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Transmission of Flower Traits and Fertility in Black Rose *x* Hybrid Tea Rose Cross Combination

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Breeding studies of scented cut roses have gained significance recently. The Black rose, a naturalized old garden rose in Türkiye, is a valuable genetic resource for breeding programs due to its pleasant scent. This study investigated the fertility of the Black rose (*Rosa odorata* cv. Louis XIV, Halfeti rose) as a seed parent and its ability to transmit flower traits to offspring. Four commercial cut rose varieties with known pollen germination rates were used as pollen parents. Each combination underwent at least 33 pollinations, and the fruit set rate, the average seed number per fruit, and the seed germination rate were examined. Both parents and one-year-old F_1 progeny were evaluated for petal number, scent, and flower color traits during two flowering periods. Cross-pollination resulted in fruit set rates ranging from 70.21% to 100.0%, average seed numbers per fruit ranging from 5.0 to 10.0, and seed germination rates ranging from 4.42% to 21.69%. Morphological characterization revealed that 5.05% of the progeny exhibited moderate to intense scent, 42.42% had sour cherry-colored petals, and petal numbers ranged from 9 to 54. Considering the overall efficiency of rose breeding programs, the findings demonstrate the promising fertility of the Black rose and its relative success in forming efficient combinations with hybrid tea roses. However, it also suggests that the Black rose is not as successful in transmitting the scent to its offspring as in transmitting the flower color.

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Introduction

Rose (*Rosa* sp.) is often referred to as the queen of flowers and is the most traded cut flower in the world (Kazaz et al., 2020). Thousands of new cut rose varieties have been developed from the past to the present, and the development continues to meet constantly changing consumer expectations through breeding studies. Previous breeding programs aimed to improve traits such as flower stem length, flower diameter, yield, disease tolerance, and vase life (Karagüzel et al., 2013). In recent years, due to increased consumer demands for scented cut roses, breeders have focused their efforts on transmitting scent to cut roses by including scent as one of the selection criteria (Gudin, 2003; Cherri-Martin et al., 2007).

Since most commercial cut rose varieties are scentless, breeders have turned to expand the gene pool by incorporating scented wild species. Most of the old garden roses and wild species found worldwide are scented. Among these species, *Rosa odorata* XIV Louis, which has had limited studies, is an important genetic resource with sour-cherry color flowers close to black as well as its scent trait (Baytop, 2001; Özçelik and Korkmaz, 2015; Hatipoğlu and Ak, 2021). Known as the Black Rose or Halfeti Rose (In Turkey, it is called Halfeti Rose because it is found naturalized in the Halfeti district of Şanlıurfa), this species is not directly used as a cut flower in the global market as it does not meet commercial quality criteria as a cut flower. Furthermore, little is known about its effectiveness in breeding programs for developing new rose varieties.

Parent selection is the most crucial stage in increasing the success of rose breeding programs, where the success rate of developing new varieties decreases to 0.002% due to the challenges in sexual reproduction (Chaanin, 2003; Kılıç, 2023). Genotypes to be used as pollen parents are expected to have high pollen quality, whereas genotypes to be used as seed parents require more fruit and seed sets and a high seed germination rate. Knowing the compatibility between parents is also crucial for success. In addition, parental selection should be based on the maternal or paternal control of desired traits in hybridization to increase the chance of obtaining a promising genotype (Xing et al., 2014). This study aimed to determine the fertility of the Black rose as a seed parent, its ability to transmit its characteristics to offspring, and its compatibility with commercial cut rose varieties for breeding scented cut roses.

Material and Method

In this study, conducted between 2018 and 2020 to determine the fertility of the Black rose as a seed parent and its ability to transmit its flower characteristics to the progenies, cross-pollination studies were carried out and quantitative and qualitative traits of flowers were measured in a plastic greenhouse of the Department of Horticulture, Faculty of Agriculture, and Ankara University. The pollen germination rates of the pollen parents were determined in the cytology laboratory of the same department.

Plant Material, Planting the Parents, and Cultural Practices

The plant material for this study consisted of four commercial hybrid tea roses of the species *Rosa x hybrida*, which were used as cut roses, and *Rosa odorata* cv. Louis XIV, also known as the Black rose, is an old garden rose. The Black rose was used as the seed parent, while the commercial cut roses Myrna, Annakarina, First Red, and Magnum were chosen as the pollen parents, all produced in Türkiye. All genotypes were tetraploid, with a chromosome number of 2n=4x=28 (Kazaz et al., 2020; unpublished data). Some flower traits of the parents are given in Table 1.

All parents were obtained as one-year-old plants from a company in Şanlıurfa. The planting of plants, greenhouse growing conditions, and cultural practices such as irrigation, fertilization, and pest control were carried out following the procedures described by Kılıç (2023).

Cross-pollination and Evaluation of Fertility

The period of cross-pollination was from May 1 through June 30. A total of 4 combinations were created, and at least 33 crossings were performed in each combination. The emasculation of the seed parent's blossoms took place when approximately one-third of the flowers were open in the early morning. The following emasculation, the flowers were covered with a paper bag (Chimonidou et al., 2007). Simultaneously, anthers were collected from the pollen parents. Pollen grains were expected to be dispersed from the anthers for one day in a growth chamber with a temperature of 24ºC and a humidity level of 60%. The following day after emasculation and anther collection, when the pollen was dispersed, pollination was performed by applying the pollen to the stigmas using a brush. The pollinated plants were then covered with paper bags for four days. Both the apical bud and the blooms developed on the shoots of the axillary buds were pollinated.

Fruits that had reached the harvest maturity stage, indicated by a change in color from green to orange-red and the onset of browning on the flower stalk, were collected on average 150 days after pollination. The total number of fruits was recorded, and the fruit set rate (FSR) per combination was calculated as a percentage. Subsequently, the harvested fruits were transported to the laboratory, carefully sliced using a sharp knife to extract the seeds. The average number of seeds per fruit was determined for each combination (SNpF). The sorted F_1 progeny seeds obtained from the fruits were subjected to a period of warm stratification at 20–24°C for 5 weeks, followed by cold, moist stratification at 4±1°C for 100 days (Haouala et al., 2013). Following the cold, moist stratification period, the stratified seeds were placed in vials filled with peat and placed in a plastic-covered greenhouse with temperatures ranging from 18°C to 21°C. During the germination process, the seeds were irrigated with fog irrigation. According to the criteria set by Nadeem et al. (2015), seeds were considered germinated when the cotyledon and hypocotyl were visibly above the growing medium. The number of germinated seeds was recorded, and the seed germination rate (SGR) was calculated as a percentage using the following formula:

$$\text{SGR (\%)} = \frac{Number\ of\ seeds\ germinated}{\text{Number of seeds sown}} x\ 100$$

In addition to indexes indicating the combination efficiency, and fertility of the seed parent, such as fruit set rate, seed number, and seed germination rate; the pollen germination rate, which directly demonstrates the fertility of the male parent, was also determined. The germination rates of the pollen were determined using the petri dish method (Imrak, 2010; Kazaz et al., 2020). Plastic Petri plates were filled with a germination medium consisting of 20% sucrose, 10% boric acid, and 1% agar solution. The medium was poured into the plates to a thickness of 2 mm. After dispersing the pollen onto the divided germination medium in four sections, the Petri dishes were incubated at 24ºC and a humidity of 60%. After 8 h, the pollen grains were counted under a microscope and those that formed a pollen tube longer than 1.5 times their diameter were considered germinated. The pollen counts were conducted using a Leica DM1000 microscope equipped with 40x and 100x magnification lenses, and imaging equipment.

Table 1. Flower characteristics of parents

Genotypes	Scent	Petal number (pcs/per flower)	Flower color (RHS)
Myrna	Scentless	63 / very full blooms	Red – N45B
Annakarina	Scentless	39 / full blooms	Pink – NN137B
Magnum	Scentless	28 / full blooms	Red – N45A
First Red	Scentless	29 / full blooms	Red – N45A
Black rose	Strong	27 / full blooms	Dark Red / Cherry rot – 187B

All traits of the parents were determined using the method described in the ‘Qualitative and Quantitative Traits Examined in F_1 Progenies’.

Table 2. Fruit set rate (FSR), seed number per fruit (SNpF) and seed germination rate (SGR) of cross-combinations

♀ x ♂	PN	FSR (%)	SNpF	SGR (%)
Black rose *x* Myrna	33	100.0	8	4.42
Black rose *x* Annakarina	36	100.0	5	15.31
Black rose *x* Magnum	47	70.21	7	29.39
Black rose *x* First Red	43	100.0	10	21.69
CV (%)	-	0.139	0.240	0.517

PN: number of pollination, CV: variation coefficient, P≤0.05.

Qualitative and Quantitative Flower Traits Examined in F_1 Progenies

Traits were examined in one-year-old F_1 progenies under standardized conditions during two flowering periods. The specific details of the traits examined are as follows:

Scent: The scent of the F_1 progenies was assessed using the magnitude estimation procedure, described by Brattoli et al. (2011). The evaluation occurred in a well-ventilated and isolated room in the morning when the flowers were fully bloomed. The scent intensity was categorized into four classes. The first class was categorized as "scentless and barely perceptible" This class represented flowers that with no discernible scent or had a very faint scent that was barely detectable. These flowers lacked a noticeable fragrance. The second class was labeled as "slightly scented". Flowers in this class had a mild scent that was noticeable but not particularly strong or overpowering. While the fragrance was present, it was not prominently featured and had no a significant impact. The third class was classified as "moderately scented" Flowers in this category exhibited a moderate level of scent, where the fragrance was present and easily noticeable. These flowers emitted a distinct aroma that could be recognized without much effort. The fourth and final class was named "strong-scented" Flowers in this class emitted a strong and highly noticeable scent that was potent and distinct. The fragrance was powerful and could be easily perceived, even from a distance. To assess the scent intensity, the scent of the seed parent was used as a reference or standard for comparison.

Petal number: The number of petals was counted for at least two flowers in each genotype when the stigma and anthers were visible in the flowers of F_1 progenies (Koning-Boucoiran et al., 2012). Only the petals of terminal blooms were considered. The F_1 progenies were classed using the American Rose Society's criteria: singled (4-8 petals), semi-double (9-16 petals), double (17-25 petals), full (26-40 petals), and very full (41 petals) petals. These standards were deemed more extensive than UPOV's and more appropriate than commercial quality requirements (ARS, 2020).

Flower color: The color scale (RHS2015 123A - 6th Edition) prepared by the 'Royal Horticultural Society' and accepted as a standard reference was used to define flower colors in flowers belonging to F_1 genotypes (from Tucker et al., 1991, as cited in Stolker, 2009). Color definitions were made in daylight, and the inner surface of three petals in the second row from the outside was measured in each flower. For this objective, at least two flowers were used.

Data Analysis

The experiments on pollen germination rate were conducted using a completely randomized design with four replicates. The counting process involved selecting two random slices from each petri dish, and four different areas on each slice were examined. On average, 250 pollen grains were counted per area. To analyze the data, IBM SPSS Statistics version 20.0 was used. The angular transformation was applied to the data, and an analysis of variance was performed. Mean differences were determined using Duncan's test with a significance level of $p < 0.05$. For recording and processing the fertility index data, measuring flower traits, and calculating the variation coefficient, Microsoft Office Excel 2021 and XLSTAT were utilized. These software tools were used to manage and analyze the data related to the mentioned parameters. The distributions of the flower trait data were evaluated as percentages and agglomerative hierarchical cluster analysis (AHC) was performed with XLSTAT to evaluate the proximity of offspring to their seed or pollen parents.

Results

Fertility of Parents and Cross-Combinations

Based on the analysis of variance, a significant difference was observed among the pollen germination rates of the pollen parents. The First Red exhibited the highest rate of germinated pollen (31.87%), whereas the lowest rate was observed in Annakarina (7.96%) (Figure 1).

In combinations of the Black roses with four different hybrid tea roses, the fruit set rate and the seed number per fruit were relatively stable, with a coefficient of variation of 13.9% and 24.0%, respectively. The fruit set rate ranged from 70.21% to 100.0%, and the average seed number per fruit ranged from 5 to 10. In terms of seed germination rate, a high variability (CV: 51.70%) was determined between the combinations. The highest seed germination rate was determined with a value of 29.39% in the combination in which the Magnum variety was the pollen parent. The lowest germination rate was obtained from the hybrid combination with Myrna (Table 2).

Transmission of Flower Traits to Progenies

The seedlings resulting from the combinations in which Myrna was used as the pollen parent did not survive, and thus, no data on flower traits could be obtained. Percentages of scent, flower color, and petal number per combination of F_1 progeny obtained from other crosses are presented in Figures 2, 3, and 4. Regarding scent traits, most progeny (84.85%) were classified as scentless and barely perceptible. The hybrid genotype with a strong scent was determined only in the combination of Black rose x First Red (4.35%). The percentage of hybrid progeny with scent intensity similar to the seed parent was determined to be 2.02% in the combinations of Black rose x hybrid tea rose (Figure 2).

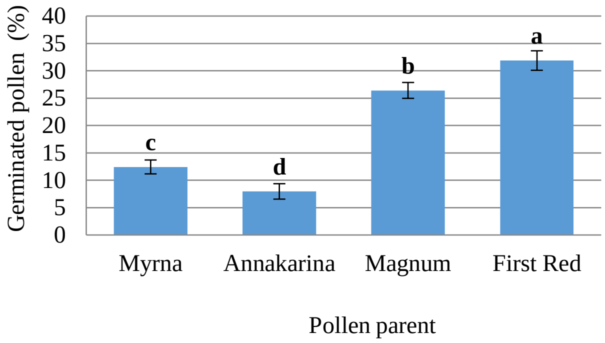

Figure 1. Pollen germination rates of pollen parents (P≤0.05)

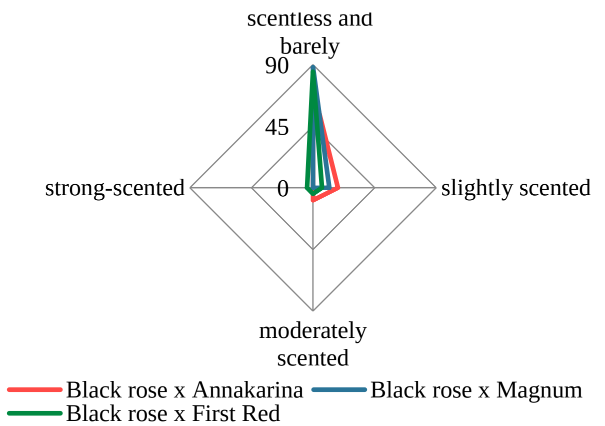

Figure 2. Radar chart showing scent distributions per cross combination

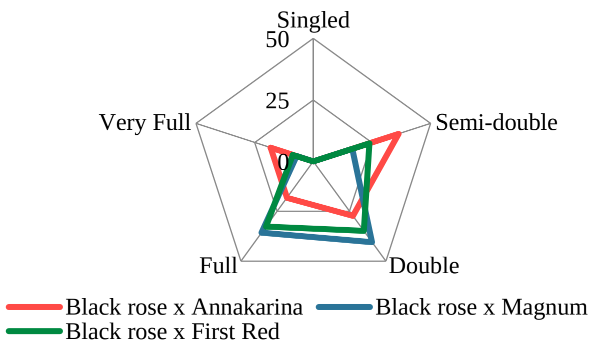

Figure 3. Radar chart showing petal number distributions per cross combination

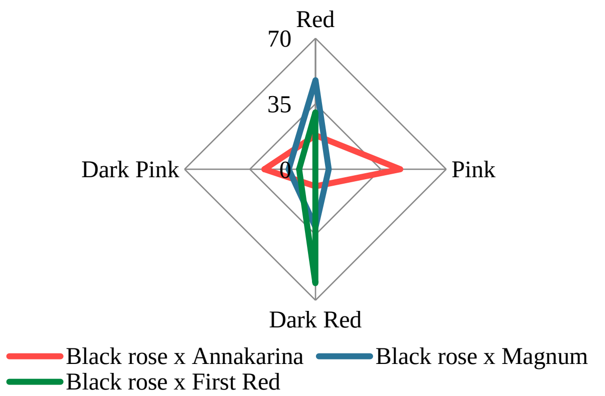

Figure 4. Radar chart showing flower color distributions per cross combination

The petal number of F_1 progenies in Black rose x hybrid tea rose combinations varied between 9.0 and 54.0. No progeny was in the single-petal class. However, the percentage of hybrid progenies with fewer petals than both seed and pollen parents was 22.22%. A positive heterosis and a negative heterosis were observed, and the petal number in 9.09% of F_1 progenies was more than the petal number in both parents. 36.36% of the progeny were in the double petal class and 32.32% in the full petal class (Figure 3).

The flower color of the F_1 progeny varied in four different groups, ranging from pink to cherry bruise (dark red). In the Black rose x hybrid tea rose cross, 42.42% of the progeny had petal colors similar to the seed parent. The rate of obtaining progeny with cherry bruise-colored petals increased due to pollinating Black rose with pollen parents of red petal color (Black rose x Magnum: 30.95%, Black rose x First Red: 60.87%). When pollinated with a pink-colored pollen parent (Annakarina), a high rate of pink (45.45%) and dark pink-colored (27.27%) hybrid progenies were obtained (Figure 4).

The results of the hierarchical cluster analysis evaluating the relationship between the F_1 progeny and the parents indicate the formation of two main clusters and a between-cluster difference of 43.51%. The first main cluster includes 56.56% of the F_1 offspring and the seed parent, while the second main cluster consists of 43.44% of the offspring and the pollen parents (Figure 5). In the first cluster, genotypes exhibited a similarity of 21.05% in scent traits, 40.35% in petal number, and 98.25% in flower color. In the second cluster, there is a similarity of 91.30% in scent, 50.0% in petal number, and 100.0% in flower color.

Discussion

In this study, the fertility of the Black rose as a seed parent and its ability to transmit its traits to offspring were investigated. The fruit set rate, the seed number per fruit, and the seed germination rate varied according to the pollen parent. Previous research conducted by several researchers has reported variations in fruit and seed set rates and seed germination rates based on different combinations resulting from hybridizations among various rose species and/or varieties (Abdolmuhammadi et al., 2014; Nadeem et al., 2015; Farooq et al., 2016; Doğan, 2022). In this study, under the same conditions, the differences among the combinations may be attributed to variances in the genetic makeup and pollen quality of the pollen parents, and differences in parental compatibility.

It has been reported that the average fruit set rate in modern roses is generally below 50% (Gudin, 2003), and the average number of seeds per fruit varies between 0 and 50 in different rose genotypes (Zlesak, 2007). In this study, which used the Black rose as the seed parent, the average fruit set rate was 92.55%, and the average number of seeds per fruit was 7.5. When comparing these results with previous studies, it was observed that the fruit set rate was significantly higher, while the seed set rate was lower than the general average. The lower seed set rate of the Black rose may be attributed to its lower number of stigmas compared with many modern roses. In roses, each stigma is expected to produce at least one seed.

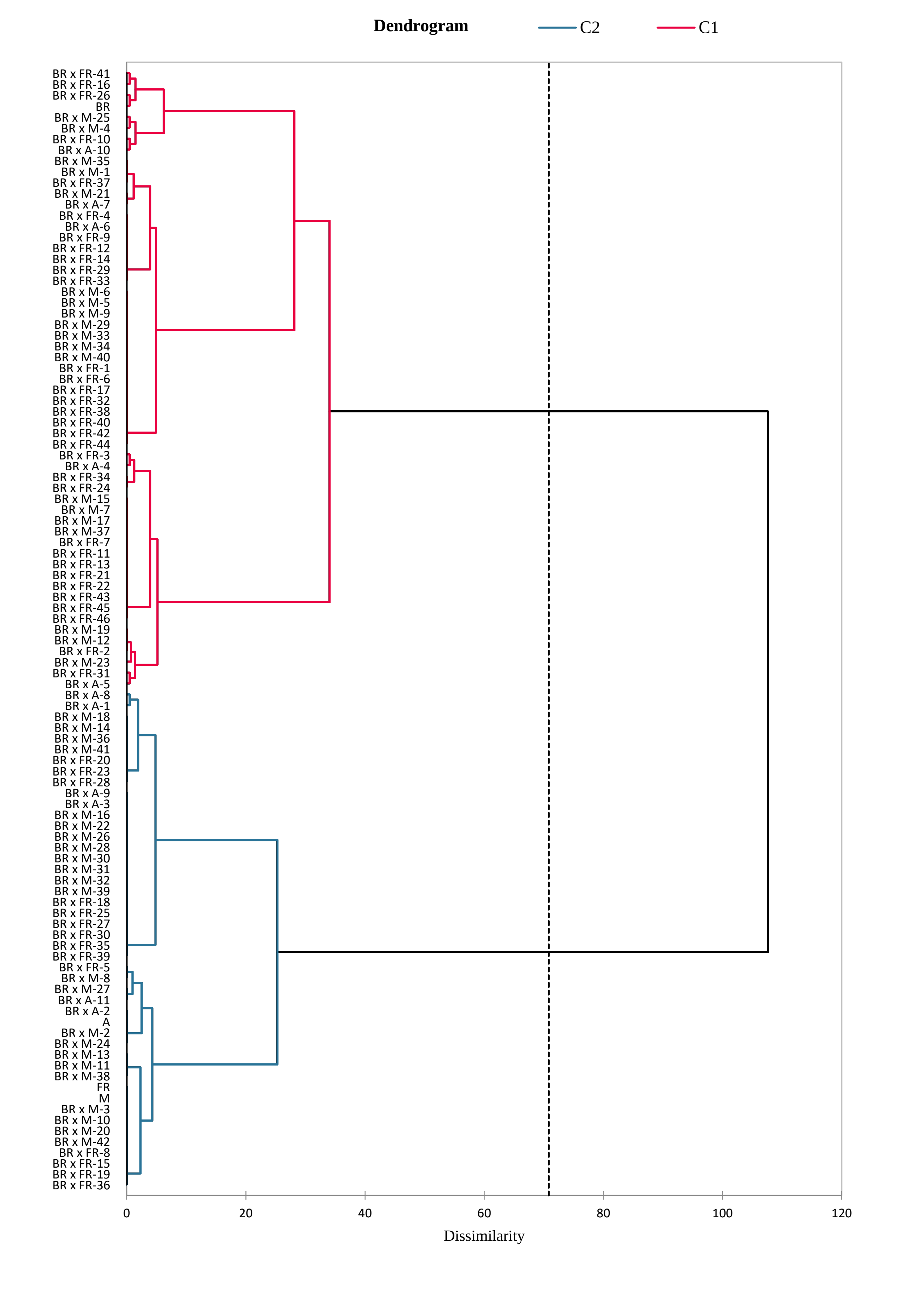

Figure 5. The dendrogram was generated through an Agglomerative Hierarchical Clustering (AHC) analysis, considering the flower traits of the rose genotypes. BR: Black rose, A: Annakarina, M: Magnum, FR: First Red.

Some modern and old garden roses were found to have at least 3.4 times more stigma than the Black rose (Doğan et al., 2020; Kılıç, 2023). Therefore, in crossbreeding studies involving genotypes with fewer stigmas, the number of seeds is expected to be lower compared with genotypes with more stigmas. However, this balance can be influenced by various factors. It has also been reported that there are certain combinations where fruit set and seed production cannot be achieved in pollination studies (Abdolmuhammadi et al., 2014; Farooq et al., 2016). Hence, the Black rose is considered relatively fertile, and by increasing the number of plants, it is possible to increase the seed set and achieve success in breeding programs. This finding suggests that using a larger population of Black rose plants could be beneficial in improving overall seed production. This, in turn, can enhance the potential for successful breeding programs aiming to develop new rose varieties with desired traits.

Incompatibility is a common phenomenon observed in the Rosaceae family. Although limited studies have been conducted on sexual incompatibility in roses, Mohaved et al. (2017) and Fibrianty and Kurniati (2020) emphasized its importance. When compatibility is promised, higher fruit and seed sets can be achieved through pollination with pollen parents, which generally exhibit a high pollen germination rate. Pipino et al. (2011) reported that the pollen germination rate among 11 different hybrid tea rose genotypes ranged from 0% to 46.3%, while the average number of seeds per fruit varied from 1.1 to 21.3. They found a strong correlation (r=0.74) between pollen germination rate and the average number of seeds per fruit. Nadeem et al. (2013) observed that the variety with the lowest pollen germination rate (1.33%) among 9 different hybrid tea roses had the lowest average number of seeds per fruit (0) when used as the pollen parent in the crosses. Conversely, combinations involving the variety with the highest pollen germination rate (46.55%) as the pollen parent achieved the highest average number of seeds per fruit (35). In the present study, a lower fruit set and a seed number below the average were observed when hybridizations were performed with Magnum, which had the highest pollen germination rate. Similar relationships were observed with other pollen parents, indicating pre-pollination barriers, i.e., incompatibility or meiotic abnormalities.

Sometimes, the germination ability of pollen determined under in vitro conditions may not fully reflect its germination ability in vivo, as observed in this study. One factor is the occurrence of meiotic abnormalities during the division of pollen mother cells. Roses with tetraploid pollen parents are expected to produce pollen grains with a *2n* (diploid) genome. However, sometimes abnormalities can produce *n* (haploid) and *3n* (triploid) pollen grains alongside diploid pollen grains. Haploid pollen grains have thinner callus plates than diploid ones, making them more likely to move easily and quickly in the stigma, increasing their chances of fertilizing the egg. On the other hand, haploid pollen grains have shorter pollen tubes, which may not reach the ovary within the required time for fertilization (Gao et al., 2019). Another factor is gametophytic incompatibility. In some cases, pollen grains may fail to germinate on the stigma, or even if they do germinate, they may not be able to develop in the stigma and reach the ovary. This can lead to a lack of successful fertilization and seed production (Karaağaç and Kar, 2016). Therefore, it should be understood that not every pollen grain that germinates in roses will necessarily result in seed production. This can be attributed to both meiotic abnormalities and gametophytic incompatibility.

In general evaluations, the seed germination rate ranges between 30% and 45% (Leus et al., 2018). However, the average seed germination rate in this study was 18.37%. It is known that the characteristics of the seed coat in hybrid individuals are inherited from the seed parent. Physical dormancy in roses causes a decrease in seed germination (Hartmann et al., 2002). The difference in seed germination rates observed between studies may be attributed to different seed parents. It could be related to variations in the needed cold temperatures associated with changes in seed coat thickness. However, the difference in seed germination rates among the combinations created with a single seed parent but different pollen parents, as observed in this study, suggests that the issue is more likely related to embryonic development than seed coat impermeability. The varying severity of post-pollination barriers among the combinations may have led to differences in embryo degeneration, thus impacting the seed germination rates among the different combinations.

It is crucial to know about trait inheritance to make progress in breeding programs and effectively transfer desired traits to offspring. While there is limited research on trait inheritance in roses, the scent trait is particularly challenging to inherit (Shi and Zhang, 2022). Indeed, in this study, despite having a scented seed parent, the number of scented offspring was quite low. Additionally, most offspring exhibited scent traits similar to those of the pollen parents. Several studies have reported that the scent profile and intensity can vary based on the composition and quantity of volatile compounds (Joichi et al., 2005; Chimonidou et al., 2007; Ritchie and Rosarian, 2012). Considering that the production of volatile compounds may occur at low levels in scentless genotypes (Baudino et al., 2019), it is likely that the composition and quantities of volatile compounds in hybrid genotypes may differ depending on the parental contributions, leading to variations in scent intensity due to changes in the volatile compound profile. It can be speculated that the Black rose used in this study may not have successfully transferred its volatile compounds to the progeny. However, a more accurate assessment would require an examination of the specific compositions of the volatile compounds.

The petal number in cut roses is a crucial commercial quality parameter. In this study, most offspring exhibited a similar petal number as both parents. Since the parental genotypes used in the study had a range of 27 to 63 petals and were classified as full or very full blooms according to ARS standards, it is expected that the F_1 progeny would predominantly display the double-flowering trait. However, negative heterosis was also observed, indicating a decrease in the petal number compared to the parents. Although the influence of climatic conditions on petal number is known (Debener, 1999; Debener, 2003; Shupert, 2005), it would be helpful to evaluate the trait under different environmental conditions. The precise categorization of the doubleness petal trait has been reported as challenging, particularly under varying climatic conditions (Bendahmane et al., 2013).

While cherry rot color is not commonly observed in roses, it is noteworthy that approximately half of the offspring in this study exhibited a dark red color similar to the seed parent. The success of the Black rose as a seed parent in transmitting its petal color to the progeny varied depending on the pollen parent. Dark-colored offspring were obtained When crossed with red-colored pollen parents, whereas this rate significantly decreased with pink-colored genotypes. This finding highlights the significance of selecting red genotypes to achieve the goal of obtaining dark red flower color when using the Black rose as the seed parent. Therefore, the Black rose successfully transferred its color to offspring through crosses with red genotypes.

Although the specific results of the study were not provided, it was observed that most hybrid progenies exhibiting cherry rot color displayed petal browning and darkening, which resemble symptoms of Botrytis gray mold disease. Preliminary investigations suggest that this browning and darkening can be attributed to Botrytis gray mold disease. Hybrid progenies with cherry rot colors may be more susceptible to this disease. The observation that Botrytis gray mold symptoms are only seen in hybrid progenies with cherry rot color leads to the hypothesis that there might be a relationship between this disease and anthocyanin accumulation. Some studies have indicated a positive correlation between anthocyanin accumulation in fruits and vegetables and resistance to Botrytis gray mold disease (Bassolino et al., 2013; Liu et al., 2018). However, further scientific investigation is needed to establish and validate this relationship.

Conclusion

In this study, which aimed to evaluate the effectiveness Black rose as a seed parent in rose breeding programs, it was found that the Black rose demonstrated relatively good fertility and successfully transferred the traits of petal number and petal color to the offspring. However, the study highlights the need to explore the Black rose's potential as a pollen parent in terms of scent traits. It is recommended that future studies employ advanced techniques to transfer the scent traits effectively. This study's findings are expected to guide interspecies hybridization in roses and contribute to breeding programs aiming to obtain diverse hybrid populations. Using available data on parental performance, cross combinations can be selected more strategically, increasing the likelihood of success compared with random selections. Selecting parents and identifying of cross-breeding combinations in breeding programs requires significant costs, effort, and time. Expanding the seed parent gene pool through this study's insights would greatly benefit breeders and contribute to advancing rose breeding programs.

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