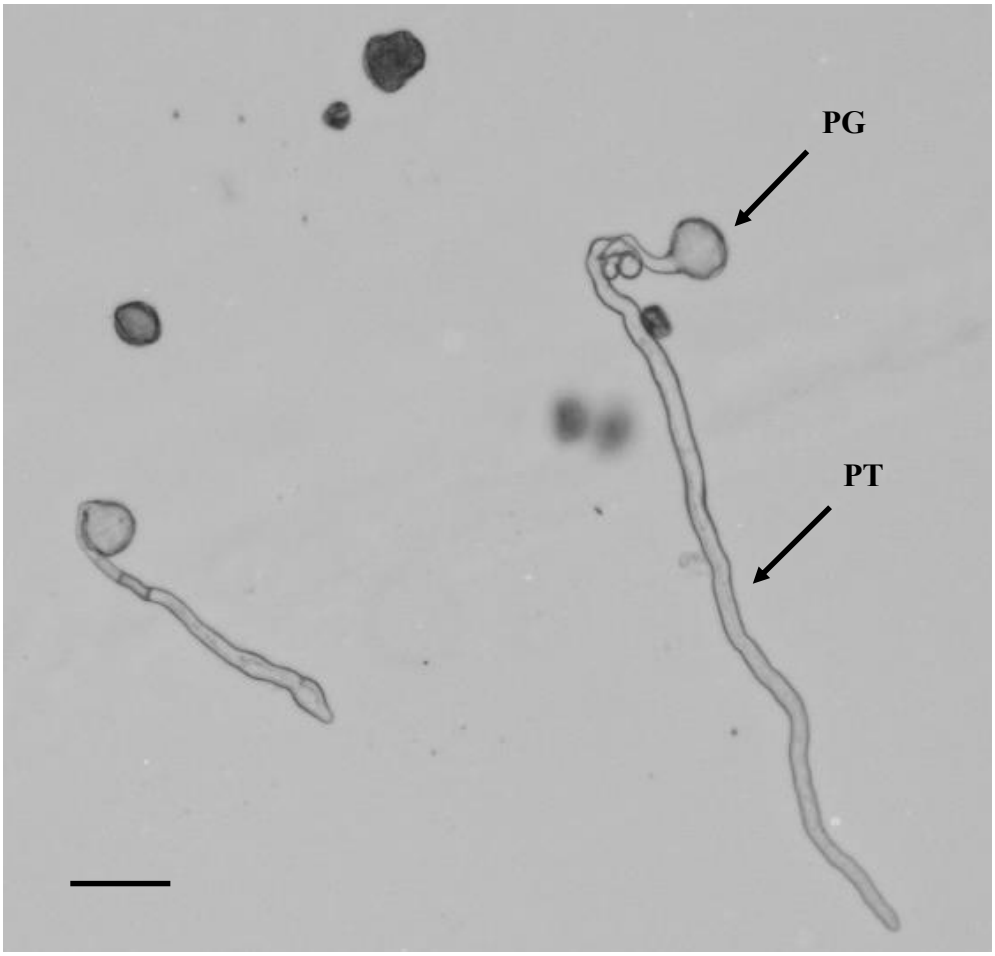

Fig. 2-2 Observation of pollen germination in germination medium at 50x magnification. Bar = 50μm. PG: pollen grain; PT: pollen tube.

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CONCLUSION

In chapter 1, I conducted studies on relationships between morphological characteristics of flower organs and incidence of fruit malformation in 'Maehyang' and compared them with those of other major cultivars mainly used for forced cultivation. Flowers of 'Maehyang' had uniquely distinctive characteristics such as higher height to width ratio of the receptacle, shorter pistil, smoother surface with more beaks on the stigma, and smaller number of pollen grains in the secondary flower clusters compared with those of the other cultivars. These results suggest that those distinctive characteristics of flower organs in 'Maehyang' could be a cause of the higher incidence of fruit malformation in this cultivar.

In chapter 2, I found that pollen viability and percentage of pollen germination of 'Maehyang' were lower than those of 'Seolhyang' in all the tested night temperature treatments. Especially in the secondary flower cluster of 'Maehyang', the percentage of pollen germination decreased when night temperature was lower than 20°C. These results suggest that the higher sensitivity to lower night temperature in 'Maehyang' could be another cause of the higher incidence of fruit malformation in this cultivar during winter.

The research results of the present study could be used for developing new

cultivation techniques to produce high-quality strawberry fruits in winter cultivation, so called forced cultivation.

ABSTRACT IN KOREAN

본 석사학위 논문은 딸기 품종별 화기 구조의 차이와 재배 중 야간 기온이 기형과 발생에 미치는 영향을 구명하는 두 개의 장으로 이루어졌다. 제1장에서는 주요 수출 품종인 '매향'의 기형과 발생 원인을 구명하기 위한 연구의 초기 단계로 '설향', '금향', '아끼히메' 등 국내 주요 재배 품종의 화기 구조와의 차이를 비교 분석하였다. '매향'의 2화방 꽃의 주두는 다른 품종과 비교하여 보다 매끄럽고 돌기가 많았으며, 암술이 짧고, 화분 수가 적었다. 이러한 품종 간 차이는 '매향'에서 기형과 발생률이 높은 원인의 하나가 될 수 있다고 사료된다. 제2장에서는 국내 주요 육성 품종인 '매향'과 '설향'을 식물공장에서 주간 기온 25°C 조건에서, 야간 기온을 각각 5, 10, 15 및 20°C로 설정하여 재배한 후 1, 2, 3 화방의 꽃에서 채취한 화분의 활성과 발아율을 acetocarmine 염색법 등을 통해 조사하였다. 그 결과 '매향'은 화분 활성과 발아율 모두 '설향'보다 낮았다. 또한 화방 별 및 야간 기온 별 유의한 차이를 발견할 수 있었는데 5 °C와 10 °C 저온 처리구에서는 화방과 품종에 관계없이 모두 낮은 화분 활성이 관찰되었고 고온 20 °C-2화방 처리구에서 화분 발아율이 낮았다. 이는 기존의 야간 최저 기온 유지의 재배법도 중요하지만 촉성 재배 시 문제 될 수 있는 높은 야간 기온 조건에의 조우를 회피하는 재배법 등 새로운 딸기 재배 기술을 제안할 수 있는 이론적 배경을

제시할 수 있는 발견이다. 본 연구를 통해 ‘매향’ 딸기의 기형과 원인은 품종적 특성뿐 아니라 야간 기온 등 환경적 차이에 기인함을 알 수 있었다.