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Effects of Short Term Pollen Storage on Fruit Set, Seed Number and Seed Germination Rate in Cut Rose Hybridization

Gülşah TURNA[1], Soner KAZAZ[1], Tuğba KILIÇ[2*]

Highlights:

- Seed set in roses is key to hybridization success
- There is a positive correlation between pollen germination rate and seed set
- Roses' pollen can be used for up to 3 days by keeping it at +4°C

Keywords:

- Hybridization
- Cut flower
- Pollen germination
- Seed set
- IKI

ABSTRACT:

Roses are known for their low pollen quality. It remains unclear how many days of pollen should be used in order not to reduce crossbreeding success. The study was conducted to determine whether short-term pollen storage is effective on the crossing success of roses comparatively in vivo and in vitro. The study was conducted in a greenhouse and a cytology laboratory at Ankara University, Turkey. The 'Avalanche' rose variety was used as the seed parent, and 'Magnum' was the pollen parent. A total of 210 crosses were made. Pollens were used immediately (day 0) and stored at +4°C for 6 days after collection. The pollen viability by IKI and the germination rates by the petri dish method, the fruit and seed set, the fruit and seed weight, and the seed germination rate were recorded. All examined traits showed a decreasing trend as the storage time increases. The pollen germination rates were recorded at 1.63% for stored pollen at six days and 15.27% for fresh pollen. The highest fruit and seed set rates were obtained from crossing with pollen stored for one day. The fruit set decreased below 10% as of the 5th day, and the seed numbers decreased 1.8 times compared to one day of storage. No germination was observed in seeds obtained from crosses with pollen stored for 5 or 6 days. A positive correlation was found between seed number and pollen germination rate. The study concluded that successful crossing could be made with pollen stored at +4°C for up to 3 days.

[1]Gülşah TURNA (**Orcid ID:** 0000-0002-4297-1070), Soner KAZAZ (**Orcid ID:** 0000-0002-6644-9690), Ankara Üniversitesi, Ziraat Fakültesi Bahçe Bitkileri Bölümü, Ankara, Türkiye
[2]Tuğba KILIÇ (**Orcid ID:** 0000-0002-0528-7552), Yozgat Bozok Üniversitesi, Ziraat Fakültesi Bahçe Bitkileri Bölümü, Yozgat, Türkiye

***Sorumlu Yazar/*Corresponding Author*:** Tuğba KILIÇ, e-mail: tuğba.kilic@yobu..edu.tr

Bu çalışma Gülşah TURNA'nın Yüksek Lisans tezinden üretilmiştir.

INTRODUCTION

The global cut flower industry shows rapid change every year in line with consumer and market demands. Cut flower breeding companies, which want to maintain their place in the industry, constantly offer different colors, shapes, and types, high yield and quality cultivars to the market (Kılıç, 2020). Until today, more than 37.000 cultivars have been developed and brought to the market by breeding studies in roses, which are one of the most popular cut flower species traded in the world (Anonymous, 2019).

Crossbreeding is the most commonly utilized breeding technique in developing new rose cultivars. However, low seed number per fruit and low seed germination rate are two of the most common challenges in cut rose hybridization, and modern roses are frequently known to have low fertility (Pipino et al., 2011; Bosco et al., 2015). Incompatibility, meiotic abnormalities, heterozygous polyploid parents, and the accumulation of deleterious recessive alleles are the causes of poor fertility (Nadeem et al., 2013). Thus, parental selection is critical for a successful crossbreeding program (Zlesak, 2007). The success rate in pollination and fertilization can be increased by using pollen with high viability and germination rates (Farooq et al. 2016).

The quality of pollen, which indicates its viability and germination rate, varies according to the genotype and is highly affected by climatic conditions. The viable pollen rate at room temperature is initially high on the first day and then gradually decreases in the following days (Giovannini et al., 2017). Visser et al. (1977) reported that the rate of fruit set rate decreased with pollen stored at room temperature for more than three days in some rose species, while some breeders stated they used the rose pollen for up to seven days (Gülbağ et al., 2021). Considering that repeated pollination is typically performed within 1-2 days or up to 5 days immediately after emasculation in rose breeding programs, it is essential to know the effective duration for using pollen parent. In addition, pollen must be preserved to enable controlled pollination between rose species and varieties blooming at different times or among cross combinations where geographical distance is a consideration. For these reasons, pollen holding time and pollen storage are critical factors affecting success in rose breeding. It is believed that rose pollen stored for a short time can be used more effectively and for longer periods than pollen kept at room conditions in terms of fruit and seed set rates. However, it is not known how many days the pollen can be used effectively with short-term storage. This study investigated, using both in-vivo and in-vitro methods, how long pollen stored at +4°C and fresh pollen for six days can be used in crossing studies.

MATERIALS AND METHODS

Cross-pollinations were carried out in the greenhouse at Ankara University in the Ankara province of Turkey (39°57'40.2"N 32°51'51.7"E). The determination of pollen viability and germination rates of the pollen parent was carried out in a cytology laboratory.

Plant Material

The 'Avalanche' and 'Magnum' cultivars belonging to *Rosa x hybrida* L. species were used as plant materials. 'Avalanche' was used as the seed parent (the stigma number is between 70 and 180) in the hybridization and the 'Magnum' was used as the pollen parent. Both of them are tetraploid by 2n=4x=28 chromosome numbers (Kılıç, 2020). The plants were grown in pots with coco peat as the growing medium. The greenhouse temperature was monitored and maintained between 23ºC and 30ºC, and the relative humidity was stabilized between 60% and 70% during the vegetation season. A heat-shade curtain with 55% shade was used to protect the plants from excessive light intensity. The drip

irrigation system was used for watering and nutrient supply, and the it was automatically regulated by a fertigation computer. The nutrient solutions' electrical conductivity (EC) given to the plants was 1.5-1.7 mS/cm in April and May, and 1.8-2.0 mS/cm between June and October. The pH of the solutions was kept between 5.5-5.8 during the vegetation period. Both pesticide and biological control were used against diseases and pests. However, no pesticides were applied to the plants between 7 days before and 15 days after crossing.

Methods

Cross-pollination and storage of pollen

Cross-pollination (hybridization) was performed from July 12 to July 18. First, the blooms of 'Avalanche' were emasculated, and the anthers of 'Magnum' were collected when 1/3 of the flowers were opened at 09.00-10.00 am. Then emasculated flowers were covered with a paper bag for one day (Crespel & Mouchotte, 2003; Chimonidou et al., 2007). For one day, the anthers were placed in an incubator at 24°C and 60% humidity to release pollen. Twenty-four hours after anthers were collected, pollen was divided into seven different glass bottles. Six of them were stored at +4°C in the fridge for six days. One of them was used immediately (control). One day after the emasculation of flowers, the fresh pollen (control) was rubbed onto the stigma by a brush and again covered with a paper bag for four days (De Vries & Dubois, 1983; Gudin, 2003). Crosses were continued for six days with pollen taken daily from the fridge, and stored pollen was rubbed onto the stigma of the seed parent emasculated the day before. The stored pollen was taken out of the fridge at one-day intervals and then kept for two hours in a climate cabinet with a temperature of 20-24°C and a relative humidity of 60-65%, and it was used in hybridization studies. The pollen viability and germination rates were recorded simultaneously. The detailed methods for assessing them are given under the section titled 'Evaluation of viable pollen and germinated pollen rate'. Hybridizations were only made in the flowers of the axillary buds. Both the apical buds and the flowers on the weakly growing shoots were not used for crosses.

The fruits were harvested between November 30 and December 2, 2020, when they reached harvest maturity (the fruit color changed from green to orange and red). The fruit set rate (%) was calculated by dividing the total number of hybridizations by the number of harvested fruits. The average fruit weight per hybridization that day was determined by weighing the fruits and expressed as grams (g). The seed number per fruit was recorded by removing seeds from the fruit, and the average seed weight, as expressed in milligrams (mg), was determined by weighing the seeds. The seeds were placed in storage in sealed bags containing moist perlite to determine the germination rate. After being subjected to moist stratification at 3±1°C for four months, the seeds sown in vials containing peat were considered to have germinated when hypocotyls were seen (Roy, 2010).

Evaluation of viable pollen and germinated pollen rate

The iodine potassium iodide (IKI), a chemical pollen viability method, was used to determine viable pollen rate under in vitro conditions, while the petri dish, a biological pollen viability method, was applied to determine germinated pollen rate.

The IKI method was modified according to Eti (1990). Counts were made under the microscope within 5 minutes after the samples was prepared. Pollen grains dyed black and dark brown were considered 'viable', pollen grains dyed orange, red, or light brown were considered 'semi-viable', and pollen grains dyed yellow or colorless were considered 'non-viable'. Theoretically, 50% of the pollen grains counted as semi-viable were accepted as viable.

The petri dish method was modified according to Imrak (2010) and Kılıç (2020). Germination media containing 20% sucrose, 10 ppm boric acid, and 1% agar solution were poured into plastic petri dishes with a thickness of 2 mm. The agar solution in petri dishes was divided into four separate areas, and pollen was sprinkled lightly on each area with the help of the brush. The preparation, which was incubated for 8 hours at 24ºC and 60% humidity, was taken from petri dishes and then counted with a microscope. Pollen grains that form a pollen tube longer than their diameter was considered germinated.

Both procedures used the Leica DM1000 version microscope and imaging system with x40 and x100 magnifying objectives for pollen grain count.

Experimental design and statistical evaluation

The hybridization and pollen quality experiments were established in a completely randomized design with three replicates. Ten crosses were made in each replication of the hybridization, and a total of 210 crosses were made during the seven days. Two coverslips were utilized in the pollen viability test, and four distinct areas on each coverslip were counted. In each petri dish, two slices were randomly selected, and pollen grains were counted in four distinct areas for the pollen germination test. On average, 350 pollen grains were counted per area using both techniques. IBM SPSS Statistics version 22.0 was used for the statistical analysis. The angularly converted data were subjected to an analysis of variance. Duncan's test revealed mean differences ($p \leq 0.05$). A correlation matrix was also created to ascertain the connection between the attributes investigated. For the estimation of the effects of storage time on fruit set, seed number per fruit, seed weight, fruit weight, seed germination rate and pollen germination rate, linear regression was performed.

RESULTS AND DISCUSSION

Analysis of variance showed that the fruit set rate, seed number per fruit, and seed germination rate differed statistically according to the time of pollen storage. However, the pollen storage times did not have a statistically significant effect on fruit weight and seed weight. Moreover, the pollen quality decreased as the storage time increased, and there was a statistically significant decrease in both in vitro conditions and in vivo conditions ($p \leq 0.05$).

Cross-pollination

The highest fruit set rate was obtained from hybridization with pollen stored for one day at 4ºC by 23.33%. There was no statistical difference between the fruit set rates of pollen stored for 1, 2, and 3 days at 4ºC and pollen stored for 0 days at 24ºC. The lowest fruit set rate was obtained from crossing pollen stored at 4ºC for 6, 5, and 4 days, respectively. Similar to the fruit set rate, the highest value of average seed number per fruit was recorded in pollen stored for one day at 4ºC (5.01), which was in the same statistical group as the values obtained from crossbreeding with pollen stored at 4ºC for 1, 2, and 3 days, as well as fresh pollen. The least number of seeds (1.00) was obtained from 6 days of storage at 4ºC, followed by storage at 4ºC for 5 and 4 days, respectively (Table 1).

The fruit weights varied between 1.26 g and 1.79 g, and the seed weights were between 120 mg and 260 mg in the combination of 'Avalanche' x 'Magnum'. The variation among the seed weights was statistically insignificant, as was the variation among the fruit weights. While there was no statistical difference in the seed germination rate between fresh pollen and the first four days of short-term storage, a significant difference emerged as of the 5^{th} day, and no germination was observed in the seeds obtained as a result of hybridization with pollen stored for the 5^{th} to 6^{th} day. Seed germination rates in the first five days, including day 0, varied between 19.44% and 27.78% (Table 1).

Between day 0 and day 6, the fruit set decreased by 75%, the average number of seeds per fruit by 75.66%, and the seed germination rate by 100%. On the 4th day, when a statistically significant difference was observed, the fruit set rate decreased by 16.66%, the seed set rate by 25.55%, and the seed germination rate by 25.50%. As of the 5th day, the decrease in fruit set rate, the number of seeds per fruit, and the seed germination rate were almost 50% and above.

Table 1. Crossability indices data obtained from crossing pollen stored at different temperatures and times.

Storage Temperature	Storage Time (Days)	FSR (%)	SNpF (pcs/fruit)	FW (g)	SW (mg)	SGR (%)
24ºC	**0**	13.33 ± 3.85 ac	4.11 ± 0.81 ab	1.26 ± 0.40 ns	220 ± 0.06 ns	26.11 ± 3.89 a
4ºC	**1**	23.33 ± 3.33 a	5.01 ± 0.72 a	1.32 ± 0.29	260 ± 0.06	25.65 ± 3.84 a
	2	18.89 ± 1.11 ab	3.42 ± 0.45 ab	1.39 ± 0.22	210 ± 0.03	27.78 ± 5.55 a
	3	12.22 ± 5.55 ac	3.60 ± 0.46 ab	1.44 ± 0.11	160 ± 0.03	26.11± 3.88 a
	4	11.11 ± 4.84 bc	3.06 ± 0.06 b	1.57 ± 0.09	140 ± 0.02	19.44 ± 10.0 a
	5	6.67 ± 1.92 c	2.77 ± 0.36 b	1.62 ± 0.03	210 ± 0.05	0.00 ± 0.00 b
	6	3.33 ± 0.10 c	1.00 ± 0.20 c	1.79 ± 0.03	120 ± 0.02	0.00 ± 0.00 b

The difference between the means shown with the same letters in same column is insignificant at the 0.05 level, ± standard errors, ns: not significant. FSR: fruit set rate, SNpF: seed number per fruit, FW: fresh weight, SW: seed weight, SGR: seed germination rate.

In this study, the fruit set rate, the seed number per fruit, and the seed germination rate varied depending on the storage period of the pollen, and the longer the pollen storage period, the lower the fruit set rate, fewer seeds were obtained, and the germination ability of the seeds was lost. Similar findings were reported by Visser et al. (1977), who observed a decrease in the fruit set rate in roses with pollination made using pollen stored at room temperature for more than 3 days. Veeresha et al. (2018) reported that the seed yield per plant in *Solanum melongena* L. decreased with longer pollen storage times, and they also found a higher seed germination rate for seeds obtained from hybridizations with fresh pollen compared to pollen kept for 1 and 2 days. Pereira et al. (2014) suggested that short-term storage conditions and duration of pollen in *Annona squamosa* plants affect fruit set and fruit quality and that the best storage conditions and durations should be determined for each species and variety. The decrease in fruit and seed set rates observed in this study could be attributed to the decrease in pollen germination rates as the storage time increased, which is consistent with the findings of the other researchers mentioned above.

While fruit weight and seed weight were not affected by the pollen storage period in this study, Santosh & Malabasari (2014) reported that the fruit and seed weight of *Momordica charantia* L. species were affected by the storage period of the pollen, and both decreased as the storage period extended. They attributed this to the decrease in fertilization rate due to a decrease in the viable pollen rate. Pereira et al. (2014) also reported that the longer pollen storage period in *Annona squamosa* plants, the lower the fruit quality, but it was related to the number of fruits obtained. Considering that each pistil of a rose contains an ovary, it is expected to obtain as many seeds as the number of stigmas. Therefore, it can be expected to obtain 100 or more seeds from one fruit in roses. However, in this study, the average number of stigmas was 125, which is therefore less than the expected number of seeds obtained. The reason for this could be that the plant has the necessary nutrient content to feed fewer seeds than expected, which might explain why there was no change in seed and fruit weight.

The fruit set rates of roses vary according to the species and types of roses used (Zlesak, 2007). Generally, the fruit set rate is less than 50% in modern roses (Gudin, 2003). Similarly, in crosses made

between nine different hybrid tea roses, the fruit set rate was between 0% and 43.75% (Nadeem et al., 2013). Fruit set rates can be much higher in combinations where wild and old garden roses are used as parents. For instance, the fruit set rates varied between 0% and 90% in cross combinations made among 36 different modern roses and between modern rose and wild rose species (Abdolmuhammadi et al., 2014), while the fruit set rates ranged from 11.32% to 100% in cross combinations made between old garden roses and modern roses (Kılıç, 2020). In this study, the average fruit set rate was 12.69%. The differences in fruit set rates observed in various studies can be attributed to the complex genetic structures and ploidy levels of the genotypes used as parents (Ueckert, 2014), parental fertility (Nadeem et al., 2015), incompatibility between the cross combinations (MacPhail & Kevan, 2009), the climatic conditions at the study location, nutritional status of the plant, and the pollination method (Farooq et al., 2016). Kılıç (2020) revealed the effects of paternal parents on fruit set rate by reporting that the fruit set rate varied between 29.92% and 56.00% in cross combinations where the same seed parent and different pollen parents were used. It has also been reported that seed formation has effects on fruit set (Gudin, 2003). Successful seed formation combinations, may lead to increased fruit set rates. Indeed, a positive correlation was found between the fruit set rate and the seed number in this study.

The seed numbers per fruit vary between 0 and 50 depending on the genetic structure of the parents and environmental conditions in roses, although they can exceed 100 in some cross combinations (Zlesak, 2007; Gülbağ et al., 2018; Erken et al., 2018). In addition, Gülbağ et al. (2018) reported that the seed number per fruit in garden roses varied between 2.0 and 57.57, while it was found between 2.17 and 59.47 in cut roses by Erken et al. (2018), between 7.29 and 31.5 in modern roses by Doğan et al. (2019), between 7 and 25.67 in cut rose varieties by Kazaz et al. (2020), and between 0 and 14.33 in roses by Khan et al. (2021). In this study, the average seed number per fruit varied between 1 and 5.01, while it ranged between 0 and 35.33 in the other studies. The upper limit values of the findings obtained from this study are considerably lower than the studies mentioned above. The seed number per fruit can be influenced by various factors such as pollination time, pollination method, pollination number, and pollen fertility (Nadeem et al., 2013; Gülbağ et al., 2021). Moreover, factor such as the seed parent fertility, difference in the developmental status of the plants, and the incompatibility in the combinations may also contribute to a lower seed set. Farooq et al. (2016) reported that pre-pollination barriers can prevent fertilization, obstruct pollen tubes from entering styles, and subsequently lead to limited fruit and seed set.

The seed germination rate in rose breeding varies between 10% and 60% according to the combinations (Zlesak, 2007). However, Leus et al. (2018) stated that the seed germination rate could reach 80%. On the other hand, Pipino et al. (2011) reported that the germination rate of the seeds obtained from 11 different hybrid tea roses varied between 15.4% and 37.1%. Abdolmuhammadi et al. (2014) found that the seed germination rate in seeds obtained from hybridizations among hybrid tea roses and between hybrid tea roses and wild rose species ranged from 0% to 93.40%, and Kılıç (2020) determined that the seed germination rate in seeds obtained from hybridization between fragrant rose species and hybrid tea roses varied between 0% and 30.80%. Uran (2022) recorded that the seed germination rate varied between 0% and 47.5% in hybridizations between miniature roses and different commercial cut rose varieties. While seed germination rates in this study ranged from 0% to 27.78%, seed germination rates in the studies mentioned above ranged between 0% and 93.40%. Although the results obtained from this study are generally similar to those obtained from other studies, the upper limit values differ. Seed germination rate varies according to the species and variety,

the method and temperature of seed stratification, and the degree of maturation at the seed development stage (De Vries & Dubois, 1987; Gudin et al., 1990; Anderson & Byrne, 2007). Alp et al. (2009) stated that species and cultivars respond differently to the stratification methods and temperatures applied to eliminate dormancy among cross combinations. Moreover, varying levels of post-pollination barriers may exist depending on the cross combinations. Post-pollination barriers play a role in embryo abortion and/or abnormal growth of endosperm, which can lead to reduced seed germination rates (Tonosaki et al., 2016).

Evaluation of Pollen Quality

The viability rate and germination rate were determined to be 48.33% and 15.27%, respectively, in fresh pollen. The highest viable pollen rate was obtained on the 1st day of storage (50.49%), and the highest germination rate was observed in fresh pollen (15.27%). However, no statistically significant difference was found between pollen stored at 4ºC for one day and fresh pollen. A continuous decrease was recorded in pollen viability and germination rates from the 1st to the 6th day of storage. Between one day of storage and six days of storage, a decrease of 34.22% in viability and 89.29% in germination rate was observed. The least viable pollen and germination rate were determined on the 6th day of storage. There was no statistical difference between the viability rates of pollen stored for 3, 4, 5, and 6 days, and the germination rates of pollen stored for 5 to 6 days (Figure 1).

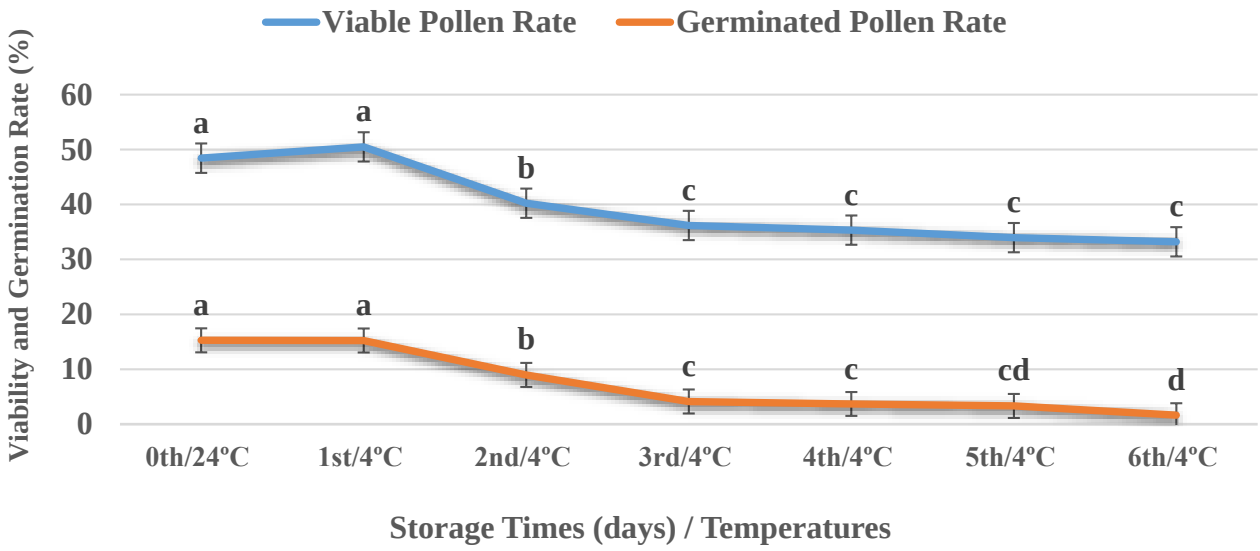


Figure 1. Pollen viability and germination rate of 'Magnum' at different storage temperatures and times (The difference between the means shown with the same letters is insignificant at the 0.05 level; error bars show standard errors.)

The storage conditions and duration of pollen significantly affect pollen quality, making pollen and pollen storage crucial factors in successful rose breeding. Numerous studies have been conducted on rose pollen viability and germination rates at different storage times and conditions. In these studies, the viable pollen rate gradually decreases as the storage period is prolonged, whether at room temperature or in short-term or long-term storage (Seyhan, 2020; Kılıç et al., 2020; Korkut et al., 2022). Kılıç et al. (2020) reported that the viable pollen rate of 'Magnum', which was kept for four days at room temperature, decreased by 30.91% and the germination rate by 68.96% on the 3rd day compared to the 0th day. Similar to their research, this study also observed a loss of viability of over 20% in pollen viability rates and over 50% in pollen germination rates as of the 3rd day. In addition, although the decrease in the viability rate in pollen stored for a short time in this study was similar, the

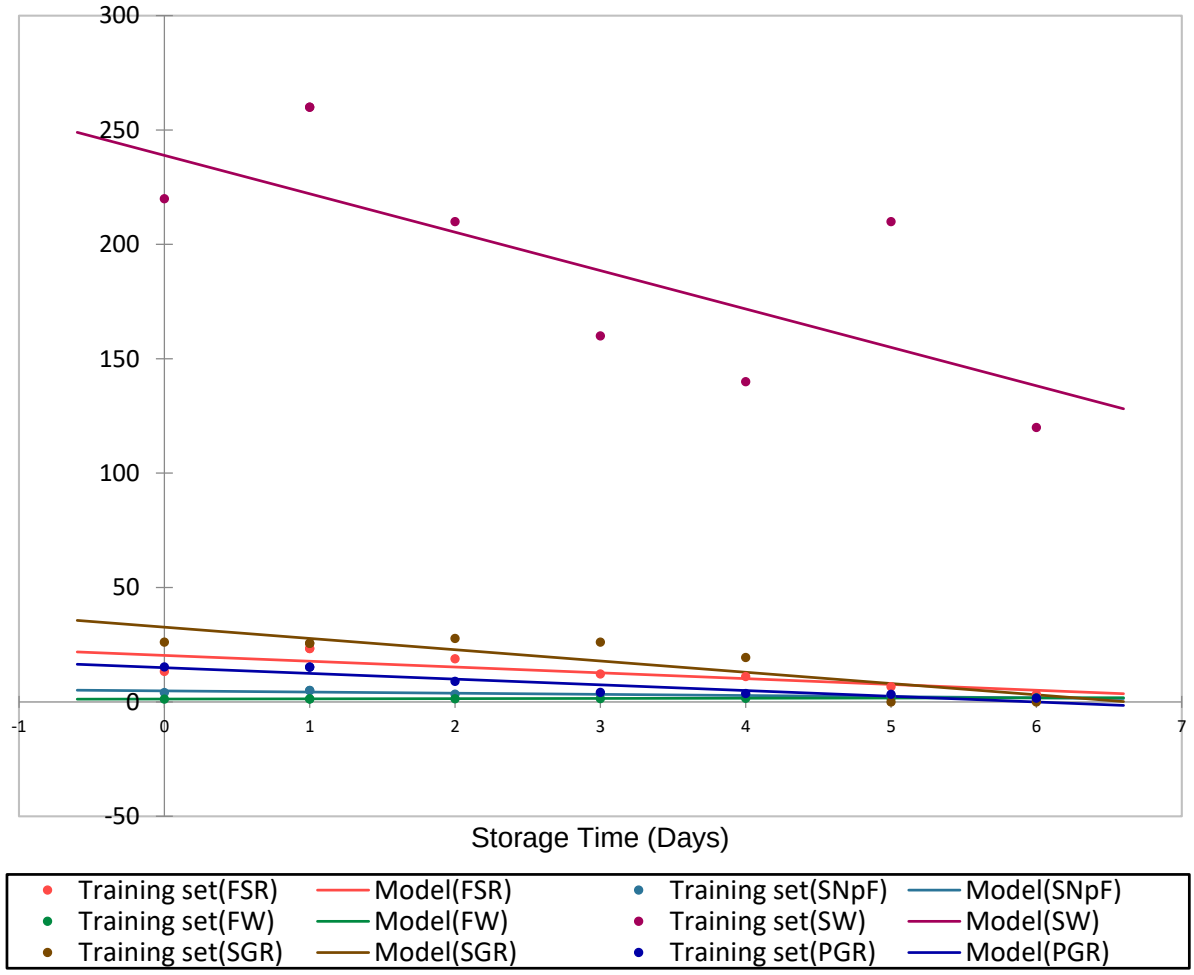
reduction in the germination rate was found to be higher than the pollen stored at room temperature in their research. This may be related to the period when pollen was collected. While the researchers collected pollen during the second flowering period in June of their study, pollen collection in this study was carried out during the third flowering period in July. Erbaş et al. (2015) reported that pollen quality decreases as the flowering season progresses.

In this study, the viable pollen rate was 48.33% and the germination rate was 15.27% in fresh pollen. However, Pipino et al. (2011) found that the germinated pollen rates of eleven different hybrid tea rose varieties were between 0% and 46.5%. Nadeem et al. (2013) showed that the viability rates of 13 different hybrid tea roses were between 35.0% and 70.0%, and the germination rates were between 1.3% and 46.5%. Erbaş et al. (2015) determined that pollen viability rates in *R. damascena* at different flowering periods were between 32.8% and 71.5%, and germination rates were between 24.2% and 57.0%. Żuraw et al. (2015) reported that the pollen viability rates of 4 different rose species ranged from 26.7% to 96.9%, and Giovannini et al. (2017) stated that the germinated pollen rates of 44 hybrid tea roses ranged from 6.0% to 99.0% based on storage conditions. Although the findings obtained from the present study are generally in line with the results obtained from the investigations mentioned above, pollen quality showed variation among the studies. Pollen viability is thought to vary depending on factors such as the genetic makeup of species or varieties, ploidy levels, the method used for assessment, climate conditions, nutritional status of the plant, collection time of pollen, storage conditions, and duration (Güneş et al., 2005; Zlesak, 2009; Sulusoglu & Cavusoglu, 2014; Martins et al., 2017).

The viable pollen rate of the 'Magnum' at chemical and biological methods differed from each other, and the viable pollen rates obtained from the IKI method were found to be higher than the agar method in petri dishes. Similarly, Parfitt & Ganeshan (1989) determined that chemical methods do not show similarities with biological methods. In general, although it is expected that there is a linear relationship between chemical and biological methods (Martins et al., 2017), pollen that has not yet matured can be dyed using chemical methods (Şensoy et al., 2003). The possibility of staining immature pollen using the IKI method may have affected the results, as the culture medium used for germination may not have provided the optimum conditions for the Magnum. Some researchers stated that the pH, sucrose, and boric acid content of the germination medium can affect the germination rate of pollen (Mert & Soylu, 2006; Fragallah et al., 2019).

Effects of Storage Time on Traits and the Relationships between Traits

The regression of FSR, SNpF, SW, FW, SGR, and PGR by storage time is shown in Figure 2. According to Figure 2, it can be concluded that storage time had significant effects on FW, SGR, PGR, FSR, and SNpF, playing a crucial role in shaping these traits. However, the impact of storage time on SW was not found to be statistically significant. The storage time explained 64.8% of the variance in FSR, 77% in SNpF, 96.7% in FW, 53% in SW, 72.7% in SGR, and 87.5% in PGR. Furthermore, it was evident that, as the storage time increased, there was a noticeable decrease in all other traits, except for FW.



	FSR	SNpF	FW	SW	SGR	PGR
R^2	0.648	0.770	0.967	0.530	0.727	0.875
F	9.204	16.757	148.203	5.638	13.287	34.985
Pr > F	0.029	0.009	<0.000	0.064	0.015	0.002
β	-0.105	-0.021	0.003	-0.699	-0.205	-0.105

Figure 2. Linear regression analysis showing the effects of storage time on FSR (fruit set rate), SNpF (seed number per fruit), FW (fruit weight), SW (seed weight), SGR (seed germination rate), PGR (pollen germination rate). β= parameter estimate

The results obtained from this study regarding the effects of storage time on various traits in plants are in line with some previous research findings. Regarding FSR, the observed significant effect of storage time is in line with previous studies showing the importance of pollen freshness in achieving higher fruit set rates. Other studies have also reported a decrease in fruit set as the storage time increases (Kadri et al., 2022). Similarly, the significant effect of storage time on SNpF is consistent with previous research highlighting the impact of pollen quality on seed production success (Parimala & Swarnalatha Devi, 2018). The significant variation in these traits, explained by storage time, indicates that maintaining pollen viability is crucial to ensure reproductive success in the plants in question. The study's findings also support previous research highlighting the critical role of storage time in influencing PGR (Aldahadha et al., 2020).

The correlation matrix given in Table 2 showed a high positive correlation between the pollen germination rate and fruit set rate (r=0.78) and between the pollen germination rate and the seed number per fruit (r=0.82). At the same time, there was a high positive correlation between fruit set rate

and seed number (r=87), between seed number and seed weight (r=0.81), between seed germination rate and seed number (r=0.79), and between seed germination rate and fruit number (r=0.82).

Table 2. Correlations among the fruit set rate, seed set rate, seed and fruit weight, seed germination rate, and pollen germination rate

Traits	FSR	SNpF	FW	SW	SGR	PGR
FSR	1	.872*	-.825*	.744	.819*	.779*
SNpF	.872*	1	-.923**	.805*	.791*	.818*
FW	-.825*	-.923**	1	-.752	-.874*	-.883**
SW	.744	.805*	-.752	1	.417	.824*
SGR	.819*	.791*	-.874*	.417	1	.657
PGR	.779*	.818*	-.883**	.824*	.657	1

*Correlation is significant at the 0.01 level.**Correlation is significant at the 0.05 level. FSR: fruit set rate. SNpF: seed number per fruit. FW: fruit weight. SW: seed weight. SGR: seed germination rate. PGR: pollen germination rate.

It is expected that there will be a similar trend in the pollen germination rate. fruit set. and seed set rates. Pipino et al. (2011) stated that there was a positive correlation between the pollen germination rate and the average number of seeds per fruit. Nadeem et. al. (2013) and Deng et al. (2022) reported that they obtained the least fruit from their crosses with parents with the lowest pollen germination rate and the most fruit from their hybridization with parents with the highest pollen germination rate. In the results of this study. pollen germination rate. fruit set. and seed set rates showed a positive correlation similar to their findings. However. the germination ability in vitro may not completely reflect the ability to germinate in vivo in some cases (Pipino et al.. 2011). and it is thought that the culture medium used in the germination method is related to the success of imitation of the stigma fluid. The positive correlation between the seed number and the fruit set rate may be related to the fact that the seed number is calculated per fruit. The positive correlation among seed number. seed weight. and seed germination rate may be related to obtaining more endosperm. larger embryos. and thinner seed coats. Germination occurs as a result of successful fertilization and healthy seed development. and it may indicate that heavier seeds in roses have a higher germination ability. Because they have had a low seed germination rate due to embryo abortion and dormancy due to a thick seed coat. However. each increase in seed weight may not necessarily correspond to the size of the embryo and endosperm. Heavier seeds can be obtained due to an increase in seed coat weight. even if the embryo and endosperm are relatively smaller.

CONCLUSION

This study investigated how many days can be successfully made crossed with pollen stored for a short time. The study results confirm the importance of considering pollen storage duration. which is a significant factor that affects various characteristics of plants. The pollen viability and germination rates decreased over time. but this decrease did not create a significant difference in fruit and seed set rates in hybridization studies carried out for up to 3 days. However. after 3 days. significant decreases were observed in fruit set. seed number. and seed germination rates. The study suggest that parents with pollen showing a germination rate of 4.00% or more on roses can be considered moderately fertile. The positive correlation between seed germination rate and seed number highlights the importance of obtaining a considerable number of seeds for successful breeding studies. Studies to increase the seed set rate are of great importance as they will increase the effectiveness of breeding programs.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

Conceived and planned the experiments: SK. Performed the experiments: SK and GT. Analyzed the data: TK. Wrote the paper: TK. Reviewed and edited the paper: SK.

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