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A Research on Determination of the Performance *Rosa damascena* Mill. as Pollen Source in Rose Breeding by Hybridization

Ezgi DOĞAN[1] Soner KAZAZ[2] Tuğba KILIÇ[3]
Hilal DURSUN[2] Hasan Talha ÜNSAL[2] Merve URAN[2]

[1]Bingöl University, Faculty of Agriculture, Department of Horticulture
[2]Ankara University, Faculty of Agriculture, Department of Horticulture
[3]Yozgat Bozok University, Faculty of Agriculture, Department of Horticulture
Sorumlu yazar: ezgidogan@bingol.edu.tr

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Abstract: Hybridization breeding is the most widely used method in the development of rose varieties in the world. Recently, with the increasing consumer demand for fragrance in cut flowers, the breeders has led to improve the scented rose in breeding. *Rosa damascena* Mill. known as oil rose, is one of the most important fragrant rose species in the world. Parental selection is one of the factors affecting success in the development of new rose varieties by hybridization breeding. The study was conducted on 01 May-30 October 2019 to determine the performance of *Rosa damascena* as a male parent in rose breeding through hybridization. In this study, the viability and germination rate of the flower were examined in *Rosa damascena*, while the number of fruit and seeds and the germination rates of hybrid seeds were determined in 10 hybrid combinations. The maximum number of seeds per fruit was determined in Freedom x *Rosa damascena* combination with 31.5 seeds, followed by Pink Akito x *Rosa damascena* combination with 26.6 seeds while the minimum seed number per fruit was 7.29 with Cherry Brandy x *Rosa damascena* combination. The highest germination rate (32.33%) was determined in Pink Akito x *Rosa damascena* when the lowest germination rate was examined in Black Magic x *Rosa damascena* combination.

Key words: breeding, fragrance, hybridization, rose, seed germination

Melezleme Yoluyla Gül Islahında Polen Kaynağı Olarak Isparta Gülü (*Rosa damascena* Mill.)'nün Performansının Belirlenmesi Üzerine Bir Araştırma

Özet: Dünyada gül çeşitlerinin geliştirilmesinde en yaygın kullanılan yöntem melezleme ıslahıdır. Son yıllarda birçok süs bitkisinde olduğu gibi güllerde de kokuya olan talebin artması, gül ıslahçılarını güllere koku karakterini kazandırmaya yönelik ıslah çalışmalarına yöneltmiştir. Ülkemizde yağ gülü ve Isparta gülü olarak da bilinen *Rosa damascena* Mill. dünyadaki en önemli kokulu gül türlerinden biridir. Melezleme ıslahı ile yeni gül çeşitlerinin geliştirilmesinde başarıyı etkileyen faktörlerden biri de ebeveyn seçimidir. Melezleme yoluyla gül ıslahında baba ebeveyn olarak *Rosa damascena*'nın performansının belirlenmesi amacıyla yürütülen bu çalışmada, 10 farklı melez kombinasyonu oluşturulmuş ve 01 Mayıs-30 Haziran 2019 tarihleri arasında kombinasyon başına 10'ar adet olmak üzere toplam 90 adet tozlama işlemi gerçekleştirilmiştir. Çalışmada, *Rosa damascena*'da çiçek tozu canlılık oranı ve çimlenme gücü belirlenirken, melez kombinasyonlarında meyve ve tohum sayısı ile melez tohumların çimlenme oranları belirlenmiştir. Meyve başına en fazla tohum sayısı 31.5 adet ile Freedom x *Rosa damascena* kombinasyonunda belirlenirken, bunu 26.6 adet ile Pink Akito x *Rosa damascena* kombinasyonu izlemiş, meyve başına en az tohum sayısı ise 7.29 adet ile Cherry Brandy x *Rosa damascena* kombinasyonunda elde edilmiştir. Elde edilen melez tohumlarda, en yüksek çimlenme oranı (%32.33) Pink Akito x *Rosa damascena*, en düşük çimlenme oranı (%1.82) ise Black Magic x *Rosa damascena* kombinasyonunda belirlenmiştir.

Anahtar Kelimeler: gül, ıslah, koku, melezleme, tohum

Introduction

Roses have been used not only as an ornamental plant, but also for perfumery, cosmetics, food and medical purposes, and known for thousands of years as the symbol of love and queen of flowers (Lawrence, 1997; Liu et al., 2015). In 2017, foreign trade volume of roses reached 6 billion 600 million 336 thousand dollars and 95% of this value was cut roses and 5% of them were landscape roses (AIPH/Union Fleurs, 2017). Rosa genus has about 150 species in the Northern Hemisphere. Many of rose cultivars come from prolong process of hybridization, involving a wide range of wild roses, domesticated species (*R. damascena, R. gallica, R. phoenicia, R. moschata*) and Chinese species (*Rosa chinensis, R. gigantea*) (Baudino, 2019).

The most widely used breeding method in the development of cut rose varieties is hybridization. During the breeding of modern roses, the fragrance properties of wild roses and domesticated species have been used and a number of extraordinary scent variations were obtained. Because of the fact that some authors assume the linkage between scent and short vase life, fragrance has been eliminated for many years in cut flowers (Barletta, 1995; Spiller et al., 2010). Hovewer, specific types of genetic fragrance roses that shorten vase life have not been studied and also physiological-molecular mechanisms of these roses have not been specified (Borda et al., 2011).

Recently, with the increasing consumer demand for fragrance in cut flowers, the breeding efforts for the scent character of roses have come to the forefront and fragrance character has become the first two ranks of selection character in cut flower species. Moreover, considering the value of rose essential oil and also for the perfume industry will be very interested in high-quality varieties with valuable fragrance profiles (Rusanov et al., 2009). Due to the intense of scents, breeders began to include *Rosa damascena* in gene pools. Furtermore, *Rosa damascena* has a rich source of pollen for pollinator because of androecium.

Growers and breeders prefer most fertile progenitors as pollen and/or seed donors (Zlesak, 2006). Especially the high rate of pollen viability and germination of fragrant rose species such as *Rosa damascena, Rosa centifolia* and *Rosa odorata* increased their uses as important pollinators in breeding programs. Roses are known for their difficult sexual reproduction from pollination to seed set and germination (Gudin, 1992; Abdolmohammadi et al., 2014). Moreover, genetic barriers to hybridization are often an obstacle to obtain interspecific hybrids (Perez and Moore, 1985; Abdolmohammadi et al., 2014).

Breeders desire to get high numbers of seeds per hips and as many seedlings as possible when make hand hybridization. Seed set, number of seed per fruit (hip) and seed germination are most important to breeders because of sexual reproduction in roses (Gudin, 1995).

The present study was aimed to make crosses between the *Rosa damascena* as a pollen source and some *Rosa hybrida* varieties and/or two rose species as female parent to examine the number of fruit set, number of seed per fruit and percentage of seed germination.

Material and Methods

Plant Material

This study was carried out on 01 May-30 June 2019 in the rose breeding greenhouse, Department of Horticulture, Faculty of Agriculture, Ankara University, Ankara-Turkey. In the study, three different fragrant rose species (*Rosa damascena, Rosa centifolia, Rosa odorata* Louis XIV) and seven different commercial cut roses (Polar Star, Pink Akito, Harmonie, Lady Rose, Moonlight, Black Magic, Freedom) were used as plant material. *Rosa damascena* was obtained from Isparta (Turkey) province as male parent while other species /varieties as female parents were obtained from the rose breeding greenhouse where the study was carried out. Some information about parents was given in Table 1.

Table 1. Quantitative characters of plant materials

Species/Variety	Fragrance	No.of petals/flower	No. of pistil/flower	No. of Anther/flower
Rosa damascena	Yes	30-35	30-40	85-90
Rosa centifolia	Yes	30-35	160-165	190-195
***Rosa odorata* Louis XIV**	Yes	25-30	25-35	35-45
Black Magic	No	30-35	100-135	130-135
Polar Star	No	60-65	150-190	120-160
Pink Akito	No	25-30	210-230	50-60
Harmonie	Yes	20-25	60-70	120-130
Lady Rose	Yes	20-35	100-130	100-120
Moonlight	No	40-50	180-190	140-160
Freedom	No	50-60	200-220	180-200

Pollen viability and germination tests

Pollen grain was collected when flowers of *Rosa damascena* were in one-third to one-half open stage. Anthers were removed from the receptacles then pollen was stored in incubator (24 ºC and 60-65 % humudity, 24h) for dusting. The pollen viability test was carried out after the method described by Eti (1991). Iodine and Potassium Iodide (IKI) was used in examined pollen viability. IKI solution was prepared by melting 0.5 g iodine and 1 g potassium iodide in 100 ml distilled water. A drop of the solution was placed on microscope slides and pollen grains of each type were spread on the stain with a brush.. Pollen grains were determined using a light microscope (x 100). They were counted after waiting for 5 minutes. The viability of pollen was determined according to staining level: pollen with dark brown or black colour as viable, with light red or orange colour as semi-viable and with yellow-green colour or colourless as non-viable (Eti, 1990). The study was conducted with a total of four replicates and randomly chosen five fields and 250 pollens were counted in each areas. Pollen germination was carried out in petri dishes by using 20% sucrose in 1% agar media having four replications for *R. damascena* at 24°C and 60-65% humudity. Pollen was sprinkled uniformly on the medium with a brush. After 12 hours incubation period, pollen germination percentage was calculated under the light microscope. Pollen was considered germinated when a pollen tube reached a length of at least 1.5 times the pollen diameter (Leus, 2005; Nadeem et al., 2013) and 200 pollens were counted in each fields.

Determination of ploidy level

In this study, the ploidy of all parents were examined using a PA-II flow cytometer in Namık Kemal University, Faculty of Agriculture, Department of Field Crops. Ploidy levels were confirmed by chromosome counting which is the classical method. The nuclear DNA contents of the plants were first determined for flow cytometry ploidy analysis then chromosomes of one of the plants with different DNA content and chromosome number were correlated (Tuna, 2016).

Polination

The petals were removed very carefully from receptacles of both parents when flowers were in 1/3 or 1/2 was open (Chimonidou et al., 2007). The anthers were take off because of prevent self or unwanted pollination and were collected in a labeled glass bottle and placed in incubator (24 ºC and 60-65 % humudity). While the dusted pollen was obtained 24 h later, seed parents (*Rosa centifolia*, *Rosa odorata* Louis XIV, Polar Star, Pink Akito, Harmonie, Lady Rose, Moonlight, Black Magic, Freedom) were prepared by emasculation. Hybridization made in the early hours of the morning. Pollen was placed on the stigma possessing the sticky exudates aiding a camel-hair brush. For each cross

combination were 10 hybridizations. After hybridization, the hips labelled. 8 weeks after the pollination, hip set started and 12 weeks after polination mature hips harvested. When all seeds were extracted from fruits, the total number of seeds (pieces) and the number of seeds per fruit (pieces) of each combination were determined. The seeds were kept moist in peat at 4ºC for a period of 4-12 weeks, after that were sown in media containing peat and cocopit. Germinating seeds of each combinations were recorded.

Results

Ploidy Levels

The core DNA contents of rose genotypes were determined by the method used in the study. Core DNA contents of rose genotypes used as parent ranged from 2.26 pg/2C to 2.54 pg/2C in this study. The ploidy levels of all rose genotypes were found 2n=4x=28 (Table 2).

Table 2. Nuclear DNA contents and ploidy contents of rose genotypes

Species/Variety	Sample Peak	Standard Peak	Amount of DNA	DNA (pg/2C)	Level of ploidy
Rosa damascena	150.94	243.72	3.65	2.26	2n=4x
Rosa centifolia	178.10	266.98	3.65	2.43	2n=4x
***Rosa odorata* Louis XIV**	152.29	237.18	3.65	2.44	2n=4x
Black Magic	148.70	224.20	3.65	2.42	2n=4x
Polar Star	158.65	240.32	3.65	2.41	2n=4x
Pink Akito	155.38	233.49	3.65	2.43	2n=4x
Harmonie	152.21	238.94	3.65	2.33	2n=4x
Lady Rose	152.84	233.51	3.65	2.39	2n=4x
Moonlight	148.87	228.21	3.65	2.38	2n=4x
Freedom	150.09	225.03	3.65	2.43	2n=4x

Pollen viability and germination

The average pollen viability rate of *Rosa damascena* was 45.63 %, though the pollen germination rate was 40.67 %. The average of morphological normal pollen was 54.46 % (Table 3). The results showed that IKI method were higher than agar plate tests.

Table 3. Pollen viability and germination

Species	Pollen viability (%)	Pollen germination (%)	Morfological normal pollen (%)
Rosa damascena	45.63	40.67	54.46

Number of fruit (hip), number of total seed and number of seed per fruit

As a male parent, *R. damascena* showed a good fruit set percentage (100 %) and number of hip (10) with *R. odorata* Louis XIV, Pink Akito, and Harmonie. while Black Magic showed the second highest value for hip set as 9 when crossed with *R. damascena*. A limited success in fruit setting (20 %) and number of hip (2) was observed in the crosses of Moonlight x *R. damascena*. While the Freedom excelled for number of seed per fruit as 31.50 cross with *R. damascena* while Pink Akito observed the second the highest number of seed per fruit as 26.60 when crossed with *R. damascena*. While Lady Rose x *R. damascena* showed the lowest number of seed per fruit with 9.14 the second least

number of seed per fruit was *Rosa centifolia* x *R. damascena* with 9.33 (Table 4). Pink Akito found the highest value for germination of seed (32.33 %) and 86 germination seed when crossed with *R. damascena*, while Polar Star x *R. damascena* observed the second highest germination of seed (22.45 %), whereas the lowest germination of seed and 2 germination seed was recorded Black Magic x *R. damascena* (1.82%).

Table 4. Comparison of different parameters in crossing of *Rosa damascena*

Combination	Fruit set (%)	No. of fruit (hip)	No. of seed per fruit	Germination of seed (%)	No. of germination seed
R. centifolia* x *R. damascena	60	6	9.33	8.93	5
R. odorata* x *R. damascena	100	10	11.90	10.08	12
Black Magic x *R. damascena*	90	9	12.22	1.82	2
Polar Star x *R. damascena*	60	6	16.33	22.45	22
Pink Akito x *R. damascena*	100	10	26.60	32.33	86
Harmonie x *R. damascena*	100	10	17.00	9.09	17
Lady Rose x *R.damascena*	70	7	9.14	6.25	4
Moonlight x *R. damascena*	20	2	13.50	22.22	6
Freedom x *R.damascena*	40	4	31.50	16.67	21

Discussion

In most modern breeding programs, the creation of new rose hybrids involves a series of steps that include controlled pollination followed by the fertilization of the ovary, fruit set and hip formation, seed maturation, germination and surviving of new plant (Nadeem et al., 2013). Therefore fertile progenitor (male or/and female) has a very important role in hybridization breeding. On the other hand, generating broader populations in terms of number of seedlings is only one of the direction that attend to all efficiency of a rose breeding programme. Identifying all the parameters related to fertility, increases the overall efficiency of a breeding program. Many same studies have been carried out in different rose groups (Visser et al., 1977a, deVries and Dubois, 1996; Ogilvie et al., 1991; Crespel et al., 2006). Rosa genus has a polyploid complex with a base chromosome number of seven. Many species are between diploid (2n=2x=14) and octaploid (2n=3x, 4x, 5x, 6x, or 8x, respectively). In most plant species with same ploidy level, the seed parent and the pollen parent transmit roughly the same amount of genetic material to their offspring, therefore in offspring have traits of both parents (Werlemark, 2003). All genotypes in the study, had the same ploidy level (2n = 4x). However, different hip and seed formation were observed among genotypes with the same ploidy level. This difference was due to genetic compatibility of parents. Many researches were reported that the fertility of cut roses, typically tetraploid (2n = 4x = 28) (de Vries and Dubois, 1996; Debener et al., 2000). Van Huylenbroeck et al. (2007) reported that if the tetraploid cultivar is used as a seed parent, the number of seeds can be higher.

In present study, pollen viability, morphological normal pollen and germination rates were determined according to IKI and plate-agar methods. The viability of *Rosa damascena* as a result of chemical and biological methods differed from each other. In a study by Parfitt and Ganeshan (1989), it was found that chemical methods did not demonstrate similarity with biological methods. In general, although a linear relationship between pollen viability and germination rate is expected (Martins et al., 2017), immature pollen can be dyed in chemical methods. Therefore, higher viability values can be seen compared to biological methods. Similarly, many researchers have reported that the number of seeds per fruit is due to differences in pollen viability and germination in parents (Visser et al., 1977a, b; de Vries and Dubois, 1987;

Gudin and Arene, 1991, 1992; Gudin et al., 1991a, b; Zlesak et al., 2007). Fertility of pollen donor parents are scaled 61-100% as highly fertile, 41-60% as fertile, 21- 40% as moderately fertile and 1-20% as low fertile or sterile (Nadeem et al., 2013). In parallel, *Rosa damascena* was found to be a fertile genotypes.

The succes in the percent of hip set ranged between 20-100 % in the study. A low success in hip setting (20 %) was showed in the crosses of *R. damascena* (as a male parent) with Moonlight proving that a significant variation appeared in a number of fruit setting that was mainly assign to certain physiological and genetic factors. Also Farooq et al. (2016) reported that percentage of rose hybrid success between 0.0- 83.0 % in their study. Crosses with *Rosa damascena* showed that the number of seeds per hip varied between the all female parents and changed between 9,14-31.50 seed per hip. Gudin (2007) stated that the hips containing ranged from 1 to 30 seed. It have been reported that hybridizations with *Rosa damascena* produce high levels of hip set and seed per hips (Chimonidou et al., 2007; Farooq et al., 2016) and the results were parallel with our study. The number of seeds per hip showed differences in female parents. The results may be cause meiotic abnormalities, agomospermy, interspecific derivation, the accumulation of deleterious recessive alleles in the progenies (Erlanson, 1931; Ogilvie et al., 1991; Nadeem, 2013). Seed production per hip is based on to genetic and/or physiological reasons attribute pollination, fertilization and embryogenesis.

Hand pollinated seeds are high investments and labour, hence breeders and growers desire to profit all viable embryos of each hybridization. Percentage of germination was ranged between 1.81 % and 32.33 % in this study. Some researches mentioned that hybrid tea roses produce low seeds (low 50 %) and non-uniform germination. (Gudin, 2003;2007, Ueda, 2003, Zlesak, 2005, Anderson and Byrne, 2007). Germination of rose cultivars is blocked by hardness of the seeds, which is linked to the pericarp and primary dormancy (Zlesak, 2006). Interspecific hybridization of *R. damascena* ($2n = 4x$) and tetraploid rose varieties resulted high embryo germination in tissue culture (Abdolmohammadi et al., 2014).

Conclusion

The increasing demand for fragrant roses has led the rose breeders to develop fragrant roses in recent years. *Rosa damascena* Mill. is a valuable fragrant rose species grown in order to obtain rose oil. Fertility of the parents is the one of the important factor affecting the success of rose breeding through hybridization. In the study, *Rosa damascena* was found to have high pollen viability and germination rate. The results showed that in the hybrid combinations where *Rosa damascena* was used as a source of pollen, fruit formation, number of seeds per fruit and seed germination rates varied according to rose species and/or varieties used as parent. In hybridization with *Rosa damascena*, morphological characterization studies should be carried out in addition to determining recurrent bloom of F_1 hybrids

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