

EFFECT OF DIFFERENT ROOTSTOCKS ON POLLEN FERTILITY OF INTERSPECIFIC EGGPLANT HYBRID (*SOLANUM MELONGENA* L. X *SOLANUM TORVUM* SW.) GENOTYPES

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ABSTRACT

The major bottleneck in interspecific hybridization is the sterility of the hybrid individuals. Different methods such as tissue culture techniques, molecular studies and grafting are used to overcome the sterility barriers as a result of hybridization between species. In the present study, the grafting method was used to overcome the low pollen fertility of hybrid plants obtained from the hybridization between *Solanum melongena* and *Solanum torvum*. Three different rootstocks with high fertility (Pala, Faselis F₁, and *S. torvum*) were used for grafting. Three interspecific hybrids (8/I-3, 8/II-1 and 8/IV-2) were used as the scion. Pollen viability and pollen germination rates of these genotypes were determined. According to the data obtained, it was determined that the pollen viability and germination were affected significantly by the rootstock-genotype combination. The highest rate of pollen viability and pollen germination was obtained from Pala - 8/II-1 combination. The rate of pollen stained in "red + pink" of the 8/II-1 genotype grafted on Pala rootstock increased 98.00 times and the pollen ratio stained only in red increased 77.87 times, compared to the non-grafted. The Pala was determined as the most suitable rootstock for the interspecific hybrids.

Keywords: Grafting, pollen fertility, rootstock, *Solanum melongena*, *Solanum torvum*

Published first online January 06, 2022

Published final July 30, 2022

INTRODUCTION

Cultivated eggplant (*Solanum melongena* L.) is sensitive to a lot of diseases and pests especially bacterial diseases, fungal wilts such as *Fusarium* and *Verticillium*, nematode, and some insects. However, fertile generations have not been achieved from the sexual or somatic hybridization of *S. melongena* and *S. torvum* Sw that is known as resistant to some pests and soil-borne diseases (Guri and Sink 1988; Sihachakr *et al.*, 1989; Kameya *et al.*, 1990; Bletsos *et al.*, 2000; Collonnier *et al.*, 2003; Daunay 2008; Çürük and Dayan 2018; Ata 2019). Different researchers have reported that grafting of plants is effective on nutrient intake, flowering time, determination of flower sex, flower behaviors, number of clusters, harvest, and quality (Paton and Barber 1955; Zeevaart 1958; Ikeda *et al.*, 1986; Kim and Lee 1989; Ruiz *et al.*, 1997; Pulgar *et al.*, 2000; Davis *et al.*, 2008; Turker and Ak 2010; Manazo *et al.*, 2014; Rebollo-Martínez *et al.*, 2019). Wild species are used as rootstocks for cultivated vegetables because of their resistance to some soil-borne diseases, pests, and other adverse conditions (Yetişir *et al.*, 2004). *S. torvum* and interspecific hybrids (*S. incanum* x *S. melongena*, *S. melongena* x *S. aethiopicum*) are most commonly rootstock used in the production of grafted eggplant seedlings (Çürük and Dayan 2017; Sarıbaş *et al.*, 2019).

Moreover, it has been reported that genetic material exchange occurs between the rootstock and the scion, thus, permanent genetic changes may occur in the scion (Goldschmidt, 2014). According to the histological analysis, Ohta (1991) determined that the genetic material can move from the rootstock to the scion. Furthermore, it has been put forward that plant grafting may result in the exchange of genetic information through large DNA fragments of entire plastid genomes (Stegemann and Bock, 2009). Besides, chloroplast genome transfer between stock and scion cells of grafted distinct *Nicotiana* species have been demonstrated (Stegemann *et al.*, 2012). In addition, grafting may have different effects on pollen viability, pollen germination, pollen number, and pollen tube length. Radice *et al.* (2004) reported that while some rootstocks increase pollen viability in the Forastero peach cultivar, some rootstocks decrease pollen viability. Moreover, research that was conducted to determine the effects of rootstock on seed fertility and quality in watermelon showed no significant difference between the grafting combinations in pollen viability and pollen germination (Kombo and Sari, 2019). In addition, it was stated that the grafting combination affected the pollen tube length in the pistachio (Meimand and Shamshiri, 2019). The present study aimed to investigate the impacts of different fertile eggplant rootstocks on the low pollen fertility reported in *S. melongena* x *S. torvum* hybrids genotypes.

MATERIALS AND METHODS

This research was carried out in the laboratory and greenhouses in Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Horticulture. Faselis F₁, the Pala, and *S. torvum* were used as rootstocks. Interspecific hybrid (Faselis F₁ x *S. torvum*) genotypes 8/I-3, 8/II-1, and 8/IV-2, were used as scion for bud source. The interspecific hybrids plants were obtained by germinating the seeds in MS (Murashige and Skoog, 1962) medium at the growth room conditions of 25±1°C, with 16 hours of light and 8 hours of darkness (Çürük and Dayan, 2018). Rooted interspecific hybrids seedlings were planted in 0.2 L pots filled with a mixture of peat and perlite (2:1) and grown in a glasshouse after acclimatization in the same grow room. The buds taken from the interspecific hybrids were grafted on rootstocks according to the method reported by Çürük *et al.* (2009). Non-grafted plants were propagated by rooting the cuttings of the interspecific hybrids. Three clones of the same genotype were obtained. After the base bud of the cuttings (with three budded) was removed, the cuttings in the soil mixture mentioned above were rooted. In greenhouse conditions, four buds of each genotype (8/I-3,

8/II-1 and 8/IV-2) were grafted on each rootstock. A total of 36 grafted plants were obtained from 9 grafting combinations. A total of 12 plants from non-grafted interspecific hybrids were used. The viability of the pollen (Figure 1), taken from grafted and non-grafted plants in the flowering period, was determined with 1% 2,3,5 triphenyl tetrazolium chloride (TTC). The germination rate of the pollen collected from flowers that were closed 12-24 hours before anthesis, were determined in the medium containing 5% sucrose, 50 mg L⁻¹ boric acid, and 1% agar in glass petri dishes at 25°C (Khan and Isshiki, 2008). These experiments have been carried out two times on 8 and 16 June 2012. Pollen TTC and germination trials were established according to a completely randomized design. In these experiments, there were four replicates and 50-60 pollen were observed in each replication. Pollen viability was determined by calculating the percentages of pollen from red-stained, "red stained + pink stained" pollen in TTC. Data were analyzed by ANOVA after angular transformation (Bartlett, 1947). Duncan's multiple range test was used to compare means at a 5% significance level.

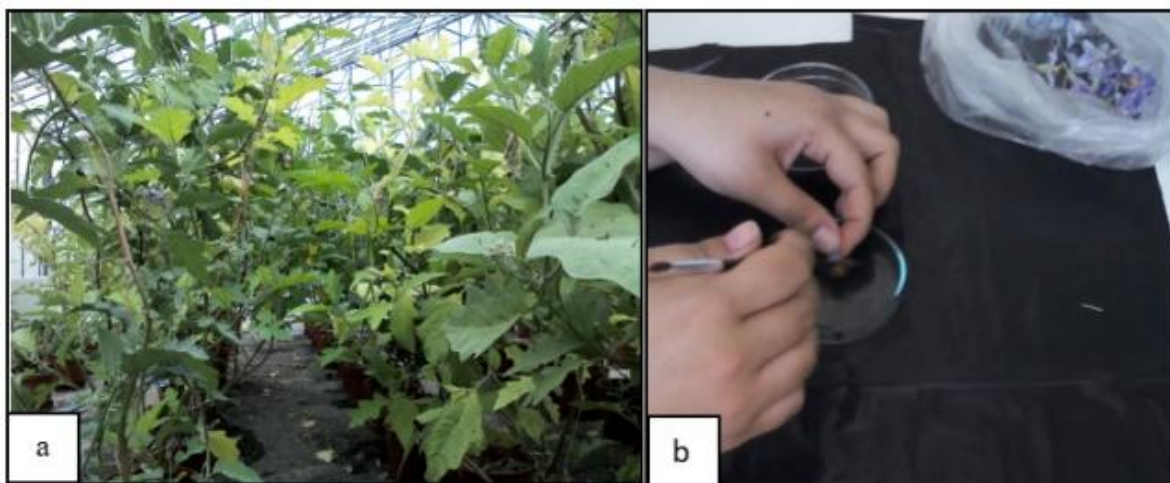


Figure 1. a) Flowering period of grafted and non-grafted plants in greenhouses b) Pollen collection for pollen viability and germination tests.

RESULTS AND DISCUSSION

The data from the first experiment (June 8, 2012) to determine the pollen viability and germination rates are presented in Table 1. According to the variance analysis performed in this period, the differences between the means of the grafting combinations were significant in pollen viability but not in terms of pollen germination rate. The obtained results showed that the rootstocks used in the 8/I-3 interspecific genotype did not affect the pollen viability. However, when the Pala was used as a

rootstock, the pollen viability of the 8/II-1 and 8/IV-2 genotypes (grafted onto the rootstock) significantly increased compared to other rootstocks and non-grafted ones, and the highest values were obtained from these combinations. Furthermore, the Pala rootstock increased the rate of pollen stained in the "red + pink" of the 8/IV-2 genotype from 0.00% to 4.96% compared to non-grafted. In addition, this rootstock increased the viability of "red + pink stained" pollen by 26.50 times, and the viability of only red-stained pollen 17.94 times in the 8/II-1 genotype.

Table 1. Viability and germination rates of pollen obtained in the first trial of some interspecific hybrids grafted on different rootstocks.

Rootstock	Genotype	Viability (red + pink stained) (%)	Viability (red stained) (%)	Germination (%)
Faselis F ₁	8/I-3	0.38 c	0.19 bc	0.00
Faselis F ₁	8/II-1	0.00 c	0.00 c	0.00
Faselis F ₁	8/IV-2	1.43 b	0.66 b	0.29
Pala	8/I-3	0.00 c	0.00 c	0.00
Pala	8/II-1	4.40 a	3.03 a	1.10
Pala	8/IV-2	4.96 a	2.58 a	0.73
<i>S. torvum</i>	8/I-3	0.00 c	0.00 c	0.00
<i>S. torvum</i>	8/II-1	1.32 b	0.60 bc	2.77
<i>S. torvum</i>	8/IV-2	0.00 c	0.00 c	0.00
Non-grafted	8/I-3	0.00 c	0.00 c	0.00
Non-grafted	8/II-1	0.16 c	0.16 bc	0.00
Non-grafted	8/IV-2	0.00 c	0.00 c	0.00

Means followed by different letters in the same column are significantly different by Duncan's multiple range test at a 5% significance level

In the second trial that was carried out on June 16 to determine whether pollen viability and germination rates changed depending on fertile rootstock usage, it was found that the parameters changed depending on rootstock-genotype combinations (Table 2). Accordingly, using Pala and *S. torvum* as rootstock was ineffective on pollen viability and germination of 8/IV-2 interspecific hybrid. Yet, when this genotype was grafted onto the Faselis F₁ variety, pollen viability was observed to increase significantly compared to the non-grafted one (8.79 times in the "red + pink stained" pollen, 7.29 times in the red-stained pollen). Furthermore, pollen viability increased significantly when the 8/I-3 genotype was only grafted onto Pala and Faselis F₁. On the other hand, while the "red + pink stained" pollen rate of the non-grafted 8/II-1 genotype was 0.00%, this rate increased to 11.43% when this genotype was grafted onto the Pala cultivar. Grafting enhanced the rates of red-stained pollen and germination from 0.00% to 9.58% and 3.65%, respectively. These were the highest values.

According to the statistical results of pollen viability and germination tests, it was determined that the differences between grafting combinations in the first experiment in terms of in vitro pollen germination were not significant (Table 1) but significant in the second trial (Table 2). Therefore, variance analysis was performed using average values of both tests (Table 3). According to this analysis, it was determined that pollen viability and germination rates varied depending on the grafting combination. The differences between the combination averages were significant in terms of these characteristics. When 8/IV-2 and 8/I-3 genotypes were grafted onto *S. torvum* rootstock, it was found that the pollen viability and germination rates did not change significantly compared to the non-grafted control. However, when these genotypes were grafted onto Faselis F₁ and Pala varieties, pollen viability rates

enhanced considerably compared to non-grafted ones. In contrast, the increment in pollen germination rates was not significant in these grafting combinations compared to their control. A similar result was obtained from the 8/II-1 genotype grafted on the Faselis F₁. However, when the Pala variety was used as rootstock for this genotype, pollen viability and germination rates significantly increased. Concerning the average data of the two experiments, it was determined that the increase in the Pala-8/II-1 combination was the highest compared to other rootstock-genotype combinations. The rate of pollen stained in "red + pink" of the 8/II-1 genotype grafted on Pala was increased 98.00 times, while the pollen ratio stained only in red increased 77.87 times. Furthermore, pollen germination enhanced from 0.00% to 2.37%.

It was found that the interspecific hybrids which were grafted on the Pala and Faselis F₁ rootstocks had higher pollen viability compared to non-grafted interspecific hybrids when the average results of the pollen viability tests were assessed. This enhancement was the highest in the 8/II-1 genotype that was grafted on the Pala rootstock. The effect of *S. torvum* rootstock on pollen viability changed depending on the interspecific hybrid grafted onto it. On the other hand, pollen germination rates in the interspecific hybrids (except 8/II-1 interspecific hybrid) grafted onto *S. torvum* rootstock were not affected significantly by grafting. However, the pollen germination rate of interspecific hybrids grafted onto the Pala rootstock was found to be increased, especially the pollen germination rate of 8/II-1 genotype, compared to other grafting combinations or non-grafted genotypes. These results show that the rootstock affect the pollen viability and pollen germination rate of interspecific hybrids. Radice *et al.* (2004), Kidman *et al.* (2014), Dziejczak *et al.* (2019), and Solmaz *et al.* (2020)'s reports support our research results, while the findings of

the studies conducted by Kombo and Sarı (2019) and Pandey *et al.* (2020) are not consistent with our results. The fact that *S. torvum* rootstock was less effective on pollen viability of interspecific hybrids may be due to lower pollen viability than Pala and Faselis F₁ rootstocks. Çürük (2012), Çürük and Dayan (2018) have reported that the pollen viability of the Pala and Faselis F₁ was higher than *S. torvum*. Regarding the grafting, the flower buds of scion displayed an excessive representation of the transcription factor genes like Homeobox, NAC, MYB, bHLH, B3, C3HC4, PLATZ, etc. (Kumari *et al.*, 2015). In the scion leaves displayed accumulation of the regulatory genes for flower development such as SEPALLATA 1–4, Jumonji C, and AHL16. Differential transcription of genes about ethylene, gibberellic acid, and other stimuli was detected between scion and rootstock (Kumari *et al.*, 2015). Moreover, it has been reported that the transcription factors related to the improvement and hormone signaling are among the genes whose mRNAs were often found in plant phloem samples

and some of which were confirmed grafting transmissible, involving *CmNACP*, *StBEL5*, a *Knotted 1*-like transcription factor, *GAI*, and a few *Aux/IAA* genes (Ruiz-Medrano *et al.*, 1999; Banerjee *et al.*, 2006; Omid *et al.*, 2007; Deeken *et al.*, 2008; Kanehira *et al.*, 2010; Xu *et al.*, 2010; Mahajan *et al.*, 2012; Notaguchi *et al.*, 2012; Yang *et al.*, 2015). Yang *et al.* (2015) have reported that some gene categories were more likely to produce mobile mRNA in grafted plants. In grafting combinations, different results may occur due to transmissible genetic material and various metabolic products, some hormones from the rootstock to the scion or the scion to the rootstock (Liu *et al.*, 2010; Goldschmidt 2014; Wang *et al.*, 2016; Sharma *et al.*, 2019). The Pala variety is a very strong rootstock (Çürük *et al.*, 2009; Çürük *et al.*, 2010) and may have been well compatible with different interspecific genotypes. Therefore, the Pala may have caused the rootstock products to pass into the scion at a high rate.

Table 2. The rates of pollen viability and pollen germination of some interspecific hybrids grafting on different rootstock in the second experiment.

Rootstock	Genotype	Viability (red + pink stained) (%)	Viability (red stained) (%)	Germination (%)
Faselis F ₁	8/I-3	4.60 bc	3.41 b	0.91 b
Faselis F ₁	8/II-1	7.08 ab	5.78 ab	0.31 cd
Faselis F ₁	8/IV-2	3.72 c	3.15 b	0.00 d
Pala	8/I-3	4.35 bc	3.32 b	0.61 bc
Pala	8/II-1	11.43 a	9.58 a	3.65 a
Pala	8/IV-2	0.00 d	0.00 c	0.00 d
<i>S. torvum</i>	8/I-3	0.00 d	0.00 c	0.00 d
<i>S. torvum</i>	8/II-1	0.84 d	0.45 c	0.00 d
<i>S. torvum</i>	8/IV-2	0.00 d	0.00 c	0.00 d
Non-grafted	8/I-3	0.25 d	0.25 c	0.00 d
Non-grafted	8/II-1	0.00 d	0.00 c	0.00 d
Non-grafted	8/IV-2	0.38 d	0.38 c	0.00 d

Means followed by different letters in the same column are significantly different by Duncan's multiple range test at a 5% significance level

Table 3. Viability and germination rates of pollen obtained according to the averages of two trials of interspecific genotypes grafted on different rootstocks.

Rootstock	Genotype	Viability (red + pink stained) (%)	Viability (red stained) (%)	Germination (%)
Faselis F ₁	8/I-3	2.49 b	1.80 bc	0.45 bc
Faselis F ₁	8/II-1	3.54 b	2.89 b	0.15 bc
Faselis F ₁	8/IV-2	2.58 b	1.90 b	0.15 bc
Pala	8/I-3	2.17 b	1.66 bc	0.30 bc
Pala	8/II-1	7.92 a	6.31 a	2.37 a
Pala	8/IV-2	2.48 b	1.29 bc	0.37 bc
<i>S. torvum</i>	8/I-3	0.00 c	0.00 d	0.00 c
<i>S. torvum</i>	8/II-1	1.08 b	0.52 cd	1.39 b
<i>S. torvum</i>	8/IV-2	0.00 c	0.00 d	0.00 c
Non-grafted	8/I-3	0.13 c	0.13 d	0.00 c
Non-grafted	8/II-1	0.08 c	0.08 d	0.00 c
Non-grafted	8/IV-2	0.19 c	0.19 d	0.00 c

Means followed by different letters in the same column are significantly different by Duncan's multiple range test at a 5% significance level

Conclusion: Different interspecific hybrids (Faselis F₁ x *S. torvum*) were grafted onto three different rootstocks, Faselis F₁, Pala, and *S. torvum*. The results of the study can be summarized as follows;

- 1) The effect of the fertile rootstocks on the pollen viability and germination rates of the interspecific genotypes was different. The highest pollen viability and germination rates were obtained from Pala rootstock.
- 2) Interspecific hybrid (scion) also affected pollen viability and germination rate.
- 3) It was assessed that the Pala variety could be a candidate rootstock for increasing the very low pollen fertility in interspecific hybrids.
- 4) For interspecific hybrids, except for the rootstocks used within the content of the study, different rootstocks can be examined, and more effective rootstocks can be determined by investigating the effects on pollen viability and germination rates.

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