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Effects of temperatures on pollen germination and pollen tube growth in apple

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ABSTRACT: Apple cultivars in Korea have been experiencing poor fruit sets due to early spring low temperatures and global warming. Therefore, the present study aimed to investigate the effects of temperature on fruit setting and to select apple cultivars tolerant to extreme temperatures based on the temperature response of their pollen. The effect of temperature on pollen germination percentage (PGP) and on pollen tube length (PTL) was determined under laboratory conditions using the pollen of ten apple cultivars. The two parameters were evaluated using a pollen germination medium, at temperatures ranging from 5 to 45 °C, at 5 °C intervals. The temperature variations considerably affected pollen germination and tube growth in all the examined cultivars. The maximum pollen germination percentage ranged from 61.5 ("Green Ball") to 99.9% ("Shinano Gold") with a mean of 85.2%, whereas the maximum pollen tube length ranged from 716.5 ("Tsugaru") to 989.8 μm ("Arisoo") with mean of 855.1 μm. There were differences in the cardinal temperatures (T_{min}, T_{opt}, and T_{max}) of PGP and PTL between cultivars. Mean cardinal temperatures T_{min}, T_{opt}, and T_{max} computed using the modified bilinear model were 4, 27.7, and 44.9 °C and 6.3, 28.6, and 43.7 °C for PGP and PTL, respectively. Our preliminary results may be used to select low-temperature and global warming-friendly apple cultivars for farmers and can also be exploited to develop temperature-tolerant apple cultivars during flowering.

Key words: *Malus domestica* Borkh., modified bilinear model, cardinal temperature, principal component analysis.

INTRODUCTION

Apple (*Malus domestica* Borkh.) is the most commonly cultivated tree fruit in temperate climates because fresh fruits can be consumed, and it is a raw material for producing processed foods (Cornille et al. 2014, O'Rourke 2021). In Korea, apple fruits are one of the major sources of income for producers and affect the national economy significantly through exports and domestic markets (KSIS 2022).

Currently, climate change is overly affecting the productivity and quality of apples in Korea. In traditional areas of Korea, where apple trees are widely cultivated, global warming adversely affects production (Jeong et al. 2021). In response to these problems, the apple production areas in Korea are increasingly shifting to higher elevations or towards the northern part of the Korean Peninsula.

Due to global warming, the climate in Korea's northern regions would be ideal for apple cultivation, especially during vegetative growth and fruit development (Yun et al. 2020). However, it should be noted that in these areas, temperatures during the flowering period are low, and climate change frequently creates anomalous temperature conditions, such as unseasonably low and high temperatures (KMA 2019)[3].

[3] [KMA] Korea Meteorological Administration (2019). Abnormal report. (in Korean).

Plant reproductive development is more susceptible to low and high temperatures than vegetative development (Díez-Palet et al. 2019, Guo et al. 2019, Radović et al. 2019). Previous studies on olive trees (Benlloch-González et al. 2018), tomato (Vijayakumar et al. 2021), hazelnut (Cetinbas-Genç et al. 2019), and lentil (Baidya et al. 2021) have shown that high temperatures during flower development can dramatically decrease fruit set. In several crop species, decreased pollen viability and germinability at extreme temperatures have been implicated in a decline in the fruit set (Tolessa and Heuvelink 2018, Yang et al. 2019, Shenoda et al. 2021). Pollination of apples is also temperature-dependent and one of the important factors determining fruit productivity (Ramírez and Davenport 2013, Alvarez et al. 2018). The occurrence of extreme temperatures can lead to low fruit-bearing during the flowering period. Cultivars containing pollen that can withstand the constantly changing climate should be screened for sustainable apple cultivation in Korea to address this climatic issue.

The *in-vitro* pollen germination screening has been successfully used to select extreme temperature-tolerant cultivars in several plants (Sorkheh et al. 2017, Hebbar et al. 2018, Bheemanahalli et al. 2021). Additionally, differences in pollen germination ability under extreme temperatures vary between and within species (Milatovic and Nikolic 2017, Cetinbas-Genç et al. 2019, Thuy et al. 2020). Such differences have enabled researchers to group cultivars into heat-tolerant or heat-sensitive, and cold-tolerant or cold-sensitive types based on the temperature response of the pollen (Sorkheh et al. 2017).

Recently, several apple cultivars have been introduced in northern Korea (Geleta et al. 2023). Because these cultivars have different genetic backgrounds, their pollen germination and pollen tube growth may respond differently to various temperature levels. Thus, the objectives of this study were to investigate the effects of temperature on pollen germination and pollen tube growth in different apple cultivars under *in-vitro* conditions and to categorize them into heat and cold-tolerant or sensitive based on their temperature response.

MATERIALS AND METHODS

Plant material

In this study, ten apple cultivars were used, six recently developed and four traditional cultivars from Korea and Japan (Table 1). The apple trees selected for this investigation were 6 years old and growing at a well-managed experimental orchard of Jeongseon Agricultural Technology Center (37°43'N and 128°66'E) situated in the Gangwon-do province, northern Korea.

Table 1. The name, origin and harvest season of apple cultivars used in this study.

Cultivars name	Origin	New/ Traditional	Harvest season
Arisoo	Korea	New	Early to mid (early Sept)
Fuji	Japan	Traditional	Late (late Oct)
Gamhong	Korea	Traditional	Early to mid (early Oct)
Hongro	Korea	Traditional	Early to mid (early Sept)
Honggeum	Korea	New	Early to mid (early Sept)
Picnic	Korea	New	Early to mid (late Sept)
Shinano Gold	Japan	New	Mid. October
Tsugaru	Japan	Traditional	Early to mid (late Aug)
Summer King	Korea	New	Early to mid (late Aug)
Green Ball	Korea	New	Early to mid (early Sept)

Pollen collection, medium preparation, and temperature treatments

Ten completely pink flowers for each cultivar were harvested randomly from five trees at the end of April 2022. The flowers were immediately put in polythene bags, then placed into a cooler and transported to a laboratory. The anthers of all flowers of each cultivar were released using forceps and placed in a sterile Petri dish. They were then exposed to artificial light for 24 h to allow drying. The pollen grains were then collected by shaking the anthers above the Petri dishes, packed with silica gel, and stored at -80 °C until be used.

Prior to the experiment, trials were conducted to optimize the incubation time using a growth medium containing 100 g sucrose, 75 mg boric acid, and 10 g agar in a liter of deionized water at pH 7. The pollen tubes reached their maximum length in 4 h at 25 and 30 °C and began to burst beyond that time. Therefore, the incubation time for the experiment was set at 4 h. For each treatment replication, 5-mL growth medium was poured into a Petri dish. The pollen was dusted onto the Petri dishes containing 5-mL growth medium and incubated for 4 h at 5, 10, 15, 20, 25, 30, 35, 40, and 45 °C in a proportional integral derivative (PID) microprocessor-controlled biological oxygen demand (BOD) incubator (HB-101S, Bucheon, Korea). Three Petri dishes per cultivar were used at each temperature.

Pollen germination and pollen tube growth measurement

When the length of the germinated pollen tube equals or exceeds the pollen grain's diameter, the pollen grain is considered to have germinated *in vitro*. Petri dishes with germinated pollen were observed under a microscope at a 10× magnification power (Kern Optics, Kern and Sohn, Balingen, Germany). Ten microscopic fields per Petri dish were photographed randomly. Pollen germination percentage was determined by counting 200 pollen grains from the randomly photographed images. For each replication, pollen germination was recorded at 5-min intervals. Using an ocular micrometer mounted on the microscope's eyepiece, pollen tube length was measured and converted to micrometers after calibration with a stage micrometer. In each replication of the treatment, 20 pollen tubes were selected based on the length of the pollen tube from the captured images and measured. The average was used to analyze the tube length. The Eq. 1 was used to compute the percentage of the germinated pollen:

$$\text{Pollen germination percentage} = \frac{\text{number of germinated pollens}}{\text{total number of pollens}} \times 100 \quad (1)$$

Curve estimation and fit model selection

To quantify the developmental response to temperature, linear and nonlinear regression models were used to evaluate the maximum pollen germination percentage and pollen tube length, 4 h after incubation. The best-fit model was determined by applying quadratic, cubic, or higher-order polynomials and modified bilinear equations to the data. For estimating the developmental response to temperature as well as for computing the cardinal temperature, the modified bilinear model was the most appropriate and best fit for this data (Kakani et al. 2002, 2005). The optimum temperature was calculated using SAS (Version, 9.4) and the modified Gauss-Newton method. The following modified bilinear equations were applied to compute pollen germination percentage, pollen tube growth, and maximum and minimum temperatures (Eqs. 2, 3 and 4):

$$\text{Pollen germination percentage (PGP) or Pollen tube length (PTL)} = a + b_1(T - T_{opt}) + b_2 \times \text{ABS}\,(T_{opt} - T) \quad (2)$$

$$T_{min} = \frac{a_1 + (b_2 - b_1) \text{ x } T_{opt}}{(b_1 - b_2)} \quad (3)$$

$$T_{max} = \frac{a_1 - (b_2 + b_1) \text{ x } T_{opt}}{(b_1 + b_2)} \quad (4)$$

in which: a, b_1, and b_2 = the parameter estimates of the bilinear equation; ABS = the absolute value; T = the actual temperature at which PGP and PTL were determined; T_{opt} = the optimum temperature for PGP and PTL.

Principal component analysis

Principal component analysis (PCA) is one of the most reliable statistical tools for detecting trends, clusters, and outliers in multivariate data (Granato et al. 2018). In this study, the responses of cultivars' pollen to temperature were investigated using PCA, which was carried out using the Statistical Package for the Social Sciences (SPSS) software (Version 25, SPSS Inc., Chicago, IL, United States of America). The responses of ten apple cultivars to temperature were classified as tolerant, moderately tolerant, moderately susceptible, and susceptible based on maximum pollen germination percentage, maximum pollen tube growth, and cardinal temperatures (minimum, optimum, and maximum) for pollen germination and pollen tube growth.

RESULTS

Pollen germination percentage

The pollen germination percentage was evaluated 4 h after inoculation. The results showed that the maximum pollen germination percentage differed considerably among the ten cultivars and their responses to temperature (Table 2 and Fig. 1). The mean value of pollen germination percentage was 85.2%; "Shinano Gold" (99.9%) had the highest and "Green Ball" (61.5%) had the lowest percentage. Moreover, distinct genotypic responses to temperature treatments were also observed for the ten apple cultivars evaluated in this study (Fig. 1). Both low and high temperatures reduced pollen germination. In this study, no pollen grain germination was observed at 5 and 45 °C in any of the ten apple cultivars studied. The cultivars with maximum pollen germination percentages were "Shinano Gold" (99.9%), "Arisoo" (99.8%), "Fuji" (94.8%), "Hongro" (86.4%), "Tsugaru" (85.2%), "Summer King" (84%), "Gamhong" (77.8%), "Honggeum" (64.8%), and "Green Ball" (61.5%). When comparing cultivars, "Shinano Gold" had the highest pollen germination percentage under lower temperatures, i.e., 10 (58.8%) and 15°C (70.6%) (Suppl. Table 1), indicative of its adaptability to low temperatures during blooming.

Table 2. Maximum pollen germination percentages, modified bilinear equation constants, and cardinal temperatures of ten apple cultivars.

Cultivars	PGP_{max}	a	b1	b2	R^2	$PGPT_{min}$	$PGPT_{opt}$	$PGPT_{max}$
Arisoo	99.80	112.3	-0.9867	-5.5267	88.62	3.01	27.75	44.99
Fuji	94.80	107.7	-0.9183	-5.3583	92.43	3.69	27.95	45.11
Gamhong	77.79	83.617	-0.9283	-4.5617	92.89	5.93	28.94	44.17
Green Ball	61.50	68.4078	-0.2517	-3.6683	87.36	7.00	27.02	44.47
Hongro	86.41	97.302	-1.1417	-4.8417	86.74	1.88	28.18	44.44
Honggeum	64.84	72.0974	-0.2567	-4.0933	83.20	8.10	26.89	43.46
Shinano Gold	99.93	110.3	-0.7533	-4.74	78.75	0.07	27.60	47.68
Summer King	84.04	86.9182	0.2167	-4.1567	95.54	4.40	24.27	46.33
Tsugaru	85.22	89.3761	-2.0543	-5.1124	90.33	2.13	31.36	43.83
Picnic	97.64	107.8	-0.7233	-5.2767	89.41	3.56	27.23	45.20
Mean	85.20					3.96	27.72	44.97

PGP_{max}: maximum pollen germination; $PGPT_{min}$: minimum temperature for pollen germination; $PGPT_{opt}$: optimum temperature for pollen germination; $PGPT_{max}$: maximum temperature for pollen germination.

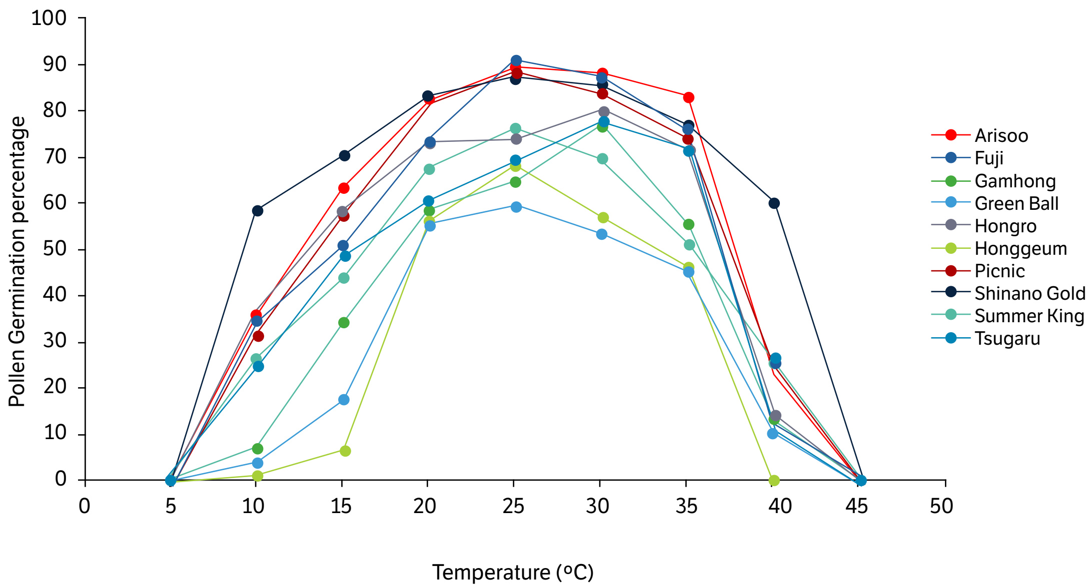

Figure 1. The influence of temperature on pollen germination percentage in vitro at 5, 10, 15, 20, 25, 30, 35, 40, and 45 °C in ten apple cultivars (the cultivars are represented by different colors).

Cardinal temperatures for pollen germination

The cardinal temperatures (T_{min}, T_{opt}, and T_{max}) for the maximum pollen germination were calculated using a modified bilinear model. Among the ten cultivars studied, there were considerable differences in the cardinal temperatures for pollen germination (Table 2). The average cardinal temperatures (minimum, optimum, and maximum) calculated were 4, 27.7, and 45 °C, respectively. The optimum temperature for pollen germination ranged from 24.3 ("Summer King") to 31.4 °C ("Tsugaru"). The minimum temperature for pollen germination ranged from 0.1 ("Shinano Gold") to 8.1 °C ("Honggeum"), whereas the maximum temperature ranged from 43.5 ("Honggeum") to 47.7 °C ("Shinano Gold"). "Shinano Gold" had the widest temperature range ($T_{max} - T_{min}$ = 47.7 °C), followed by "Hongro" ($T_{max} - T_{min}$ = 42.6 °C), indicating wider temperature adaptability. Moreover, "Honggeum" (35.4 °C) had the smallest temperature range, indicating narrow temperature adaptability for pollen germination.

Pollen tube growth

Similar to pollen germination, pollen tube growth was also assessed 4 h after inoculation. Considerable differences in pollen tube length were calculated among the cultivars (Table 3). Figure 2 illustrates the variation between the ten studied apple cultivars in response to temperature on pollen tube growth. The mean value of the pollen tube length was 855.1 μm; "Arisoo" (989.8 μm) and "Tsugaru" (716.5 μm) showed the maximum and minimum values, respectively. Furthermore, different genotypic responses to temperature treatments were also observed in the ten apple cultivars (Fig. 2). Both high and low temperatures slowed the development of pollen tubes (Suppl. Table 2). In this investigation, none of the ten apple cultivars evaluated exhibited any pollen tube development at temperatures of 5 and 45 °C. The cultivars with maximum pollen tube growth were "Arisoo" (989.8 μm), "Picnic" (976.9 μm), "Summer King" (783 μm), "Fuji" (911.5 μm), "Shinano Gold" (890.3 μm), "Hongro" (876.3 μm), "Gamhong" (781 μm), "Honggeum" (737.1 μm), "Green Ball" (728.7 μm) and "Tsugaru" (716.5 μm). "Green Ball" had the highest optimum temperature for pollen tube growth (31.3 °C), whereas "Summer King" had the lowest optimum temperature (26.5 °C). However, the highest maximum pollen tube length was calculated at 30 (989.8 μm) and 27.1 °C (976.9 μm) for the cultivars "Arisoo" and "Picnic", respectively.

Table 3. Maximum pollen tube length, modified bilinear equation constants, and cardinal temperatures of ten apple cultivars.

Cultivars	PTL_{max} (μm)	a	b1	b2	R^2	$PTLT_{min}$	$PTLT_{opt}$	$PTLT_{max}$
Arisoo	989.80	989.8	-15.0994	-56.7437	92.85	6.23	30.00	43.78
Fuji	911.55	989.3	-11.9174	-55.6914	92.08	6.25	28.85	43.48
Gamhong	781.02	886.5	-8.4412	-50.156	90.75	6.95	28.20	43.33
Green Ball	728.73	768.6	-14.6591	-46.0477	85.78	6.78	31.27	43.93
Hongro	876.35	932.8	-13.2177	-52.4159	90.91	5.34	29.14	43.35
Honggeum	737.14	826.7	-7.3185	-47.9668	78.93	8.04	28.38	43.33
Shinano Gold	890.28	980.9	-10.6299	-52.3003	96.43	5.02	28.56	44.15
Summer King	942.40	1028.5	-2.8862	-55.2874	92.88	6.85	26.48	44.16
Tsugaru	716.54	814.4	-7.9154	-43.3224	92.69	5.09	28.09	43.98
Picnic	976.99	1084.7	-7.4627	-59.496	92.27	6.22	27.07	43.27
Mean	855.08					6.27	28.60	43.67

PTL_{max}: maximum pollen tube length; $PTLT_{min}$: minimum temperature for pollen tube growth; $PTLT_{opt}$: optimum temperature for pollen tube growth; $PTLT_{max}$: maximum temperature for pollen tube growth.

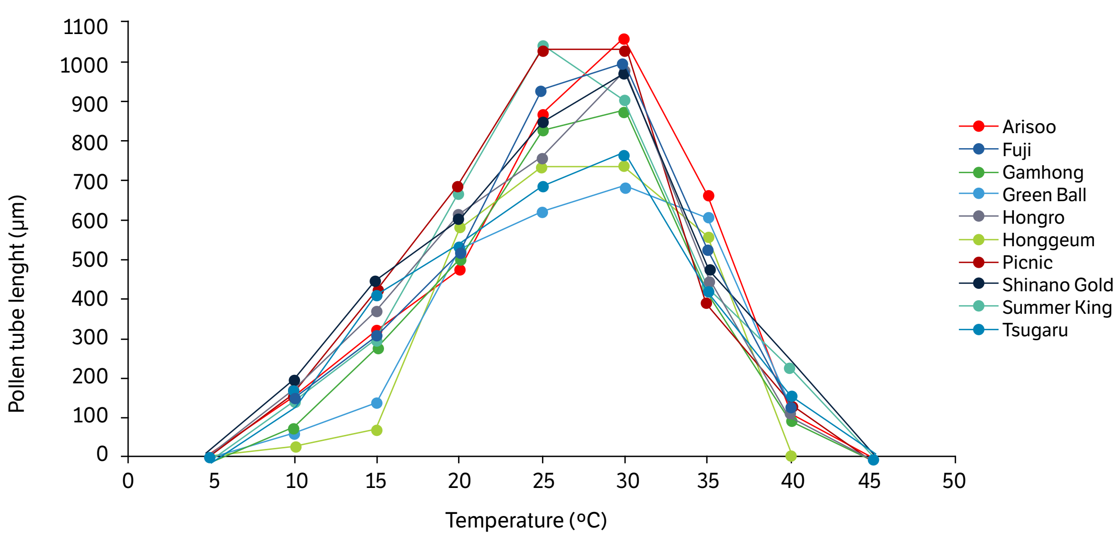

Figure 2. The influence of temperature on pollen tube length in vitro at 5, 10, 15, 20, 25, 30, 35, 40, and 45 °C in ten apple cultivars (the cultivars are represented by different colors).

Cardinal temperatures for pollen tube growth

A modified bilinear model was used to determine the cardinal temperature for pollen tube growth. The cardinal temperatures for pollen tube growth varied considerably among the evaluated cultivars (Table 3). The average cardinal temperatures (minimum, optimum, and maximum) for pollen tube length were 6.3, 28.6, and 43.7 °C. The optimum temperature for pollen tube growth ranged from 26.5 ("Summer King") to 31.3 °C ("Green Ball"). The minimum temperature for pollen tube growth ranged from 5 ("Shinano Gold") to 8 °C ("Gamhong"), whereas the maximum temperature ranged from 43.3 ("Picnic") to 44.2 °C ("Arisoo"). The widest temperature range was calculated for "Shinano Gold" ($T_{max} - T_{min}$ = 38.3 °C) followed by "Tsugaru" (38.2 °C), indicating wider temperature tolerance. However, "Honggeum" (36.3 °C) exhibited the narrowest temperature range, indicating a limited tolerance to temperature changes for pollen tube development.

Assessment of low- and high-temperature tolerant cultivars using principal component analysis

PCA was used to identify the key pollen parameters in this investigation. Therefore, eight PCAs were generated using values of maximum pollen germination percentage, pollen tube growth, and the cardinal temperatures of both maximum pollen germination percentage and pollen tube growth. Among the eight principal component vectors generated, three (PC1, PC2, and PC3) had eigenvalues > 1 (Table 4). These three PCs contributed 85.94% of the total variation among the cultivars assessed for pollen parameters in response to temperature. To identify cold tolerant cultivars, PC1 and PC3 were used because in PC1 all the parameters showed positive factors loading, except T_{min}, which showed highly negative factor loading for both pollen germination and pollen tube growth. As a result, cultivars with high values for PC1 tend to have low values of T_{min} and high values of maximum pollen germination percentage (PGP_{max}) and maximum pollen tube length (PTL_{max}). In PC3, T_{max} for both pollen germination percentage and pollen tube growth showed the highest positive factor loadings, whereas PGP_{max} and PTL_{max} showed negative factor loadings. From this, as Tmax decreased, both PGP_{max} and PTL_{max} increased. Consequently, the scores of PC1 and PC3 showed a greater inclination towards the separation of cultivars for low-temperature tolerance because, as both T_{min} in PC1 and T_{max} in PC3 decreased, both pollen germination percentage and pollen tube length increased.

Table 4. Principal component (PC) analysis eigenvectors of PC1, PC2, and PC3 of ten apple cultivars for maximum pollen germination (PGPmax), maximum pollen tube length (PTLmax), and their respective cardinal temperatures (Tmin, Topt and Tmax), and the variation accounted for by each eigenvector

Parameter	PC1	PC2	PC3
PGP_{max}	0.906	-0.026	-0.348
$PGPT_{min}$	-0.925	0.344	0.021
$PGPT_{opt}$	-0.014	-0.945	-0.222
$PGPT_{max}$	0.798	0.390	0.343
PTL_{max}	0.704	0.494	-0.331
$PTLT_{min}$	-0.767	0.596	-0.074
$PTLT_{opt}$	-0.285	-0.410	0.417
$PTLT_{max}$	0.419	0.023	0.843
Variation	45.68	24.17	16.09

$PGPT_{min}$: minimum temperature for pollen germination; $PGPT_{opt}$: optimum temperature for pollen germination; $PGPT_{max}$: maximum temperature for pollen germination; $PTLT_{min}$: minimum temperature for pollen tube growth; $PTLT_{opt}$: optimum temperature for pollen tube growth; $PTLT_{max}$: maximum temperature for pollen tube growth.

To identify high-temperature tolerant cultivars, PC1 and PC2 were used because T_{max} showed positive factor loading on pollen germination percentage and pollen tube length in PC1. As T_{max} increased, both pollen germination percentage and pollen tube length increased because both pollen germination percentage and pollen tube length had positive relationships with T_{max}. In PC2, most of the highest contributor parameters showed positive factor loadings except T_{opt}. Accordingly, PC1 and PC2 scores showed a more pronounced tendency to separate cultivars for high-temperature tolerance.

Based on the relative positions, in the quadrants of the PCs, two distinct classifications were generated, one for low-temperature tolerance and the other one for high-temperature tolerance. The ten apple cultivars were classified into four groups cold-tolerant, moderately cold-tolerant, moderately cold-susceptible, and cold-susceptible. Similarly, the categorization for high-temperature tolerance was high-temperature tolerant, moderately high-temperature tolerant, moderately high-temperature susceptible, and high-temperature susceptible based on the PCs. The classifications were conducted using the maximum pollen germination percentage, maximum pollen tube growth, and cardinal temperature (minimum, optimum, and maximum) of pollen tube growth and pollen germination percentage. Accordingly, "Arisoo," "Summer King," "Fuji" and "Picnic" were tolerant to high temperature, whereas "Gamhong" and "Greenball" were susceptible to high temperature (Table 5 and Fig. 3). Moreover, "Shinano Gold," "Tsugaru" and "Summer King" cultivars could tolerate low temperatures, whereas "Honggeum" and "Gamhong" were susceptible (Table 6 and Fig. 4).

Table 5. Classification of apple cultivars based on principal components (PC) 1 and 2 for high-temperature tolerance.

Tolerant (+PC1 and +PC2)	Moderately tolerant (+PC1 and -PC2)	Moderately susceptible (-PC1 and +PC2)	Susceptible (-PC1 and -PC2)
Summer King	Shinano Gold	Honggeum	Green Ball
Picnic	Hongro		Gamhong
Arisoo	Tsugaru		
Fuji			

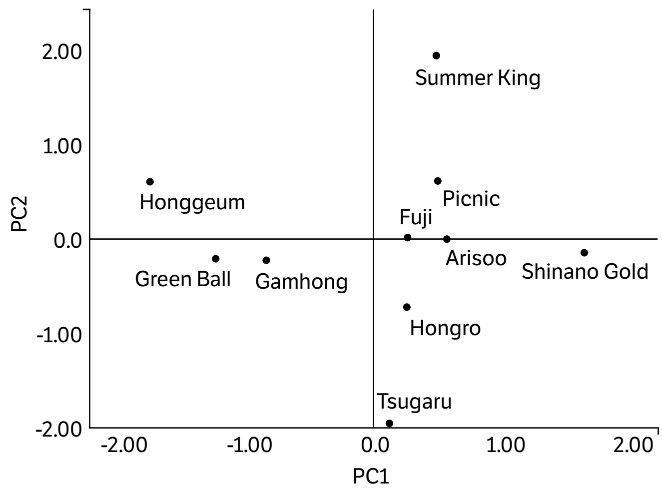

Figure 3. Principal component analysis (PCA) biplot for the first two principal components (PC) scores, PC1 vs. PC2, related to the classification of ten apple cultivars for high-temperature tolerance.

Table 6. Classification of apple cultivars based on principal components (PC) 1 and 3 for low-temperature tolerance.

Tolerant (+PC1 and +PC3)	Moderately tolerant (+PC1 and -PC3)	Moderately susceptible (-PC1 and +PC3)	Susceptible (-PC1 and --PC3)
Summer King	Fuji	Green Ball	Honggeum
Shinano Gold	Arisoo		Gamhong
Tsugaru	Hongro		
	Picnic		

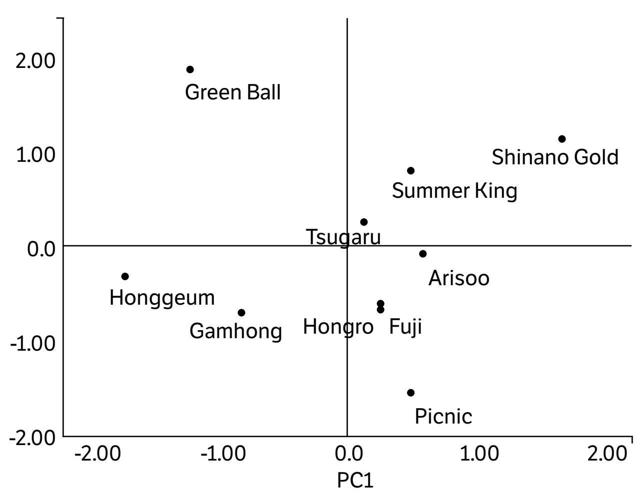

Figure 4. Principal component analysis (PCA) biplot for principal components (PC) scores of PC1 vs. PC3, related to the classification of ten apple cultivars for low-temperature tolerance.

DISCUSSION

In this study, the effects of temperature on pollen germination and pollen tube growth were studied in ten apple cultivars *in vitro*. We found that the pollen germination percentage and tube length differed among the cultivars studied. The lowest and highest temperatures prevented the germination and tube growth of the pollen in the apple cultivars used in this study. Extreme temperatures have a greater impact on the plant reproductive processes that lead to the formation of flowers and seeds (Yang et al. 2019). A crucial element in getting the sperm cell to the ovary is the pollen grain's potential to germinate and develop pollen tubes.

The effects of temperature on pollen germination and tube length have been previously reported in argan, wheat, and mango (Zahidi et al. 2017, Bheemanahalli et al. 2019, Pérez et al. 2019). As reported by Shi et al. (2018), low pollen germination percentage and tube length are highly correlated with lower fruit sets. These results strongly indicate that fruit production in apples used in this study can also be vulnerable to low- or high-temperature conditions during pollination periods. The temperature surrounding the plant determines its rate of growth and development. Each plant species has a defined temperature range represented by a minimum, maximum, and optimum (Hatfield et al. 2011).

In this study, the modified bilinear model best describes the response of pollen germination and pollen tube growth to temperature. It led to the determination of the cardinal temperatures (T_{min}, T_{opt}, and T_{max}) for pollen germination and pollen tube length. These results also indicate a considerable difference in the pollen germination percentage and tube growth depending on cultivars. Pollen germination and pollen tube growth were significantly reduced above and below the optimum temperature. This is because a slight temperature variation from well-suited to a certain range, even for a short time, can change the biochemical activities of the pollen grains (Djanaguiraman et al. 2018, Hebbar et al. 2018). The observed differences in cardinal temperatures among the apple cultivars were consistent with findings reported for almond (Ranasinghe et al. 2018), shrub rose (Bheemanahalli et al. 2021), and coconut (Hebbar et al. 2018). The variation in pollen germination percentage and tube growth length may have been due to cultivar variability. A difference in the pollen protein and carbohydrate content can result in genetic variation among cultivars because pollen protein and carbohydrates are detrimental factors for pollen germination and pollen tube growth (Djanaguiraman et al. 2018, Hebbar et al. 2018).

PCA is regarded as an efficient statistical method for highlighting similarities and contrasts in multidimensional data. Cardinal temperatures, pollen germination, and pollen tube length have been used earlier to successfully categorize, tolerant and susceptible genotypes in canola (Singh et al. 2008) and coconut (Ranasinghe et al. 2018). The cardinal temperatures for pollen germination and pollen tube length, as well as pollen germination and pollen tube development, were employed in the current study as screening tools to distinguish apple cultivars that could resist extremely high and low temperatures during the time of flowering from those that could not. As a result, we found that "Shinano Gold," "Summer King" and "Tsugaru" were low-temperature tolerant, whereas "Gamhong" and "Honggeum" were susceptible to low temperatures. Moreover, "Summer King," "Arisoo," "Fuji" and "Picnic" cultivars were tolerant to high temperatures. Importantly, because of temperature changes (low and high) throughout the pollination period, the identified low- and high-temperature-tolerant apple cultivars can be used for sustainable apple production in Korea. Additionally, apple cultivars with pollen that can withstand low and high temperatures can be utilized to enhance low and high-temperature vulnerable cultivars. However, there may be a variety of mechanisms by which plants can withstand both low and high temperatures. Hence, studies are needed to understand the mechanisms of cultivar tolerance to low and high temperatures using biochemical and molecular methods, so that suitable cultivars can be developed and identified for the current and future climates.

CONCLUSION

The ten apple cultivars evaluated in this study showed different *in-vitro* levels of pollen germination and pollen tube growth at different temperatures. In addition, the minimum, maximum and optimum temperatures for each cultivar were identified using a modified bilinear model. Furthermore, low and high-temperature tolerant apple cultivars were selected based on the temperature response of their pollen. Accordingly, “Shinano Gold,” “Summer King” and “Tsugaru” were low-temperature tolerant cultivars, whereas “Summer King,” “Arisoo,” “Fuji,” and “Picnic” cultivars were high-temperature tolerant cultivars.

It is expected that the use of these apple cultivars will reduce production losses associated with low and high temperatures during pollination time in Korea. In addition, they may be used to develop cultivars that are resistant to cold and heat during flowering.

AUTHORS’ CONTRIBUTION

Conceptualization: Zebro, M. and Heo, J. Y.; **Methodology:** Zebro, M. and Heo, J. Y.; **Investigation:** Zebro, M.; **Data analysis:** Kang, J. S. and Zebro, M.; **Writing – Original Draft:** Zebro, M.; **Writing – Review and Editing:** Heo, J. Y.; **Supervision:** Heo, J. Y.

DATA AVAILABILITY STATEMENT

We will be able to provide data upon request.

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Not applicable.

REFERENCES

Alvarez, H. C., Salazar-Gutiérrez, M., Chaves, B. and Hoogenboom, G. (2018). Modeling pollen tube growth of ‘Gala’and ‘Fuji’apples. Scientia Horticulturae, 240, 125-132. https://doi.org/10.1016/j.scienta.2018.05.032

Baidya, A., Pal, A. K., Ali, M. A. and Nath, R. (2021). High-temperature stress and the fate of pollen germination and yield in lentil (Lens culinaris Medikus). Indian Journal of Agricultural Research, 55, 144-150. https://doi.org/10.18805/IJARe.A-5440

Benlloch-González, M., Sánchez-Lucas, R., Benlloch, M. and Ricardo, F. E. (2018). An approach to global warming effects on flowering and fruit set of olive trees growing under field conditions. Scientia Horticulturae, 240, 405-410. https://doi.org/10.1016/j.scienta.2018.06.054

Bheemanahalli, R., Gajanayake, B., Lokhande, S., Singh, K., Seepaul, R., Collins, P. and Reddy, K. R. (2021). Physiological and pollen-based screening of shrub roses for hot and drought environments. Scientia Horticulturae, 282, 110062. https://doi.org/10.1016/j.scienta.2021.110062

Bheemanahalli, R., Sunoj, V. S. J., Saripalli, G., Prasad, P. V. V., Balyan, H. S., Gupta, P. K., Grant, N., Gill, K. S. and Jagadish, S. V. K. (2019). Quantifying the impact of heat stress on pollen germination, seed set, and grain filling in spring wheat. Crop Science, 59, 684-696. https://doi.org/10.2135/cropsci2018.05.0292

Cetinbas-Genç, A., Cai, G., Vardar, F. and Ünal, M. (2019). Differential effects of low and high temperature stress on pollen germination and tube length of hazelnut (Corylus avellana L.) genotypes. Scientia Horticulturae, 255, 61-69. https://doi.org/10.1016/j.scienta.2019.05.024

Cornille, A., Giraud, T., Smulders, M. J., Roldán-Ruiz, I. and Gladieux, P. (2014). The domestication and evolutionary ecology of apples. Trends in Genetics, 30, 57-65. https://doi.org/10.1016/j.tig.2013.10.002

Díez-Palet, I., Funes, I., Savé, R., Biel, C., Herralde, F. de, Miarnau, X., Vargas, F., Avila, G., Carbo, J. and Aranda, X. (2019). Blooming under Mediterranean climate: Estimating cultivar-specific chill and heat requirements of almond and apple trees using a statistical approach. Agronomy, 9, 760. https://doi.org/10.3390/agronomy9110760

Djanaguiraman, M., Perumal, R., Ciampitti, I. A., Gupta, S. K. and Prasad, P. V. V. (2018). Quantifying pearl millet response to high-temperature stress: thresholds, sensitive stages, genetic variability and relative sensitivity of pollen and pistil. Plant, Cell & Environment, 41, 993-1007. https://doi.org/10.1111/pce.12931

Geleta, B. T., Lee, J.-C. and Heo, J.-Y. (2023). antioxidant activity and mineral content in unripe fruits of 10 apple cultivars growing in the northern part of Korea. Horticulturae, 9, 114. https://doi.org/10.3390/horticulturae9010114

Granato, D., Santos, J. S., Escher, G. B., Ferreira, B. L. and Maggio, R. M. (2018). Use of principal component analysis (PCA) and hierarchical cluster analysis (HCA) for multivariate association between bioactive compounds and functional properties in foods. Trends in Food Science and Technology, 72, 83-90. https://doi.org/10.1016/j.tifs.2017.12.006

Guo, L., Wang, J., Li, M., Liu, L., Xu, J. and Cheng, J. (2019). Distribution margins as natural laboratories to infer species' flowering responses to climate warming and implications for frost risk. Agricultural and Forest Meteorology, 268, 299-307. https://doi.org/10.1016/j.agrformet.2019.01.038

Hatfield, J. L., Boote, K. J., Kimball, B. A., Ziska, L. H., Izaurralde, R. C., Ort, D., Thomson, A. M. and Wolfe, D. (2011). Climate impacts on agriculture: implications for crop production. Agronomy Journal, 103, 351-370. https://doi.org/10.2134/agronj2010.0303

Hebbar, K. B., Rose, H. M., Nair, A. R., Kannan, S., Niral, V., Arivalagan, M., Gupta, A., Samsudeen, K., Chandran, K. P., Chowdappa, P. and Prasad, P. V. (2018). Differences in in vitro pollen germination and pollen tube growth of coconut (Cocos nucifera L.) cultivars in response to high-temperature stress. Environmental and Experimental Botany, 153, 35-44. https://doi.org/10.1016/j.envexpbot.2018.04.014

Jeong, J. H., Han, J. H., Ryu, S., Cho, J. G. and Lee, S.-K. (2021). Analysis of Freezing Injury Rate, Hormone and Soluble Sugars between 'Fuji' and 'Hongro' Apple Trees in Flowering Period [Internet]. Vol. 30, Journal of Bio-Environment Control. The Korean Society for Bio-Environment Control, 30, 320-327. https://doi.org/10.12791/ksbec.2021.30.4.320

Kakani, V. G., Prasad, P. V. V., Craufurd, P. Q. and Wheeler, T. R. (2002). Response of in vitro pollen germination and pollen tube growth of groundnut (Arachis hypogaea L.) genotypes to temperature. Plant, Cell & Environment, 25, 1651-1661. https://doi.org/10.1046/j.1365-3040.2002.00943.x

Kakani, V. G., Reddy, K. R., Koti, S., Wallace, T. P., Prasad, P. V. V., Reddy, V. R. and Zhao, D. (2005). Differences in in-vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. Annals of Botany, 96, 59-67. https://doi.org/10.1093/aob/mci149

[KSIS] Korean Statistical Information Service. (2022). Census of agriculture, forestry, and fisheries. Seoul, Korea: KSIS.

Milatovic, D. and Nikolic, D. (2017). The effect of temperature on pollen germination and pollen tube growth in vitro of sweet cherry cultivars. International Society for Horticultural Science, 1161, 401-404. https://doi.org/10.17660/ActaHortic.2017.1161.64

O'Rourke, D. (2021). Economic Importance of the World Apple Industry. In S. S. Korban (Ed.). The Apple Genome (p. 1-18). Switzerland: Springer Nature. https://doi.org/10.1007/978-3-030-74682-7_1

Pérez, V., Herrero, M. and Hormaza, J. I. (2019). Pollen performance in mango (Mangifera indica L., Anacardiaceae): Andromonoecy and effect of temperature. Scientia Horticulturae, 253, 439-446. https://doi.org/10.1016/j.scienta.2019.04.070

Radović, A., Nikolić, D., Cerović, R., Milatović, D., Rakonjac, V. and Bakić, I. (2019). The effect of temperature on pollen germination and pollen tube growth of quince cultivars. International Society for Horticultural Science, 1289, 67-72. https://doi.org/10.17660/ActaHortic.2020.1289.10

Ramírez, F. and Davenport, T. L. (2013). Apple pollination: a review. Scientia horticulturae, 162, 188-203. https://doi.org/10.1016/j.scienta.2013.08.007

Ranasinghe, C. S., Kumarathunge, M. D. P. and Kiriwandeniya, K. G. S. (2018). Genotypic differences in cardinal temperatures for in vitro pollen germination and pollen tube growth of coconut hybrids. Experimental Agriculture, 54, 731-743. https://doi.org/10.1017/S0014479717000357

Shenoda, J. E., Sanad, M. N., Rizkalla, A. A., El-Assal, S., Ali, R. T. and Hussein, M. H. (2021). Effect of long-term heat stress on grain yield, pollen grain viability and germinability in bread wheat (Triticum aestivum L.) under field conditions. Heliyon, 7, e07096. https://doi.org/10.1016/j.heliyon.2021.e07096

Shi, W., Li, X., Schmidt, R. C., Struik, P. C., Yin, X. and Jagadish, S. K. (2018). Pollen germination and in vivo fertilization in response to high-temperature during flowering in hybrid and inbred rice. Plant, Cell and Environment, 41, 1287-1297. https://doi.org/10.1111/pce.13146

Singh, S. K., Kakani, V. G., Brand, D., Baldwin, B. and Reddy, K. R. (2008). Assessment of cold and heat tolerance of winter-grown canola (Brassica napus L.) cultivars by pollen-based parameters. Journal of Agronomy and Crop Science, 194, 225-236. https://doi.org/10.1111/j.1439-037X.2008.00309.x

Sorkheh, K., Azimkhani, R., Mehri, N., Chaleshtori, M. H., Halász, J., Ercisli, S. and Koubouris, G. C. (2017). Interactive effects of temperature and genotype on almond (Prunus dulcis L.) pollen germination and tube length. Scientia Horticulturae, 227, 162-168. https://doi.org/10.1016/j.scienta.2017.09.037

Thuy, T. L., Lee, C. K., Jeong, J. H., Lee, H. S., Yang, S. Y., Im, Y. H. and Hwang, W. H. (2020). Impact of heat stress on pollen fertility rate at the flowering stage in korean rice (Oryza sativa L.) cultivars. Korean Journal of Crop Science, 65, 22-29. https://doi.org/10.7740/kjcs.2020.65.1.022

Tolessa, K. and Heuvelink, E. P. (2018). Pollen viability and fruit set of tomato introgression lines (Solanum esculentum x L. chmielewskii) at moderately high temperature regimes. World Applied Sciences Journal, 36, 29-38. https://doi.org/10.5829/idosi.wasj.2018.29.38

Vijayakumar, A., Shaji, S.., Beena, R, Sarada, S., Sajitha, Rani, T., Stephen, R., Manju, R. V. and Viji, M. M. (2021). High temperature induced changes in quality and yield parameters of tomato (Solanum lycopersicum L.) and similarity coefficients among genotypes using SSR markers. Heliyon, 7, e05988. https://doi.org/10.1016/j.heliyon.2021.e05988

Yang, Q., Liu, E., Fu, Y., Yuan, F., Zhang, T. and Peng, S. (2019). High temperatures during flowering reduce fruit set in rabbit eye blueberry. Journal of the American Society for Horticultural Science, 144, 339-351. https://doi.org/10.21273/JASHS04650-19

Yun, E., Kim, J. H. and Moon, K. H. (2020). Future projection of climatic zone shifts over Korean Peninsula under the RCP8. 5 scenario using high-definition digital agro-climate maps. Korean Journal of Agricultural and Forest Meteorology, 22, 287-298. https://doi.org/10.5532/KJAFM.2020.22.4.287

Zahidi, A., Benlahbil, S., Bani-Aameur, F. and El Mousadik, A. (2017). The effects of temperature and humidity on in vitro pollen germination of Argania spinosa (L.) Skeels. Morocan Journal of Biology, 14, 36-46.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Pollen germination percentages of ten apple cultivars at each temperature level with standard deviation among the replicates

Cultivars	Temperatures						
	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C
Arisoo	35.6 ± 6.12	63.6 ± 6.18	83.0 ± 1.67	89.8 ± 1.94	88.5 ± 4.41	83.5 ± 5.24	23.3 ± 5.08
Fuji	34.5 ± 4.96	51.3 ± 2.73	73.8 ± 5.52	91.3 ± 3.98	88.0 ± 2.36	76.5 ± 6.97	26.6 ± 6.68
Gamhong	7.0 ± 3.89	34.2 ± 2.85	58.6 ± 3.77	65.0 ± 5.36	77.3 ± 6.88	55.8 ± 7.98	13.3 ± 4.88
Green Ball	4.0 ± 2.28	17.3 ± 7.89	55.8 ± 9.53	59.5 ± 4.32	53.6 ± 4.80	45.3 ± 8.54	10.3 ± 4.88
Hongro	36.6 ± 7.42	58.5 ± 3.88	73.3 ± 3.98	74.2 ± 4.07	80.3 ± 4.76	72.0 ± 5.32	13.8 ± 6.33
Honggeum	1.2 ± 0.40	6.5 ± 1.37	56.3 ± 4.32	68.3 ± 2.06	57.2 ± 2.40	46.3 ± 5.71	0
Picnic	31.6 ± 4.71	57.8 ± 5.03	82.6 ± 4.84	88.3 ± 3.77	84.0 ± 2.28	74.3 ± 7.14	26.3 ± 7.36
Shinano Gold	58.8 ± 3.60	70.6 ± 1.86	83.5 ± 5.31	87.3 ± 5.71	86.0 ± 3.16	77.2 ± 4.40	60.5 ± 8.96
Summer King	29.5 ± 6.41	44.2 ± 6.36	68.0 ± 2.82	76.3 ± 3.32	70.0 ± 3.28	51.3 ± 7.20	25.6 ± 7.06
Tsugaru	24.8 ± 4.16	48.8 ± 4.02	60.8 ± 5.42	69.5 ± 6.28	77.8 ± 2.14	71.6 ± 5.27	10.8 ± 2.14

Supplementary Table 2. Pollen tube growth of ten apple cultivars at each temperature level with standard deviation among the replicates

Cultivars	Temperatures						
	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C
Arisoo	156.2 ± 6.85	312.5 ± 6.02	475.4 ± 8.96	863.8 ± 8.30	1057.5 ± 8.96	659.5 ± 4.71	112.7 ± 3.77
Fuji	173.0 ± 5.10	306.9 ± 8.93	506.9 ± 3.55	927.3 ± 3.81	989.6 ± 3.68	520.3 ± 7.00	108.7 ± 5.23
Gamhong	71.0 ± 6.46	278.2 ± 1.88	500.7 ± 7.80	827.9 ± 3.52	876.8 ± 7.27	394.8 ± 7.51	95.5 ± 5.54
Green Ball	57.3 ± 7.81	135.7 ± 9.64	521.4 ± 5.70	621.4 ± 2.45	683.1 ± 8.71	607.1 ± 3.00	108.0 ± 5.43
Hongro	159.6 ± 4.02	367.8 ± 7.80	611.5 ± 8.50	753.9 ± 4.17	977.3 ± 4.08	454.0 ± 8.49	104.3 ± 6.37
Honggeum	24.2 ± 3.68	67.4 ± 6.42	582.8 ± 7.51	736.8 ± 5.73	735.5 ± 9.15	557.4 ± 4.67	0
Picnic	144.7 ± 7.00	424.4 ± 5.88	681.2 ± 8.27	1032.5 ± 6.97	1029.7 ± 4.73	387.2 ± 3.18	128.4 ± 5.85
Shinano Gold	194.8 ± 6.37	445.1 ± 2.70	598.9 ± 3.03	839.7 ± 3.44	968.9 ± 7.18	472.2 ± 4.89	232.6 ± 4.41
Summer King	129.8 ± 5.99	293.8 ± 5.85	670.4 ± 5.24	1039.7 ± 4.21	901.7 ± 2.65	426.2 ± 3.66	222.5 ± 3.65
Tsugaru	129.6 ± 3.09	408.0 ± 4.89	531.2 ± 7.32	684.3 ± 4.87	766.6 ± 5.30	412.8 ± 3.37	150.9 ± 6.16