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Melezleme Islahı ile Halfeti Gülünün *(R. odorata Louis XIV)* Islah Performansının Belirlenmesi

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Anahtar Kelimeler

Halfeti gülü, Karagül, melezleme, Tohum tutumu, Meyve oluşumu, Polen çimlenmesi, Kesme gül

Öz: Melezleme yoluyla gül ıslahında başarıyı etkileyen en önemli faktörlerin başında ebeveyn seçimi gelmektedir. Güllerin ploidi seviyesi, polen kalitesi, meyve oluşumu, meyve başına tohum sayısı ve tohum çimlenme oranı ıslahçının ıslah başarısını belirlemektedir. Bu çalışma melezleme ıslahında ana ebeveyn olarak kullanılan Halfeti gülünün (*R. odorata* Louis XIV) ıslah performansını belirlemek amacıyla yürütülmüştür. Baba ebeveyn olarak altı ticari gül çeşidi (Inferno, Moonlight, Tineke, Black Baccara, Lady Rose, Speelwark) kullanılmıştır. Polen canlılık ve çimlenme oranları, meyve tutum oranı, meyve başına tohum sayısı, tohum ve meyve ağırlığı, tohum çimlenme oranı gibi parametreler incelenmiştir. Ticari kesme güllerde polen çimlenme oranı %7.82 ile %23.55 arasında değişmiştir. En yüksek polen çimlenme oranı sırasıyla Tineke ve Black Baccara çeşitlerinde gözlenmiştir. Kombinasyonların meyve tutumu oranı %63.75, meyve başına düşen tohum sayısı 5.05 ve tohum çimlenme oranı %31.21 olarak belirlenmiştir. PCA ve Heatmap analizlerine göre, Halfeti x Speelwark, Halfeti x Black Baccara ve Halfeti, x Lady Rose en başarılı kombinasyon olarak belirlenmiştirr. Halfeti Gülü, gül ıslah çalışmalarında meyve tutum oranı ve tohum çimlenme oranı bakımından genel ortalamaya yakın değerler sergilemiştir. Gül ıslahı çalışmaları içerisinde yer alan parametreler, Halfeti gülünün ana ebeveyn olarak kullanılma potansiyeline sahip olduğunu göstermektedir.

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Determination of Breeding Performance on Halfeti Rose (*R. odorata* Louis XIV) In Crossbreeding

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Halfeti rose, Black Rose, Hybridization, Seed set, Fruit set, Pollen germination, Cut rose

Abstract: One of the factors influencing rose crossbreeding success is parental selection. The success rate of a breeder is influenced by factors such as ploidy level, pollen quality, seed number per fruit, and seed germination rate, which indicate parental productivity. This study was conducted to determine the productivity of Halfeti Rose (*Rosa odorata* Louis XIV) as a seed parent and its compatibility with the commercial rose varieties in terms of combination. Six commercial-cut roses (Inferno, Moonlight, Tineke, Black Baccara, Lady Rose, Speelwark) were used as pollen parents. Fruit set rate, seed number per fruit, seed and fruit weight, seed germination rate, and pollen germination rate were recorded. Among the commercial-cut roses, the pollen germination rate ranged from 7.82% to 23.55%. The highest pollen germination rate was observed in the Tineke and Black Baccara varieties, respectively. The fruit set rate of combinations where Halfeti Rose was used as the seed parent was determined as 63.75%, the number of seeds per fruit was 5.05, and the seed germination rate was 31.21%. Based on PCA and Heatmap analysis, Halfeti x Speelwark, Halfeti x Black Baccara, and Halfeti, x Lady Rose were found to be the most successful combinations. Halfeti Rose exhibited values close to the overall average in terms of fruit set rate and seed germination rate among rose breeding studies. The parameters mentioned in studies on rose breeding show that the Halfeti rose has the potential to be used as a seed parent.

1. Introduction

Turkey is an important gene center for the rose (*Rosa L.*) genus. Approximately 25% of the roses, which spread over a wide range of altitudes such as 0 to 3000 m, are native to Turkey. Anatolia is inhabited by many different varieties of wild roses, making it challenging to categorize them properly. The flowers of some rose species such as *R. gallica, R. damascena* and *R. alba* have been used in the production of rose oil and rose water for a long time in Anatolia. The fruits of some other species (rosehip) have economic value and are also used for medicinal purposes (Ercisli, 2005; Korkmaz and Özçelik, 2015).

R. odorata species has recently been used in the production of domestic needs such as jam and rose water. This genotype, often known as an "old garden rose," is typically planted for dry rose and rose water manufacturing (Özçelik, 2013). *R. odorata* 'Louis XIV' species, known as 'Halfeti Rose' or 'Black Rose', is a fragrant landscape rose with large flowers, usually single, with a white line in the middle of the petals, dark as buds, and the color of the anthocyanin pigment decreases with the effect of temperature after the flowers open (Baytop, 2001; Özden, 2013; Özçelik, 2018; Özçelik and Koca, 2021). Halfeti Rose, which is the cultivar of this species, is identified with the Halfeti district of Şanlıurfa province because it takes the darkest color in the ecological conditions of this region (Özden, 2013).

In addition to the millions of roses grown in parks, gardens, or pots each year, a large number of cut roses are also used for commercial purposes.Every year, the market is presented with hundreds of different rose varieties by keeping consumer demand front and center. Only if the

desired characteristic is present in the breeding gene pool, breeding can be considered for a specific aim. As a result, preserving genetic resources and providing access to them is a necessary first step.

Breeders and growers prefer to utilize the most fertile parents as seed or pollen donors (Zlesak, 2006). Especially the high rate of fertility as a pollen parent of fragrant rose species such as Rosa damascena, Rosa centifolia and Rosa odorata increased their uses as important genotypes in breeding programs (Dogan et al., 2020). However, there is currently limited knowledge regarding their productivity when used as seed parents. The present study aimed to make crosses between *R. odorata* 'Louis XIV', which is known as Halfeti rose, as a seed parent and some Rosa x hybrida varieties as a pollen parent to evaluate the productivity as a seed parent and determine the compatibility with commercial roses as combinations.

2. Material and Method

The present study was carried out on 15 May 2019 to 30 April 2020 in the rose breeding greenhouse, Department of Horticulture, Faculty of Agriculture, Ankara University, Ankara-Turkey. Halfeti Rose was used as a seed parent in the study, and six different commercial cut roses (Inferno, Moonlight, Tineke, Black Baccara, Lady Rose, and Speel Work) were used as pollen parents. Halfeti Rose and the cut flower varieties were obtained from the rose breeding greenhouse. All varieties of plants received similar quantities of irrigation, and the content of the nutrient solution applied to the plants was given as in Mercurio (2007).

The genotypes of roses utilized as parents have core DNA levels that range from 2.34 to 2.47 pg/2C (Table 1). All of the parents were tetraploids (2n=4x=28) when chromosome counts were performed using the traditional approach to correlate the parents' nuclerar DNA contents and ploidy levels (Kazaz et al., 2022, unpublished data).

Table 1. Nuclear DNA contents and level of ploidy some Rose genotypes

Genotypes	DNA (pg/2C)	Level of ploidy
Halfeti Rose	2.44	2n=4x=28
Inferno	2.47	2n=4x=28
Moonlight	2.38	2n=4x=28
Tineke	2.36	2n=4x=28
Black Baccara	2.34	2n=4x=28
Lady Rose	2.39	2n=4x=28
Speelwark	2.42	2n=4x=28

*Ploidy levels of all species and cultivars were determined by using PARTEC (CyFlow Space) brand flow cytometry device in the Plant Genetics and Cytogenetics laboratory of Namık Kemal University, Faculty of Agriculture, Department of Field Crops. Core DNA analyses were carried out using fresh leaf (full size and shape) tissues from plants. Ploidy levels were also confirmed by the classical method of chromosome counting (Tuna, 2016).

2.1. In vitro pollen viability and germination test

Blooms of pollen parents were harvested when they were between one-third and one-half open and immediately anthers were removed from the receptacles, and pollen was held for dusting at 24 °C and 60-65% humidity for 24 h. The IKI (iodized potassium iodide) test was used to determine the viability of pollen. For this purpose, IKI solution was prepared by dissolving 1 g of potassium iodide (IKI) and 0.5 g of iodine (I) with 100 ml of distilled water. After 1 drop of the solution was dropped on the slide, pollen was sprinkled on each drop and counted under the microscope after 5 m. Pollen grains dyed black and dark brown were considered "absolutely viable", pollen grains dyed orange, red or light brown were considered "semi-viable", and pollen grains stained yellow or appearing colorless were considered "non-viable" (Eti, 1990), and viable pollen rate calculated.

Pollen germination was conducted in Petri dishes using 20% sucrose and 10 ppm boric acid in 1% agar media with four replications at 24 °C and 60 % humidity. A brush was used to evenly apply pollen to the medium. Under a light microscope, the pollen germination rate was determined after the 8-hour incubation period. A pollen tube was determined to have germinated when its length was at least 1.5 times the pollen's diameter with a light microscope (x 100) and 300 pollen was calculated for each field.

2.2 Hybridization

In the hybridization stage, when the flowers blooms were opened at a rate of 50%-60%, the petals were removed by hand and anthers were taken in order to prevent self-fertilization, emasculation was performed. The emasculation process was carried out 1 d before pollination, and immediately after emasculation, the top of the stigma was covered with a paper bag. When the flowers of the varieties selected as the male parents at the same time with the seed parent's emasculation opened 1/2-1/3, the petals were removed and the anthers were taken and placed in glass Petri dishes. Anthers were kept in a climate cabinet at +24^{o}C and 60% humidity without closing the petri dish overnight. Pollination was carried out by applying the pollen to the stigma of the plants to be used as the seed parent with the help of a brush. After dusting, the top of the flower was insulated with a light-transmitting paper bag, and the paper bag was removed 4 d after dusting. Along with the dusting process, labeling was done after each dusting. In November 2019, mature fruits (hips) were gathered and the seeds were taken out of the fruits. In order to break dormancy and promote germination, seeds were placed moistened peat at 4°C for 4–12 weeks of cold stratification. After the stratification, the seed was sown in a mixture of peat and perlite (1:1)

Data collection: Data on fruit set rate (%), seed number per fruit, seed set rate, fruit weight (g), seed weight (mg) and seed germination rate were calculated for each cross starting with cross-pollination.

Data analysis: IBM SPSS Statistics version 20.0 software was used for conducting the statistical analysis. The data were subjected to angular transformation before performing an analysis of variance (ANOVA).

To assess mean differences, Duncan's test was employed with a significance level of $p \leq 0.05$. Additionally, a heat map was generated to visualize hierarchical clustering, and the values for each combination were standardized. Principal component analysis (PCA) was also conducted, and a biplot was created using XLSTAT to provide a more accurate approximation of the relationships among the traits and combinations, surpassing the coefficient of correlation.

3. Results

3.1. In vitro pollen viability and pollen germination rate

In this study, where the quality characteristics of pollen were examined, the pollen viability rate (%) showed statistically significant differences ($p<0.05$). The pollen viability rate of the varieties ranged from 52.76% to 58.92% (Table 2).

In the study, the difference between pollen germination rates of some cut rose cultivars ($p<0.05$) was found to be statistically significant. The Tineke variety had the highest pollen germination rate (23.55%), while the Inferno had the lowest rate (7.82%) (Table 2).

Table 2. Pollen viability and pollen germination rate of some cut rose cultivars

Genotypes	Pollen viability rate (%)	Pollen germination rate (%)
Inferno	31.03 c	7.82 d
Moonlight	10.69 d	12.85 bc
Tineke	32.97 c	23.55 a
Black Baccara	47.75 b	19.76 ab

Lady Rose	35.70 bc	11.57 cd
Speelwark	63.31 a	15.98 bc

($p<0.05$)

3.2 Crossability index of combinations

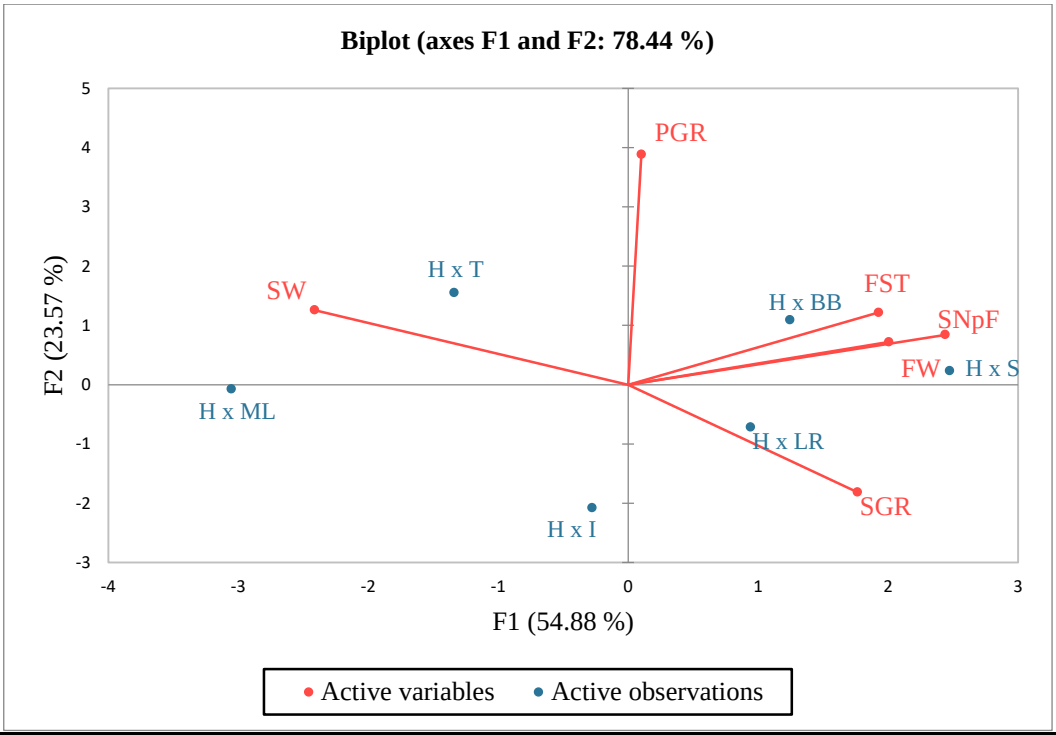
In the study, the combination of Halfeti x Blak Baccara (100%) produced the most fruit (hip) development. Halfeti x Speelwark (78.57%) and Halfeti x Inferno (67.24%) came in second and third, respectively, this combination. Halfeti x Moonlight was found to have the lowest fruit set (20%). The Halfeti x Speelwark hybrid produced the maximum seed per fruit. This combination was followed by the Halfeti x Lady Rose with 7.0 seeds and the Halfeti x Black Baccara combinations with 6.35 seeds, respectively, while Halfeti x Moonlight (1.50) was shown to have the fewest seeds per fruit (Table 3). In the study, the average fruit weight ranged between 2.36 g (Halfeti x Inferno) to 4.35 g (Halfeti x Speelwark), and the average seed weight changed from 68.58 mg (Halfeti x Speelwark) to 133.45 mg (Halfeti x Tineke). Halfeti x Inferno hybrid combination had the highest seed germination rate of 39.27%, while the Halfeti x Moonlight hybrid combination had the lowest seed germination rate of 20% (Table 3).

Table 3. Fruit set rate, seed set rate, seed number per fruit, and seed germination rate of combinations

Combination	FSR (%)	SNpF (pcs/fruit)	FW (g)	SW (mg)	SGR (%)
Halfeti x Inferno	67.24 c	2.67 cd	2.36 c	85.52 b	39.27 a
Halfeti x Moonlight	20.00 e	1.50 d	2.82 bc	132.78 a	20.00 e
Halfeti x Tineke	66.67 c	3.63 c	2.64 bc	133.45 a	30.00 d
Halfeti x Black Baccara	100.0 a	6.35 b	3.40 ab	74.76 b	29.69 d
Halfeti x Lady Rose	50.00 d	7.00 b	3.88 a	77.16 b	32.63 c
Halfeti x Speelwark	78.57 b	9.18 a	4.35 a	68.58 b	35.64 b
Avarage	63.75	5.05	3.24	95.38	31.21

($p<0.05$), FST: Fruit set rate, SNpF: Seed Number per Fruit, FW: Fruit weight, SW: Seed weight, SGR: seed germination rate

Principal component analysis (PCA) was carried out and a biplot was established for a better approximation than the coefficient of correlation to describe the crossability success. Given an eigenvalue larger than 1, the first (F1), second (F2), and third (F3) principal components accounted for 54.88%, 23.57%, and 17.73% of the total variation, totaling 96.18%. The PCA-biplot results indicated a positive correlation among the pollen germination rate, fruit set rate, seed number per fruit, and fruit weight because they were placed on the same side and had similar vector lengths. Seed weight and seed germination rate had a negative correlation. According to the PCA analysis, the combinations, in order from the most successful to the least: Halfeti x Speelwark > Halfeti x Black Baccara > Halfeti x Lady Rose > Halfeti x Inferno > Halfeti x Tineke > Halfeti x Moonlight olarak sıralanabilir(Figure 1).



	F1	F2	F3	F4	F5
Eigenvalue	3.293	1.414	1.064	0.225	0.005
Variability (%)	54.878	23.566	17.726	3.744	0.087
Cumulative %	54.878	78.443	96.169	99.913	100.00

Figure 1. Eigenvalues for principal components, and PCA-biplot of cross combinations and examined parameters. ML: Moonlight, H: Halfeti Rose, LR: Lady Rose, I: Inferno, S: Speelwark, BB: Black Baccara, T: Tineke; FST: Fruit set rate, SNpF: Seed Number per Fruit, FW: Fruit weight, SW: Seed weight, SGR: seed germination rate

Heat map analysis based on the crossability indexes of different cross combinations divided the examined parameters into two main groups. Seed weight and pollen germination rate were included in the 1st major group. The 2nd major group was divided into two subgroups. The first subgroup included the number of seeds per fruit and the fruit weight, while the second subgroup included the fruit set rate and seed germination rate. Combinations were divided into 2 main groups and 1 main group included Halfeti x Moonlight and Halfeti x Tineke combinations. The two main groups were divided into 2 subgroups and the 1st subgroup included the Halfeti x Inferno combination, while the 2nd subgroup included Halfeti x Black Baccara, Halfeti x Lady Rose, and Halfeti x Speelwark. The combinations of Halfeti x Black Baccara, Halfeti x Lady Rose, and Halfeti x Speelwark were good combinations in terms of seed germination rate, fruit set, fruit weight, and the average number of seeds per fruit. The combinations of Halfeti x Moonlight and Halfeti x Tineke stood out in terms of seed weight and pollen germination rate. Similar to the PCA results, it was determined that Halfeti x Moonlight was the combination with the lowest success in terms of other characteristics except for seed weight, and Halfeti x Speelwark was the most successful combination in general in terms of other characteristics except seed weight.

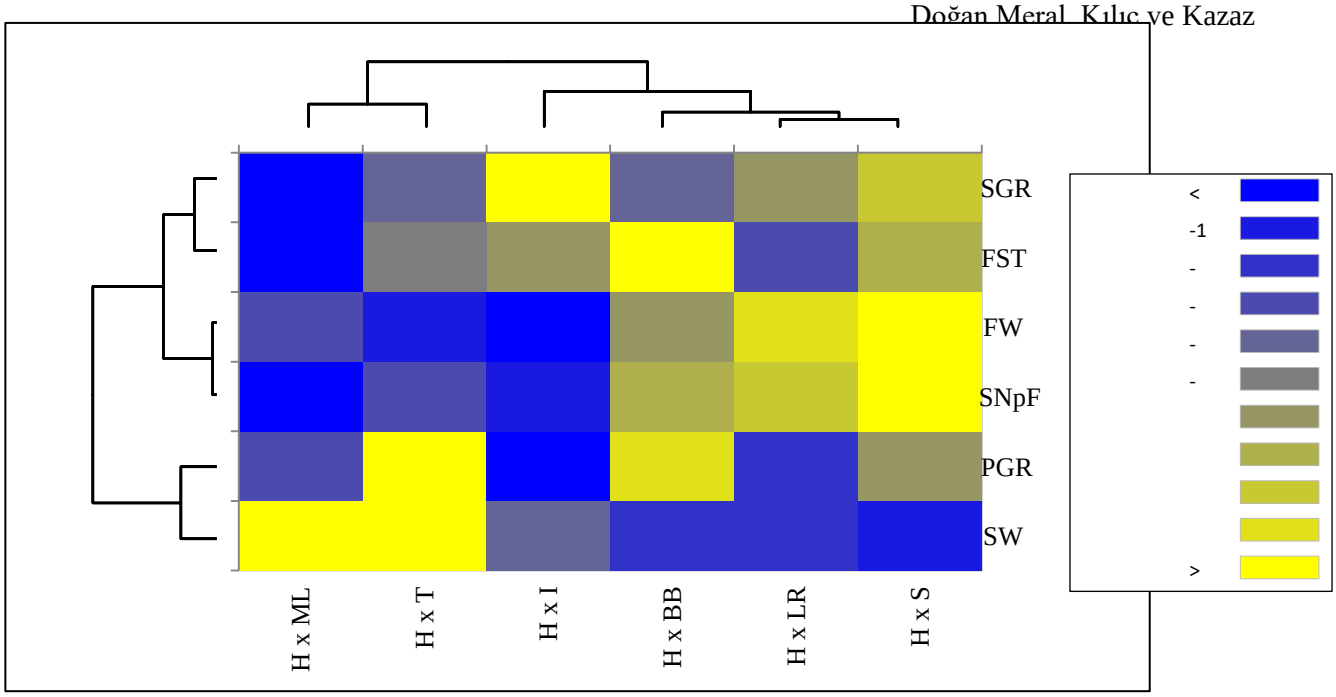


Figure 2. Hierarchical clustering heat map of the examined parameters for each of the cross combinations. The color-coded scale indicates an increase from blue to yellow ML: Moonlight, H: Halfeti Rose, LR: Lady Rose, I: Inferno, S: Speelwark, BB: Black Baccara, T: Tineke

4. Discussion

A modern hybrid rose varieties are the product of breeders' tenacious efforts over a long period of time. Due to the fact that modern hybrid rose types can manifest as triploids and tetraploids, cross-breeding can also cause changes at the ploidy level (Rout et al., 1999). The likelihood of seeds and viable embryos developing successfully decreases as the number of chromosomes rises. In this study, it was determined that ploidy levels would not have an effect on obtaining sterile seeds, because all parents have same ploidy level. According to Spethmann and Feuerhahn (2003), rose breeders often perform suitable crosses between tetraploid roses (garden and cut-flower roses). Between *R. damascena* and *R. gallica,* as well as between *R. centifolia* and *R. rugosa,* successful cross-breeding has been accomplished. Better pollinators are continuously needed by rose breeders for cross-breeding. The rate of successful cross-pollination increases even though there are many phases involved in developing a new cultivar, from pollination to seed development and ultimately seed germination. Because male parent pollen grains are more viable and have a higher rate of germination. The highest pollen viability rate was Speelwark, while the highest pollen germination rate was Tineke in the study. Chemical and biological techniques resulted in variations in each cultivar's viability. Chemical approaches did not show similarities with biological methods, according to research by Parfitt and Ganeshan (1989). Although immature pollen can be chemically colored, there is typically a linear link between pollen viability and germination rate (Martins et al., 2017). Therefore, compared to biological approaches, higher viability levels can be observed. Similarly, some researchers have claimed that variations in pollen viability and germination in parents account for the number of seeds per fruit (Visser et al., 1977; de Vries and Dubois, 1987; Gudin and Arene, 1992; Zlesak et al., 2007). One factor in the development of fruits following pollination is the quality of the pollen. The development of rose hips at the seed parent is positively correlated with the pollen fertility of the pollination parent. The included species determines the extent of the association. Pollen described as fertile in the literature has a germination ability of over 25% (Spethmann and Feuerhahn, 2003). Accordingly, although all pollen donors showed low fertility, the combinations produced a very successful fruit set rate (except the Halfeti x Moonlight hybrid combination). In this case, it can be said that the Halfeti rose, which is used in hybrid combinations, shows very fertile properties and increases the success of fruit and seed formation. Combination of Halfeti and Moonlight could not produce well-formed fruits and seeds. Incompatibility in hybrid combinations, dormancy, irregular meiotic divisions, weak zygote, and embryo forms, differences in

the fertility of the types employed as the main parents, or an accumulation of deleterious alleles could all be contributing factors in this circumstance. (Anderson and Byrne, 2007; Dogan, 2022, Meral et al., 2022). The number of seeds obtained as a result of pollination with paternal parents with high pollen germination rate; a combination with a paternal parent with a lower pollen germination rate than a lower seed count is thought to indicate incompatibility. In general, the number of fruits in the ranges specified in rose breeding studies and the seed germination rate show that the Halfeti rose has the potential to be used as a seed parent. The rather low average seed number is thought to be due to the generally low stigma number of the Halfeti rose. In addition, it is thought that more seeds can be obtained with more productive and more compatible paternal parents. Love et al. (2016) stated that the receptivity of parents and compatibility greatly affect the success of crossbreeding. Falque et al. (1995) reported that there is a strong relationship between the amount of pollen applied to the stigma and the number of fruit sets and seeds. In addition, it has been stated that there is a parallel relationship between the stigma diameter and fruit-bearing capacity of roses and the average number of seeds per fruit set and fruit (Atram et al., 2015). It has been reported that *R. noisettiana* achieves approximately 100% fruit set as a result of the crossing of the parent, 10 different pollen donors with different pollen viability and germination, and it has been reported that the fertile parent increases the breeding success in breeding studies (Doğan, 2022). The variation in achene count between genotypes and the dormancy trait of roses with hard seed coats may be the causes of the observed variations in seed germination rates in the study. These features prevent the germination of seeds and reduce success by reducing genetic variation (Zlesak, 2006). Additionally, the presence of abscisic acid (ABA) in the pericarp of rose seeds inhibits germination (Bo et al., 1993). Rose seed germination rates have been recorded to range from 0 to 100% by Grossi and Jay (2002), 15.4% to 37.1% by Pipino et al. (2011), 0% to 93.40% by Abdolmohammadi et al. (2014), and 10.6% to 62% by Ueckert (2014). In our study, germination rates were between 20-39.27% and showed similar results with related studies.

5. Conclusion

In this study, which was carried out to evaluate the effect of the use of Halfeti rose by crossbreeding breeding method on breeding performance, although the fertility of the paternal parents used in the combinations created was low, the success of the crossing breeding increased due to the fertile property of the Halfeti rose used as the seed parent. The Halfeti rose has the potential to be utilized as a seed parent, as evidenced by the number of fruits that fall within the ranges described in studies on rose breeding and the seed germination rate. Considering that resistance to diseases and pests is one of the key goals of breeding, using naturally occurring species from the area in breeding programs is crucial for the next generation and also this can be advantageous because it increases the genetic material of potential.

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