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Determination of Pollen Fertility of Some Cut Rose Varieties in Different Periods

Bazı Kesme Gül Çeşitlerinin Farklı Dönemlerdeki Polen Kalitesinin Belirlenmesi

Şüheda Basire AKÇA YILMAZ[1*], Soner KAZAZ[2]

Abstract

In rose breeding by hybridization, the viable pollen and germination rate of the male parent increases the rate of successful pollination. Therefore, a good variety of pollinators is always needed in hybridization studies. This study was carried out at Ankara University, Faculty of Agriculture, Department of Horticulture to determine pollen viability and germination rates of Magnum, Harmonie and Lady Rose cultivars at three different periods (May, June and July) during flowering. The pollen viability rate was determined by "IKI test" and the pollen germination rate was determined by " saturated petri dish method". According to the data obtained from the study, the pollen viability rate showed significant differences among cultivars and within months. The highest pollen viability rate was observed in Harmonie variety in July (51.00%) and the lowest pollen viability rate was observed in Lady Rose variety in June (16.19%). The highest pollen germination rate was obtained from Harmonie variety with 19.84%. In all three varieties, the highest values in terms of both viable pollen and germination rates were found in July.

Keywords: Cut rose, Breeding, IKI, Pollen viability

Özet

Melezleme yoluyla gül ıslahında, baba ebeveynin canlı polen ve çimlenme oranı başarılı tozlaşma oranını artırmaktadır. Bu nedenle, melezleme çalışmalarında her zaman iyi bir tozlayıcı çeşide ihtiyaç duyulmaktadır. Bu çalışma, Ankara Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümünde Magnum, Harmonie ve Lady Rose çeşitlerinin çiçeklenme döneminde üç farklı dönemde (Mayıs, Haziran ve Temmuz) polen canlılığı ve çimlenme oranlarını belirlemesi amacıyla yürütülmüştür. Canlı polen oranı "IKI testi", çimlenme oranı "doymuş petri yöntemi" ile tespit edilmiştir. Çalışmadan elde edilen sonuçlara göre en yüksek polen canlılık oranı Temmuz ayında ve Harmonie (%51.00) çeşidinde bulunurken, en düşük polen canlılık oranı ise Haziran ayında Lady Rose (% 16.19) çeşidinde gözlemlenmiştir. Polen çimlenme oranında en yüksek değer ise %19.84 ile Harmonie çeşidinden elde edilmiştir. Canlı polen oranları çeşitler arasında ve aylar içinde önemli farklılıklar göstermiştir. Üç çeşitte de hem canlı polen hem de çimlenme oranı açısından en yüksek değerler Temmuz ayında saptanmıştır.

Anahtar Kelimeler: Kesme gül, Islah, IKI, Polen canlılığı

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Address:[1]Zonguldak Bülent Ecevit University, Çaycuma Food and Agriculture Vocational School, Department of Chemistry and Chemical Processing Technologies
[1]Ankara University, Faculty of Agriculture, Department of Horticulture
E-mail: sbasire.akca@beun.edu.tr

1. Introduction

Roses are one of the most significant plant species worldwide and are used in various fields beyond the ornamental plants sector. Often referred to as the "Queen of Flowers," the rose is the most beloved flower worldwide. Today, despite the existence of more than 37,000 rose varieties that enhance and beautify the quality of life in the world, there has been significant interest and high concern in rose breeding (Salcă Roman et al. 2024). Rose breeding studies particularly aim to improve factors such as new and attractive flower colors, thornless, form, fragrance, long vase life, recurrent blooming, long flower stem length, flower diameter, propagated by cuttings, tolerant to lower temperatures, being able to grow in subtropical conditions, resistance to diseases and pests, high oil content, etc. (Gudin 2000; Gudin 2001; Datta 2018).

Most new rose cultivars are developed through artificial breeding and preservation of viable pollen, which is essential for achieving high fruit set yields (Khosh-Khui et al. 1976; Marcellán and Camadro 1996; Jeong and Park 2022). Pollen preservation directly impacts viability and is strongly influenced by storage conditions (Macovei et al. 2016; Jeong and Park 2022). While rose pollen can remain viable for extended periods under dry and low-temperature conditions, its viability deteriorates under excessive humidity and high temperatures. Specifically, pollen germination capacity declines significantly after 4 to 5 days at temperatures exceeding 25°C (Ogawa 1961; Jeong and Park 2022).

Different species and cultivars of roses exhibit varying levels of fertility (Zlesak 2009), and differences in fruit and seed set are primarily due to pollen viability rather than incompatibility (Visser et al. 1977; Nadeem et al. 2013). Breeders have selected male parents with high productivity (i.e. increasing the number of seeds formed per hybridization) and desirable traits to avoid deficient seed formation (Zlesak 2006; Pipino et al. 2011). Recognition of fertility-related parameters can improve the general efficiency of a breeding program (Pipino et al. 2011). Although there are many steps for forming a new variety after germination, pollen viability and germination rate of the paternal parent increase the rate of successful pollination. Therefore, evaluating paternal parents' pollen viability and germination rate before rose breeding studies is important. This study intended to assess the pollen viability and germination rate of three distinct rose varieties (Magnum, Harmonie, and Lady Rose) in May, June, and July.

2. Material and Method

2.1 Plant Material

The research was conducted in the Cytology laboratory of the Department of Horticulture, Faculty of Agriculture, Ankara University. Three different commercial cut rose varieties (Magnum, Harmonie, Lady Rose) were used as plant material in the study. Images of the cut rose varieties used are given in Figure 2.1 and some of their characteristics determined are given in Table 2.1.

Magnum variety

Harmonie variety

Lady Rose variety

Figure 1. Images of plant materials

Table 1. Cut rose varieties and their characteristics used in the study

Variety	Color (RHS code)	Fragrance	Number of petals/flower	Flower diameter (cm)	Number of pistil/flower	Number of anther/flower
Magnum	Red (N45A)	No	31.00	6.69	89-147	93-133
Harmonie	Salmon Pink (41C)	Yes	20.00	7.12	54-62	112-123
Lady Rose	Pomegranate blossom (40C)	Yes	21.00	7.89	85-89	95-106

2.2 Methods

Pollen viability and germination rate were assessed by collecting pollen from flowers during the 50-60% blooming stage of the varieties within the greenhouse where the research was conducted. After carefully removing the petal leaves of the flowers, the anthers were taken with the help of forceps placed in glass petri dishes, and stored in an incubator at 20°C and 60-65% humidity for 24 hours to allow the anthers to burst. Pollen viability and germination rate were determined with Leica brand DM1000 model light microscope with x20 and x40 objectives.

2.2.1 Pollen viability rate

Pollen viability rate was evaluated using the IKI (Iodine-Potassium Iodide) test. In pollen viability tests, black and brown stained pollen were considered as "absolute viable"; red, orange and light brown pollen as "semi-viable", yellow or colorless pollen as "non-viable" and morphologically deformed pollen as "abnormal pollen" (Figure 2.2). Half of the "semi-viable" pollen was added to the "absolute viable" and the other half to the "non-viable" pollen amounts, and the pollen ratio was calculated (Eti 1990).

2.2.2 Pollen germination rate

The pollen germination rate was evaluated using the 'Saturated Petri Method' (Imrak 2010). In this method, it was prepared by adding 20% sucrose and 10 ppm boric acid to 1% agar medium and the mixture was poured into 2-3 mm thick petri dishes. After solidification, the agar medium was sectioned into four parts, and pollen was applied using a brush. The petri dishes were incubated in a growth cabinet at 24°C and 60% humidity for 8 hours (Imrak 2010). Afterwards, the sections taken from the petri dishes were examined under a light microscope (Figure 2.2). Pollen grains increased to 1.5 times their original size and formed a pollen tube was regarded as germinated (Leus 2005).

Figure 2. Pollen viability and images of germinated pollen grains observed under the microscope

2.2.3 Statistical Analysis

A Randomized Plot Trial Design with four replicates was utilized to assess pollen viability and germination rate. In this study, the data obtained for determining pollen viability and germination rate were analyzed using the IBM SPSS 23 statistical software. The percentage values obtained in the experiment were subjected to angle transformation, and the differences between the means were evaluated using Duncan's Multiple Range Test.

3. Results and Discussion

The knowledge about viable pollen rate of plant materials is one of the important factors for a successful breeding plan. In our study, the data on pollen viability and germination rate of three various cut rose varieties in May, June, and July when hybridization studies were carried out are given in Table 3.1.

Table 2. Viability and germination rate of pollen in cut rose varieties. (%)

Variety	Months					
	May		June		July	
	Pollen viability rate (%)	Pollen germination rate (%)	Pollen viability rate (%)	Pollen germination rate (%)	Pollen viability rate (%)	Pollen germination rate (%)
Magnum	30.69±1.41b	0.77±0.83	45.52±7.19a	3.68±3.91	39.88±5.45b	8.78±0.74b
Harmonie	45.75±7.93a	5.01±5.54	35.20±5.57a	1.44±2.41	51.00±4.76a	19.84±4.89a
Lady Rose	16.83±2.42c	0.53±0.22	16.19±1.65b	1.43±1.98	19.95±1.25c	4.20±0.43b

*Differences between means shown with different letters in the same column are statistically significant ($p \leq 0.05$).

In this study, where the pollen viability and germination rate of some cut rose varieties were examined in different months; the differences between pollen viability rate in May, June and July were found to be significant ($p \leq 0.05$). In our study, only the differences in pollen germination rate in July were observed to be statistically significant ($p \leq 0.05$).

While the viable pollen rate of the varieties in May, June and July varied between 16.19-51.00%, the pollen germination rate varied between 0.53-19.84% (Table 3.1). According to the results, the highest pollen viability rate was recorded in July for the 'Harmonie' variety (51.00%), while the lowest pollen viability rate was observed in June for the 'Lady Rose' variety (16.19%). The highest pollen germination rate was observed in the 'Harmonie' variety, with a value of 19.84% (Figure 3.1).

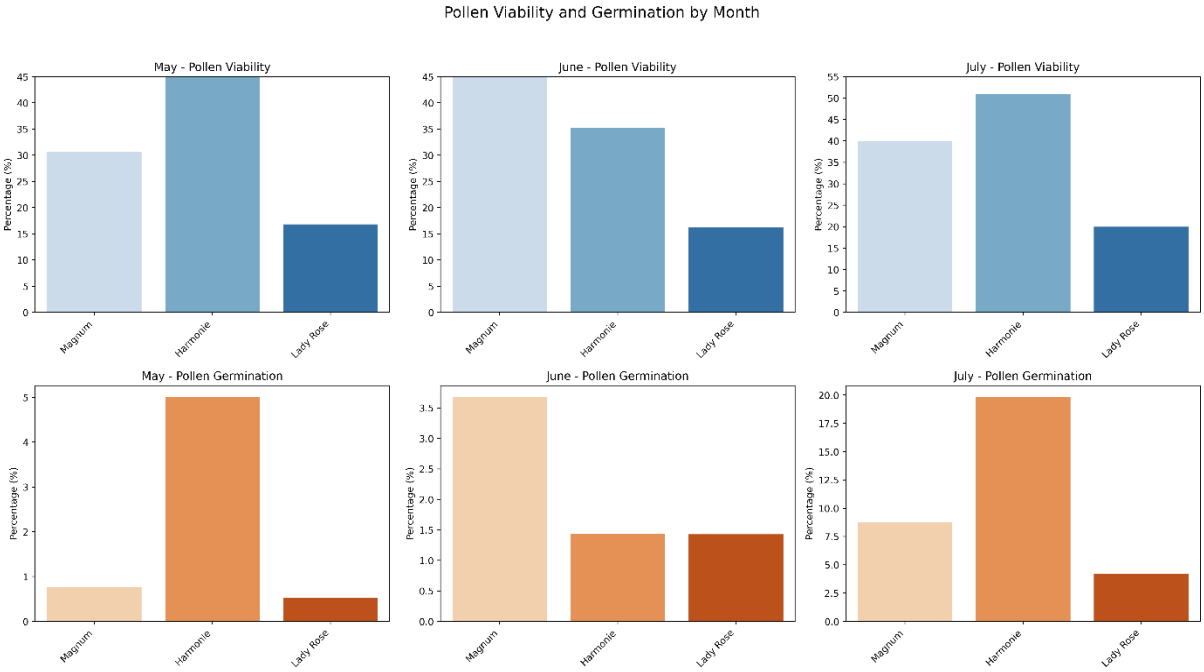

Figure 3. Viable pollen and germination rate of cut rose varieties according to months (%)

In our study, when the viability rate of pollen in cut rose varieties were evaluated in May, the viable pollen was divided into three different statistical groups. The highest pollen viability rate was recorded in the 'Harmonie' variety at 45.75%. In May, the highest pollen germination rate was also observed in the 'Harmonie' variety (5.01%). However, no statistically significant differences were found between this variety and the 'Magnum' (0.77%) and 'Lady Rose' (0.53%) varieties.

When the viability rate of pollen in cut rose varieties in June were considered, the viable pollen rate were divided into two different statistical groups. The first group was formed by Magnum (45.52%) and Harmonie (35.20%) varieties, while the second group was formed by Lady Rose variety with 16.19%. When the pollen germination rate in June was examined the highest rate was observed in the Magnum variety at 3.68%, and no statistically significant difference was noted between this variety and the Harmonie (1.44%) and Lady Rose (1.43%) varieties.

When the viability rate of pollen in cut rose varieties in our study were examined in July the viable pollen rates were divided into three different statistical groups. The highest viability rate was obtained from the Harmonie variety which was 51.00%. When the germination rates in July were examined, the highest was observed in the Harmonie variety with 19.84%, while the lowest was recorded in the Lady Rose variety with 4.20%.

This study revealed significant pollen viability and germination rate variations among the three rose varieties across different months. The 'Harmonie' variety demonstrated greater resilience to changing conditions, consistently exhibiting higher overall viability and germination rate. In contrast, the 'Magnum' variety showed a consistently lower germination rate over time.

Many studies have been conducted to observe pollen viability and germination rate in different rose species and varieties. In the study conducted to find the pollen viability and germination rate of *R. gallica, R. canina, R. elliptica,* and *R. rubiginosa* species, it was stated that pollen viability varied between 22.2-97.5% and pollen germination rate varied between 10.5-86.7% (Koncalova et al. 1976). In *R. villosa* and *R. dumalis* species, pollen viability has been reported to vary between 34.80-48.36% according to the IKI test and 33.90-47.24% according to the TTC test (Erçişli 2007). It was indicated that pollen germination in 11 different hybrid tea rose cultivars varied between 0-46.5% (Pipino et al. 2011). Lakhotia et al. (2011) reported that the pollen viability in different rose varieties varied between 6.45-78.07%. It was indicated that the pollen viability rate of four different rose varieties varied between 11.58-65.73%, and the pollen germination varied between 0-29.93% depending on the month (Anand and Raju 2016). Giovannini et al. (2017) stated that the pollen germination rate of various hybrid tea rose cultivars under different storage conditions varied between 6.0-99.0%. Khan et al, (2021) reported that in their study to find pollen viability and germination in 21 rose varieties, pollen viability ranged between 28.6-67.4%, and pollen germination ranged between 6.99-54.23%. It was observed that the pollen viability of 10 different rose varieties under different storage conditions and at different flowering stages varied between 46.4-84.2%, and the germination varied between 2.42-49.5% (Jeong and Park 2022). It could be understood from the studies reviewed above that pollen viability and germination rate might vary among rose species and varieties. In the studies reviewed, it is seen that pollen viability rate varied between 6.45-97.5%, and pollen germination varied between 0.0% and 99.0%. In our study, pollen viability rate varied between 16.19-51.00%, and pollen germination varied between 0.53-19.84%. The results obtained from our study on pollen viability and germination of some cut rose varieties in different months are generally similar to the studies mentioned above, although the lower and upper limit values differ. The pollen viability and germination rate are largely influenced by the ploidy level of the cultivars (Erçişli 2007; Zlesak, 2009), pollen productivity (Visser et al. 1977), time of flowering after vernalization (Gudin 1992), and temperature during microgametogenesis (Visser et al. 1977;

Zlesak et al. 2007), species and variety differences, climatic conditions (temperature, humidity), collection time (season, flower development period) (Gudin et al. 1991; Zlesak et al. 2007; Martins et al. 2017, Kazaz et al. 2022), method differences (biological or chemical), and pollen storage conditions and duration (Giovannini et al. 2017; Jeong and Park 2022) variable results are obtained. It is stated that the pollen viability of roses generally decreases due to male meiotic or post-meiotic deviations (Jacob and Ferrero, 2003; Macoveı et al. 2016). Differences in the lower and upper threshold values in pollen viability and germination rate may be due to these reasons. Findings from this study, it can be concluded that pollen viability and germination rate were different among various *Rosa* species and cultivars.

4. Conclusion

In the study, viable pollen rates varied significantly among cultivars and within months. The highest values in relation to both viable pollen and germination rate in all three varieties were detected in July. Pollen germination rates also varied among varieties and within months. When pollen germination rates were evaluated the Harmonie variety generally had higher rates compared to other varieties.

Viable pollen rates varied significantly among varieties and within months. The Harmonie variety generally had the highest viable pollen rate, while the Magnum and Lady Rose varieties had lower rates. Especially in July, the viable pollen rate of the Harmonie variety was determined to be 51.00%, which is a significant difference compared to other varieties. According to the data obtained, the Harmonie variety stands out due to its high pollen viability and germination rate within three months. Understanding the variations in pollen viability and germination rate is crucial for hybridization breeding programs. Further research is needed to optimize the key factors contributing to these differences.

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