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SCIENTIFIC ARTICLE

Different storage temperatures and times on pollen quality in cut rose varieties

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Abstract

Pollen parent is one of the most important factors affecting the seed set in conventional breeding. Pollen quality of pollen parents must be known for success in breeding programs. Breeders also must know how long pollen grains keep their viability to overcome geographical distance and the difference in blooming times among parents. This study was conducted to find out the viability, germination rate, and duration of the keeping viability of pollen of rose varieties being kept for 0, 4, 8, 16, 24 hours at 24 ºC and 0, 1, 2, 3, 4, 5 days at 4 ºC. The pollen of the Inferno, Layla, First Red, and Myrna varieties were used as plant material. The IKI and petri dishes methods were used to determine pollen quality. The results showed that the viable pollen rate of varieties varied between 41.1% and 49.9%, whereas the germination rate was 3.8% and 29.9% and morphological pollen rate was 71.8% and 88.7%. In all varieties, viability, germination rate and morphological normal pollen rate decreased over time both kept at 24 ºC and 4 ºC, but fresh pollen lost its quality faster than pollen stored. Fresh pollen viability rate decreased by 11.9% and 25.6% at the end of 24 hours, whereas only it decreased by 10.4%-22.6% on 1st day of storage. The reduction in germination ability in Layla, Inferno and Myrna was over 60.0% on the 5th day, while it was found less than 50.0% in First Red. The decrease in morphologically normal pollen ratio was found statistically significant in both temperature treatments, except for Layla. As it is clear, the pollen quality was significantly affected by variety, storage/holding time, and conditions. It's recommended to use stored pollen in breeding programs. Although it varies according to the varieties, the rose pollen should be use by keeping at 4 ºC between 2-5 days.
Keywords: *Rosa x hybrida.* hybrid tea rose, pollen germination, pollen storage, pollen viability.

Resumo

Diferentes temperaturas e tempos de armazenamento na qualidade do pólen em variedades de rosas para corte

O genitor do pólen é um dos fatores mais importantes que afeta as sementes no melhoramento convencional. A qualidade de pólen de seus genitores deve ser conhecida para o sucesso nos programas de melhoramento. Este estudo foi realizado para conhecer a viabilidade, taxa de germinação e tempo de sobrevivência do pólen de variedades de rosas mantidas por 0, 4, 8, 16, 24 horas a 24 ºC e 0, 1, 2, 3, 4, 5 dias a 4 ºC. O pólen retirado das variedades Inferno, Layla, First Red e Myrna foi usado como material vegetal. Os métodos IKI e ‹placas de Petri› foram usados para determinar a qualidade do pólen. Os resultados mostraram que a taxa polínica viável das variedades variou entre 41,1% e 49,9%, enquanto a taxa de germinação foi de 3,8% e 29,9% e a taxa polínica morfológica foi de 71,8% e 88,7%. Em todas as variedades, a viabilidade, a taxa de germinação e a taxa de pólen morfológica normal diminuíram ao longo do tempo tanto quanto mantidos a 24 ºC como a 4 ºC, mas o pólen fresco perdeu sua qualidade mais rapidamente do que o pólen armazenado. A taxa de viabilidade do pólen fresco diminuiu 11,9% e 25,6% ao final de 24 horas, reduzindo de 10,4%-22,6% no 1º dia de armazenamento. A redução na capacidade de germinação de pólen nas variedades Layla, Inferno e Myrna foi superior a 60,0% no 5º dia, sendo menos de 50,0% em First Red. A diminuição da razão polínica morfologicamente normal foi estatisticamente significativa em ambos os tratamentos de temperatura, exceto para Layla. Assim, a qualidade do pólen foi significativamente afetada pela variedade, tempo de armazenamento/detenção e condições de armazenamento/detenção. Embora varie de acordo com as variedades, o pólen de rosas deve ser usado mantendo-o a 4 **ºC** entre 2-5 dias.
Palavras-chave: *Rosa x hybrida*, rosa de chá híbrida, germinação polínica, armazenamento de pólen, viabilidade polínica.

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Introduction

Rose (*Rosa* spp.) is one of the most popular plants traded worldwide due to its wide range of uses as a cut flower, landscape, pot, medicinal and aromatic plant (Datta, 2018). Millions of rose bushes are used in landscape or indoor design, and billions of cut rose stems are sold each year in the world. Millions of rose flowers are also produced for highly prized essential oils. At the same time, hundreds of new rose varieties are developed every year to respond to market demands. Until today more than 30,000 rose varieties have been introduced to the market (Liu et al., 2021).

Crossbreeding is the best method for developing new rose varieties in different types, shapes, and colours (Datta, 2018). However, several problems such as a decline in fruit and seed set affect crossbreeding success. To avoid these problems, rose breeders must choose genotypes having high pollen viability and germination rate as a pollen parent (Souza et al., 2022). They must determine the pollen viability and germination rate of the genotype used as a parent because pollen viability and germination rate show variation in genotypes and environmental conditions (Giovannini et al., 2017; Pacini and Dolferus, 2019). They also must know how long pollen grains keep their viability to overcome geographical distance and the difference in blooming times among parents, considering that pollen viability and germination rate decrease rapidly from hour to hour at room temperature and 50% humidity (Pipino et al., 2011; Giovannini et al., 2017). Hence, a key aspect for rose breeders is linked to knowing the duration of the pollen keeping viability and pollen storage conditions (temperature, humidity, etc.) besides that determination of pollen viability and germination rate (Giovannini et al., 2017).

Many researches have been carried out to determine pollen viability and germination rates and to reveal the duration of pollen viability (Zlesak et al., 2007; Aldahadha et al., 2020; Iovane et al., 2022; Souza et al., 2022). In these researches, it was determined that the duration of keeping the viability of pollen differs depending on the conditions in which they are kept and the species and variety used (Rathod et al., 2018; Bheemanahalli et al., 2021; Ravindra et al., 2022; Souza et al., 2022). Temperature is one of the most important conditions affecting the viability and longevity of pollen life, and it has been reported that keeping many species and varieties of pollen at low temperatures such as -80 ºC, -20 ºC, 0 ºC, 4 ºC for certain periods provides successful results (Zlesak et al., 2007; Du et al., 2019; Aldahadha et al., 2020). -80 ºC is generally a suitable temperature for pollen storage (Zlesak et al., 2007). However, storage at -80 ºC is long-term storage and in general, long-term storage is preferred for genetic conservation. Short-term storage (+4 ºC) is preferred in breeding programs. (Aldahadha et al., 2020). This preference may be related to the usage procedure of the pollen coming out of -80 ºC and increasing the storage costs for short-term storage.

In this study, it was aimed to determine the pollen viability and germination rates of different rose varieties and to reveal how long the pollen kept in different temperatures and different times can be used.

Material and Methods

In this study, pollen of four commercial cut rose flower belongs to *Rosa hybrida* L. known hybrid tea rose used as plant material. The pollen of the Inferno, Layla, First Red, and Myrna varieties were picked from rose plants which are grown in ornamental plant breeding greenhouse of the Department of Horticulture, Ankara University (39°57′40.2′′N 32°51′51.7′′E). Rose plants were grown in a horizontal bag culture with a coco peat. During the vegetation period, the temperature of the greenhouse is 23-30 ºC and relative humidity was kept between 60%-70%. In order to prevent the plants from being damaged by the high light intensity, a heat-shade curtain providing 55% shade was used. Water and nutrients were given to the plants by drip irrigation system and the system was automatically controlled by a fertigation computer. The number and amount of irrigation were adjusted based on the 30% drainage rate during the vegetation period. Irrigation was done 2-4 times a day in April-May, including the month of May when the pollen was collected, and the amount of water per drip was generally adjusted as 80-100 cc. The nutrient solution given by Mercurio (2007) was used for fertilizing the plants.

Flower buds of rose varieties were harvested after one-third of the flowers were opened at 08.00 am, and they were brought to the laboratory. In the laboratory, the petals were removed from the buds, and the anthers were picked up with forceps. The anthers were placed in glass bottles, and then the bottles were kept overnight in a growth chamber with 24 ºC temperature and 60% humidity for anther dehiscence. Dispersed pollens from anthers were divided into two parts, and one of them was placed in the fridge to keep at 4 ºC, and the other one was placed in the growth chamber to keep at 24 ºC. Pollen grains were stored at 24 ºC for 0, 4, 8, 16, and 24 hours and at 4 ºC for 0, 1, 2, 3, 4, and 5 days for determining their viability and germination rate. Pollen viability and germination rates were measured after pollen stored in the refrigerator were kept in an incubator at 24 ºC and 60% humidity overnight whereas pollen kept in the growth chamber was measured as soon as the pollens were taking out of the chamber. The IKI (iodine potassium iodide) method was used to determine the pollen viability rates, and the petri dishes method was used to determine the germination rates.

The IKI that is a chemical pollen viability method was modified according to Kılıç et al. (2020). 1 g of potassium iodide (Sigma-Aldrich, CAS:7681-11-0) and 0.5 g of iodine (Sigma-Aldrich, CAS:7553-56-2) was weighed and dissolved in 100 mL of distilled water and the IKI solution was prepared. One drop was dropped on the slide from the IKI solution, and pollen grains were sprinkled on the drop of a brush. After five minutes, the counting of pollen was made under the microscope. Pollen dyed in black and dark brown were considered as «absolutely viable», pollen dyed in light brown, orange, and red as

«semi-viable», pollen dyed in yellow or uncolored pollen as «dead». The «viable» pollen percentage was calculated by adding the value to the «absolutely viable» pollen, assuming theoretically 50% of the pollens determined as «semi-viable» are viable. During the count, pollen grains having abnormal shapes were also counted and the quantity of morphological normal pollen was evaluated (Figure 1).

Figure 1. Pollen grains of different rose varieties considered as absolutely viable (1), semi-viable (2), dead (3) and morphologically abnormal (4).

The petri dishes method that is a biological pollen viability method was modified according to Kazaz et al. (2020). A germination medium was prepared to contain 1% agar (Sigma-Aldrich, CAS:9002-18-0), 20% sucrose (Carlo Erba-Merck, CAS: 57-50-1), and 10 ppm boric acid (Sigma-Aldrich, CAS:10043-35-3) and poured into plastic petri dishes in 2 mm thickness. When the medium cooled in petri dishes, but before it solidified, the medium was divided into four areas, and pollens were sprinkled on each of them with a sable brush. Then, the petri dishes were covered with lids, including moist filter paper and they incubated for 8 hours in a growth chamber at 24 ºC temperature and 60% humidity. After incubation, a slice was taken from areas and placed on a slide for counting under the microscope. When pollen tubes are longer (1.5 times) than pollen diameter, they were considered to be germinated (Figure 2).

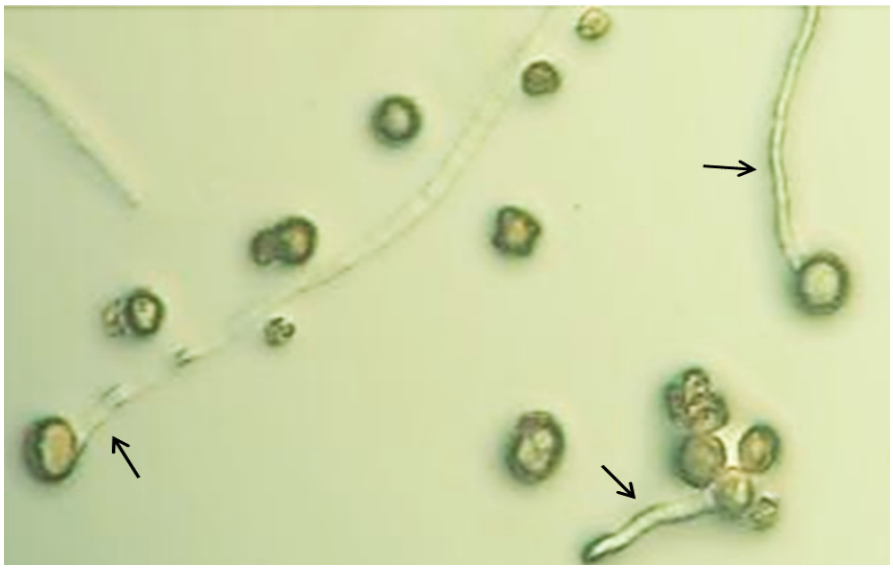

Figure 2. Germinated pollen grains in rose varieties (They are shown with arrows).

In the IKI and petri dishes method, Leica DM1000 model microscope and imaging system with x40 and x100 magnification objectives were used for pollen count. The experiment was established in a Completely Randomized Design (CRD) with four replicates. In the IKI, two coverslips for each rose variety were used, and counts were made in four areas on each coverslip. In the petri dishes, counting was made in four areas in two slices chosen randomly in each petri dish. In both methods, an average of 250 pollen grains was counted in per area. Statistical analysis was performed using IBM SPSS Statistics vs. 20.0. Analysis of variance (ANOVA) was applied to the angular transformed data. Mean differences were established by Duncan's test ($p < 0.05$).

Results

Variance analysis of factors that examined this study was given Table 1. According to the Table 1, pollen viability and morphological normal pollen rate stored at 4 ºC were significantly affected by variety and storage time ($p \leq 0.05$). Germination rates of pollen stored at 4 ºC were significantly affected by variety, storage time and variety x storage time interaction ($p \leq 0.05$). In pollen kept at 24 ºC; variety, storage time and variety x storage time interaction were significantly effective in terms of pollen viability and morphological normal pollen, while variety x storage time wasn't significant on germination rates ($p \leq 0.05$).

Table 1. Analysis of variance factors of the investigated parameters.

Parameter	Factor	Sum of Square	Mean of Square	F	Sig.*
Pollen Viability (24 ºC)	Species (S)	355.482	118.494	183.932	0.00
	Storage Time (ST)	259.891	64.973	100.854	0.00
	S x ST	25.718	2.143	3.327	0.00
Pollen Germination Rate (24 ºC)	Species (S)	5501.713	1833.904	519.094	0.00
	Storage Time (ST)	114.189	28.547	8.080	0.00
	S x ST	16.399	1.367	0.387	0.96
Morphological Normal Pollen (24 ºC)	Species (S)	1435.855	478.618	175.850	0.00
	Storage Time (ST)	112.215	28.054	10.307	0.00
	S x ST	68.730	5.728	2.104	0.00
Pollen Viability (4 ºC)	Species (S)	372.154	124.051	38112.896	0.00
	Storage Time (ST)	1863.624	465.906	60.489	0.00
	S x ST	43.196	3.600	227.183	0.07
Pollen Germination Rate (4 ºC)	Species (S)	5026.156	1675.385	932.512	0.00
	Storage Time (ST)	284.986	71.246	39.655	0.00
	S x ST	57.003	4.750	2.644	0.01
Morphological Normal Pollen (4 ºC)	Species (S)	1208.829	402.943	127.115	0.00
	Storage Time (ST)	157.862	39.466	12.450	0.00
	S x ST	21.993	1.833	0.578	0.85

* $p \leq 0.05$

Effects of storage at 4 ºC for different times on pollen viability, germination rate and morphological normal pollen rate

It is seen in Table 1 that the reduction of the amount of pollen viability stored at 4 ºC with increasing storage time does not depend on the varieties. Viable pollen, morphological normal pollen and pollen germination rates of rose varieties' pollen stored at 4 ºC for different times (0, 1, 2, 3, 4, 5 days) are given in Table 2.

Table 2. Viable pollen, morphological normal pollen and pollen germination rates of cut rose varieties stored at 4 ºC for different storage time.

Pollen Quality Parameter	Variety	Storage time (day)						Avg.
		0	1	2	3	4	5	
Viable Pollen Rate (%)	Layla	41.2 A c	36.9 B b	30.3 C b	26.9 D b	21.7 E b	16.1 F b	28.8 C
	First Red	49.9 A a	41.8 B a	36.1 C a	31.3 D a	26.2 E a	22.2 F a	34.5 A
	Myrna	41.1 A c	31.8 B c	26.6 C c	22.6 D c	18.2 E c	14.1 F b	25.7 D
	Inferno	47.7 A b	40.9 B a	36.3 C a	27.6 D b	24.2 E ab	15.5 F b	32.0 B
	Average	45.0 A	37.9 B	32.3 C	27.1 D	22.6 E	17.0 F	-
Morphologic Normal Pollen Rate (%)	Layla	75.7 A c	73.8 A b	73.5 A b	73.9 A b	69.1 A c	68.8 A b	72.5 C
	First Red	88.7 A a	82.6 B a	80.4 C a	79.3 CDa	77.6 D a	77.0 D a	80.9 A
	Myrna	71.8 A c	67.1 B c	63.1 C c	62.4 C c	62.3 C d	59.6 C c	64.4 D
	Inferno	84.4 A b	80.0 B a	75.9 C b	74.3 C b	73.7 C b	73.7 C a	77.0 B
	Average	80.2 A	75.9 B	73.2 C	72.5 CD	70.7 DE	69.9 E	-
Pollen Germination Rate (%)	Layla	3.8 A c	3.0 AB c	2.7 AB c	2.4 AB b	2.3 B b	1.0 C c	2.5 C
	First Red	29.9 A a	24.9 B a	24.4 B a	22.5 B a	21.7 B a	17.1 C a	23.4 A
	Myrna	9.4 A b	5.6 B b	5.2 B b	3.1 C b	2.4 C b	1.4 D c	4.52 B
	Inferno	7.1 A b	6.0 A b	3.2 B c	2.9 B b	2.6 B b	2.4 B b	4.03 B
	Average	12.6 A	9.9 AB	8.9 AB	7.73 AB	7.25 AB	5.48 B	-

*The differences between the means shown in the same letters are not significant at $p < 0.05$ level.

*Uppercase letters show different storage time for the same variety, lowercase letters show differences between different varieties in the same storage time.

According to Table 2, the viability rate of non-stored (0^{th}) pollen was found to be higher than stored pollen and the viable pollen rate decreased day by day during the storage in all varieties. The viable pollen rate of rose varieties varied between 41.1% and 49.9% on the 0^{th} day where it was between 31.8% and 41.8% on the 1^{st} day. On the 5^{th} day of storage, viability was varied between 14.1% and 22.2%. The highest viable pollen rate was determined on the 0^{th} day of First Red with 49.9%. Stored pollen of First Red also had the highest pollen viability among the varieties during the storage time. However, there was no statistically significant difference between viable pollen rates of Inferno and First Red on the 1^{st}, 2^{nd}, and 4^{th} days. The lowest viable pollen rate with 14.1% was obtained in Myrna on the 5^{th} day, which was in the same statistical group as the Layla (16.1%) and Inferno (15.5%). From 0^{th} to 5^{th} days, the viability rate of pollen stored decreased by 55.5% in First Red, 60.9% in Layla, 65.7% in Myrna and 67.5% in Inferno.

Morphological normal pollen rate of rose varieties varied between 71.8% and 88.7% on the 0^{th} day. On the 1^{st} day of storage, the morphological normal pollen rate of

varieties was varied between 67.1% and 82.6% where was 59.6% and 77.0% on the 5th day. The highest morphological normal pollen rate with 88.7% was determined on the 0th day of First Red. During storage, First Red had the highest morphological normal pollen rate compared to Layla and Myrna. However, there was no statistically significant difference between the morphological normal pollen rate of First Red and Inferno on the 1st and 5th days. The lowest morphological normal pollen rate with 59.6% was obtained in Myrna on the 5th day. In all varieties, the morphological normal pollen rate decreased day by day, but the decreasing of morphological normal pollen rate was not statistically significant for Layla. From 0th to 5th days, the maximum reduction was in Myrna with 16.9% where the minimum reduction was in First Red with 9.1% (Table 2).

As in viable pollen and morphological normal pollen rates, the pollen germination rate of non-stored pollen was higher than the pollen stored, and it decreased day by day throughout the storage. But there was no statistically significant difference between the germination rate of 0th and 1st days for Inferno and 0th and the first three days of storage for Layla. Among the varieties, the pollen germination rate varied between 3.8% and 29.9% on the 0th day where varied between 3.5% and 29.0% on the 1st day of storage. On the 5th day, the germination rate was varied between 1.0% and 17.1%. First Red had the highest germination rate of 29.9% on the 0th day and it also showed the highest germination rate among the varieties during the storage. However, there was no statistically significant difference between germination rates of First Red in the first 4 days. The lowest germination rate on the 0th day of pollen was obtained in Layla (3.8%) but there was no significant difference between with 0th day and the first 3 days. On the last day of storage, the lowest pollen germination rate was also determined in Layla (1.0%), which was in the same statistical group as Myrna (1.4%). Myrna was a variety with a maximum reduction in the germination rate with 75.0% from 0th to 5th days. The germination rate decreased by 31.3% in First Red, 60.0% in Inferno and 66.7% in Layla at the end of the storage (Table 2).

Effects of kept at 24 ºC in different storage times on pollen viability, germination rate and morphological normal pollen rate

Viable pollen, morphological normal pollen and pollen germination rates of rose varieties' pollen kept at 24 ºC in different storage times (0, 4, 8, 16, 24 hours) are given in Table 3.

Table 3. Viable pollen, morphological normal pollen and pollen germination rates of cut rose varieties kept at 24 ºC in different storage time.

Pollen Quality Parameter	Variety	Storage time (hour)					Avg.
		0	4	8	16	24	
Viable Pollen Rate (%)	**Layla**	41.2 A c	40.4 A b	37.9 B b	37.5 B b	36.3 B b	38.7 B
	First Red	49.9 A a	46.2 B a	43.3 C a	42.2 C a	38.3 D ab	44.0 A
	Myrna	41.1 A c	37.3 B c	33.6 B c	32.3 C c	30.6 C c	35.0 C
	Inferno	47.7 A b	46.7 A a	42.0 B a	41.8 B a	39.9 C a	43.6 A
	Average	45.0 A	42.7 B	39.2 C	38.5 D	36.3 E	-
Morphologic Normal Pollen Rate (%)	**Layla**	75.7 A c	75.0 A b	74.9 A c	74.0 A b	73.8 A b	74.7 C
	First Red	88.7 A a	85.7 B a	83.2 C a	82.4 C a	80.7 C a	84.1 A
	Myrna	71.8 A c	68.4 B c	68.7 B c	67.7 B c	67.0 B c	68.7 D
	Inferno	84.4 A b	84.5 A a	80.0 AB b	80.0 AB a	78.9 B a	81.6 B
	Average	80.2 A	78.4 A	76.7 B	76.0 B	75.1 B	-
Pollen Germination Rate (%)	**Layla**	3.8 ns	3.5 ns	3.4 ns	3.1 ns	2.7 ns	3.3 D
	First Red	29.9 ns	29.0 ns	28.3 ns	27.5 ns	24.6 ns	27.9 A
	Myrna	9.4 ns	8.1 ns	7.0 ns	5.9 ns	4.9 ns	7.1 B
	Inferno	7.1 ns	6.6 ns	6.4 ns	6.1 ns	4.2 ns	6.1 C
	Average	12.6 A	11.8 AB	11.3 AB	10.7 B	9.1 C	-

*The differences between the means shown in the same letters are not significant at $p<0.05$ level.

*Uppercase letters show different storage times for the same variety, lowercase letters show differences between different varieties in the same storage time.

ns: non-significant ($p \leq 0.05$).

According to Table 3, the viable pollen rate that immediately after all pollen had been dispersed (zero hours after pollen dispersed – 0 h) was found to be higher than pollen kept at 4-24 hours. However, for Layla and Inferno varieties, no statistically significant difference was found in terms of viability between 0 h and 4 h. Viable pollen rate of varieties varied between 41.1% and 49.9% at 0 h, 37.3% and 46.7% at 4 h where it varied between 30.6% and 39.9% at 24 h. The highest viable pollen rate was determined in First Red at 0 h with 49.9% where the lowest viable pollen rate was in Myrna at 24 h with 30.6%. During the storage times (4 h - 24 h), First Red and Inferno had the highest viable pollen rate among the varieties and Myrna had the lowest. From 0 h to 24 h, the viability rate of pollen decreased by 11.9% in Layla, 16.4% in Inferno, 23.3% in First Red and 25.6% in Myrna. Myrna was the variety that lost its viability most within one day.

Morphological normal pollen rate of rose varieties at 0 h varied between 71.8% and 88.7%. After 24 h, it decreased to 67.0% and 80.7%. There was no statistically significant difference between 0 h (75.7%) and 24 h (73.8%) in Layla, 4 h (68.4%) and 24 h (67.0%) in Myrna, 8h (83.2%, 80.0%) and 24 h (80.7%, 78.9%) in the First Red and Inferno varieties in terms of morphological normal pollen rate. The highest morphological normal pollen rate with 88.7% was obtained in the First Red at 0 h where the lowest rate with 67.0% in Myrna. From 0 h to 24 h, the morphological normal pollen rate decreased by 9.0% in First Red, 6.7% in Myrna, 6.5% in Inferno and 2.5% in Layla. First Red was the variety that the highest decreasing morphological normal pollen rate within one day (Table 3).

Pollen germination rates of rose varieties at 0 h varied between 3.8% and 29.9% where it varied between 3.5% and 29.0% at 4 h, 3.4% and 28.3% at 8h, 3.1% and 27.5% at 16 h, 2.7% and 24.6% at 24 h. Among the varieties, the highest germination rate of 27.9% was obtained from the First Red. Myrna was the second variety with the highest germination rate (7.1%) after the First Red. The lowest germination rate with 3.3% was found in Layla. In all varieties, it decreased by time, but it only showed a statistically significant difference between 0 h and 16 h-24 h. From 0 h to 24 h, the germination rate of pollen decreased by 17.7% in First Red, 29.0% in Layla, 40.9% in Inferno and 47.9% in Myrna (Table 3).

Discussion

In this study, the viability and germination rate of pollen belonging to 4 different hybrids rose varieties were investigated at different temperatures and different storage times. The results showed that the viability rates varied between 41.1% and 49.9%, and the germination rates were 3.8% and 29.9% according to the varieties. The morphological normal pollen rate of rose pollen was between 71.8% and 88.7%. Many studies have been conducted to determine the quality of pollen of rose species/varieties. Jičínská et al. (1976) found that pollen viability rates of 16 genotypes belonging to 8 rose species varied between 14.8% and 79.8%, and the morphological normal pollen rates were 66.1% and 95.3% in the first year. They also indicated that pollen viability rates varied between 26.5% and 84.2%, and morphologically normal pollen rates were 29.6% and 97.6% in the second year of the same genotypes. Pipino et al. (2011) determined that the germination rate of 11 hybrid tea roses varied between 0% and 46.5%, and morphological normal pollen rate was 9.5% and 73.1%. Anand and Raju (2016) stated that the pollen viability rate of 4 rose varieties varied between 11.58% and 65.73%, and germination rates were 0% and 29.93% according to the months. Giovannini et al. (2017) indicated that germination rates of pollen varied between 6.0% and 99.0% according to storage conditions in 44 hybrid tea roses. Although the results in this study are similar to the results obtained in previous studies, the lower and upper limit values vary from each other. The reasons for it might be since the viability and germination rates vary depending on the genotype, ploidy levels, the methods, the climatic conditions, the fertilization status of plant, pollen collection times (season, flowering period, flower development period), storage conditions and times (Martins et al., 2017; Pacini and Dolferus, 2019; Kılıç et al., 2020).

The pollen viability and germination rates decreased over time depending on the storage times at 24 ºC or 4 ºC. The viability and germination rates of pollen kept for 0 h at 24 ºC were 45.0% and 12.6%, respectively, and decreased to 36.3% and 9.1% at the end of the 24 h. The viability and germination rates of pollen stored for one day at 4 ºC were 37.9% and 9.9%, respectively, and decreased to 17.0% and 5.5% at the end of the 5th day. The highest viable pollen rate was determined in pollen kept between 0 h and 8 h just after the anther dehiscence in pollen kept at 24 ºC for 0-24 h. In pollen stored at 4 ºC, viability and germination rates were highest in fresh pollen (0th day) and pollen stored for one day. Moreover, the viability and germination rate of pollen stored at 4 ºC for one day were higher than that of pollen kept at 24 ºC for 24 h. Similar results about the decrease in pollen quality over time and different viability and germination rates at different temperatures have been reported by many researchers (Mesnoua et al., 2018; Brunet et al., 2019; Karim et al., 2021; Miler and Wozny, 2021) Brunet et al. (2019) stated that pollen viability decreased significantly over time regardless of temperature or varieties. Erbaş et al. (2015) found that pollen stored at 4 ºC preserved the viability and germination rate of pollen better than pollen stored at 25 ºC when the storage time of pollen increased. Shekari et al. (2016) indicated that the pollen viability and germination rates decreased when prolonged storage time. The researchers also indicated that the temperature played an important role in the pollen viability and germination rate, and keeping at 25 ºC caused rapid loss of quality compared to storage at 4 ºC. Khosh-Khui et al. (1976) determined that although rose pollen viability was higher at 25 ºC than 0 ºC, its decline was rapid. The reasons for the decrease in pollen quality and loss of viability are still not well defined. However, it was stated in the process of pollen ageing that intracellular integrity in which, the activity of enzymes such as cytochrome oxidase decreases, free radicals accumulate, the capacity

to enhance gene translation for polyamine biosynthetic enzymes by rehydration decreases, and the leakage of cellular components increases in rehydration through lipid peroxidation and de-esterification (Ćalić et al., 2021).

However, temperature is one of the most important factors affecting pollen quality (Paupie`re et al., 2017). High temperature accelerate the metabolic activity, respiration, the conversion of sugars into organic acids, secondary metabolic products accumulate, and dehydration (Almeida et al., 2011). The difference in the pollen quality under different temperatures in this study might be related to the metabolic activities of pollen. The pollen kept at 24 ºC might have had a strong activity of respiration and metabolism. The low temperature might have preserved the quality of pollen because it causes to decline in cellular metabolism.

As in pollen viability and germination rate, the morphological normal pollen rate decreased over time depending on the storage times at 24 ºC or 4 ºC. It has been reported that there is a positive relationship between morphological normal pollen and the viability and germination rates of pollen (Pipino et al., 2011). The results obtained in the study support this finding.

The pollen viability and germination rates of the varieties differed from each other as well as to preserve their viability differed. The least quality loss was observed in the First Red during storage at 4 ºC and the least germination rate was obtained from First Red at 24 ºC. However, First Red lost faster their viability than Layla at 24 ºC. One of the most important factors on pollen viability and germination rate after the temperature is the water content of pollen. The pollen with high water content has been reported to be sensitive to stress and is typically short-lived during storage (Du et al., 2019). But they have the advantage of germinating quickly, normally in a few minutes to less than an hour (Pacini et al., 2006). The First Red might have higher water content than the other varieties. Radev (2018) reported that the water content of pollen could change depending on the species/varieties.

The First Red had the highest values of pollen quality parameters (viable pollen rate, morphological normal pollen rate, and pollen germination rate) from the first to last days on both storage temperatures. In terms of pollen germination rate, this variety was better than other varieties. However, in terms of viable pollen rate and morphological normal pollen rate, the difference between the varieties was not so much. Although a significant decrease in pollen germination rate in this variety started from the second day, finally the amount of this trait in this variety, even on the last day, was much higher than the amount of pollen germination rate at the beginning of maintenance for the other three varieties. It is might be related that among the parameters, pollen germination rate is the most important criterion in evaluating pollen grain quality, or for the First Red, there is less concern and care in terms of storage and the negative effect of time on pollen grain quality than other varieties.

The viability rate of chemical and biological methods in the same varieties differed from each other. The rate of viable pollen by the IKI was found to be higher than the Petri dishes. Kazaz et al. (2020) found similar results that chemical methods were not similar to biological methods. Although a linear relationship is expected between pollen viability and germination rates, immature pollen can be dyed in chemical methods, and pollen viability rates can be higher than biological methods (Martins et al., 2017). However, germination medium characteristics like pH, sucrose content, boric acid concentration can affect the germination rate of pollen (Fragallah et al., 2019; Damayanti et al., 2021; Mondo et al., 2021). The germination medium used in the study might not be the optimum conditions for the varieties.

Conclusions

In the present study, results showed that pollen quality was significantly affected by variety, storage times, and temperatures. The first 8 hours after pollen scatter is most important for rose varieties pollination with fresh pollen. The pollen viability and germination rates of the varieties in the first 8 hours were significantly higher than that of pollen stored for one day. However, pollen stored at 4 ºC for one day preserved their quality better than pollen kept at 24 ºC for 24 hours. Pollen of the Inferno lost their germination ability by more than 50.0% on the 2nd day, the pollen of the Myrna on the 3rd day, and the pollen of Layla on the 5th day. The pollen germination ability of the First Red was still below 50% on the 5th day. Differences were determined between the varieties about the pollen viability and germination rates. Biological and chemical methods gave quite different results from each other. Pollen determined to be viable with the IKI didn't germinate with the petri dishes method. As a result, it can be said that studies are needed for determining appropriate storage time and temperature in roses and the optimization of methods for determining pollen viability and germination rates. Studies on these issues are of great importance to increase the success of breeders and reduce the time and cost in crossbreeding. There is a need to perform more investigations with a vaster selection of tested cultivars.

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Author Contribution

SSK.: performed the experiments; **SK**: conceived the study and planned the experiments; **TK**: Helped to carry out the experiment, analysed the data, manuscript write and review.

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