

## STUDIES ON *IN VITRO* FERTILIZATION BIOLOGY OF *MESPILUS GERMANICA* L. CV. 'İSTANBUL'

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### Abstract

This study was aim to investigate the fertilization biology of 'İstanbul' medlar variety. For this purpose, firstly pollen production capacity and morphological homogeneity were assessed (hemocytometer slide). The pollen viability was tested with TTC and IKI staining methods. Pollen germination experiments were carried out in different medium with different sucrose concentration (agar in plate method) during 2 h, 6 h, 12 h and 24 h period. The number of anthers in a flower 36.5, pollen number in an anther 160.55 and pollen number in a flower 12753.8 were found. Morphological homogeneity was high with 98%. Pollen viability rates varied between 94% (TTC) and 96% (IKI). Contents of medium V: 20% sucrose 1% agar-agar+5 ppm boric acid and 24 hours incubation duration were stated as optimum pollen germination rate and tube growth.

**Key words:** Medlar, pollen germination, morphological homogeneity, pollen tube growth, TTC.

### INTRODUCTION

Medlar (*Mespilus germanica* L.) has been cultivated for over thousands of years in temperate zones of Anatolia. The medlar, called as 'Muşmula', 'Beşbüyük' or 'Döngel' (in Turkish), is botanically classified as a pome and produces edible fruits (Atay, 2013). *Mespilus germanica* L. belongs to Rosaceae family and it grows mainly in frost-free areas, and on rocks and poor soils (Hacisferoğullari et al., 2005). The flowers are hermaphrodite, five-piece, white-pink color, and each bud has one flower. Flowers generally open in May-June. In Turkey, they are abundant particularly in north and west-Anatolia and Marmara regions. Medlar is one of the latest maturing fruits and the ripening occurs in late October before frosts in Turkey. The fruits are used as a nutrition component by the local population and are prepared by the local people as marmalade or pickle (Ercisli et al., 2012).

Fertilization success in plants is the result of processes that take place during the progamic phase (Thompson, 2004; Güçlü and Koyuncu, 2017). Pollen germination and pollen tube growth are important components of fertilization success in fruit trees (Janick and

Moore, 1996; Tosun and Koyuncu, 2007). The first condition of formation of seed and fruit is developing healthy male and female organs of the flower and cells, except for an apparent partenocarpy of some cultivars. Pollen performance, which includes pollen germination, pollen tube growth rate and pollen competition, is an important component of fertilization success in seed-producing plants. Pollen performance is clearly affected by the genotype of the pollen (Acar and Kakani, 2010; Hedly et al., 2004). There have been some studies about 'İstanbul' medlar, but apparently almost no work has been done on fertilization. The aim of this study is to examine the pollen performance of the 'İstanbul' medlar variety and determine the optimum pollen germination medium protocol.

### MATERIALS AND METHODS

In the study, pollens taken from 'İstanbul' medlar trees in the orchard of Eğirdir Fruit Research Institute were used. Pollens were obtained from flowers at balloon stage. The flowers were transferred to the laboratory immediately. Anthers were removed and placed into the dark-colored bottle to promote dehiscence at room temperature. For the pollen

performance, pollen production capacity, morphological homogeneity, pollen viability rates were investigated. In addition, optimum germination medium and incubation period were determined. The pollen production capacity and morphological homogeneity percentages of pollens were assessed with the hemocytometer (Marienfeld, Germany) slide (Eti, 1990). Imperfectly shaped pollen grains were considered as aborted pollen. The final percentage of morphological homogeneity (MH) was defined as:

$$MH = \frac{Ns - Na}{A} \times 100$$

*Ns*: Number of shaped pollen per area;

*Na*: Number of aborted pollen per area;

*A*: Total number of pollen area.

In the stain tests, pollen viability was estimated by using TTC (2, 3, 5-triphenyl tetrazolium chloride) and FDA (fluorescein di acetat) stains. Pollens were scattered onto TTC and FDA solutions, and stained pollens were counted after 2 hours and 15 minutes, respectively. To determine the pollen viability, pollens of each cultivar (of four different areas) were observed onto two slides under a light microscope ( $\times 100$  magnification). The stained pollen was considered as viable in these tests.

Four concentrations of sucrose were tested in the germination medium for the *in vitro* germination test.

- I. 5% sucrose + 1% Agar-Agar + 5 ppm  $H_3BO_3$  (Boric Acid)
- II. 10% sucrose + 1% Agar-Agar + 5 ppm  $H_3BO_3$
- III. 15% sucrose + 1% Agar-Agar + 5 ppm  $H_3BO_3$
- IV. 20% sucrose + 1% Agar-Agar + 5 ppm  $H_3BO_3$

The 'agar in plate' method was used to establish pollen germination and pollen tube growth (Koyuncu and Tosun, 2009). Pollen tube long at least as its diameter was considered to be 'germinated'. The percentage of pollen germination was determined after 2 h, 12 h and 24 h incubation period at 21°C. An ocular micrometer was used to measure pollen tube length, under a light microscope, at a magnification. Four Petri dishes were used for

germination and pollen tube growth experiments. For each assay, 2 mL of medium was placed into Petri dishes. Counts were made from 4 different microscope fields (100-150 pollen grains per field for each Petri dishes) (Hedly et al., 2004; Koyuncu, 2006). Statistical analysis was conducted using Duncan's multiple range test within the general linear model procedure of SPSS 16.0.

## RESULTS AND DISCUSSIONS

### Pollen production capacity and pollen viability

Pollen production amount and viability results were shown in Table 1. Pollen morphological homogeneity (98%) and pollen viability (96%) were found very high. IKI viability test (96%) was higher than TTC (94%) test. Similarly, researcher reported that pollen viability in the IKI test was generally higher (84.83-100%) and stable than TTC test (11.78-91.66%) (Çavusoğlu and Sülüşoğlu, 2013). Koyuncu (2006) studied strawberry pollens using TTC and reported that pollen viability ratios reached 82% (Allstar and Elvira) and 86.5% (Chandler). Koyuncu and Tosun (2009) used TTC, FDA and IKI stain tests for the same sweet cherry cultivars. They reported that the pollen viability differed according to stain methods and cultivars.

Table 1. Pollen production capacity, morphological homogeneity and pollen viability tests

Pollen production capacity and morphological homogeneity					
Cultivar	N	M	PN	MH	
Istanbul	16 %	160 %	98.56 %	84	98%
Pollen viability tests					
	TTC		IKI		
Istanbul	94 %		96%		

N: Numbers of anthers in a flower; M: Mean pollen number in an anther; PN: Pollen number in a flower; MH: Morphological homogeneity.

### Pollen germination tests

For the optimum germination medium different sucrose concentrations were tried.

A linear equation best described the relationship between the germination percentage and different concentration of sucrose ( $r = 0.966$ ). In Medium I, sucrose concentration was the lowest, pollen germination has not started in 2 hours whereas the others even low gave germination at 2

hours with increasing sucrose concentration. As seen Figure 1, pollen germination rates increased with increasing sucrose concentration. The highest ratio of pollen germination was obtained from Medium IV (20% sucrose + 1% Agar-Agar + 5 ppm H<sub>3</sub>BO<sub>3</sub>). In terms of mean pollen germination rate, it followed by Medium III (16.82%) and Medium II (7.25) (Table 2). This suggests that the increased concentration of sucrose increases the germination rate. Çavuşoğlu and Sülüşoğlu (2013) reported that pollen germination varied between 16.4%-66.67% for all germination media. Another study which was conducted in Arabidopsis emphasized sugar-dependent multilayer regulation of Arabidopsis pollen germination is supported, which makes this approach a valuable experimental system for future studies addressing sugar sensing and signaling (Hirsche et al., 2017). Güçlü and Koyuncu (2017) found the suitable germination medium 0.5% agar + 15% sucrose + 5 ppm H<sub>3</sub>BO<sub>3</sub> (Boric acid) for apple's pollen.

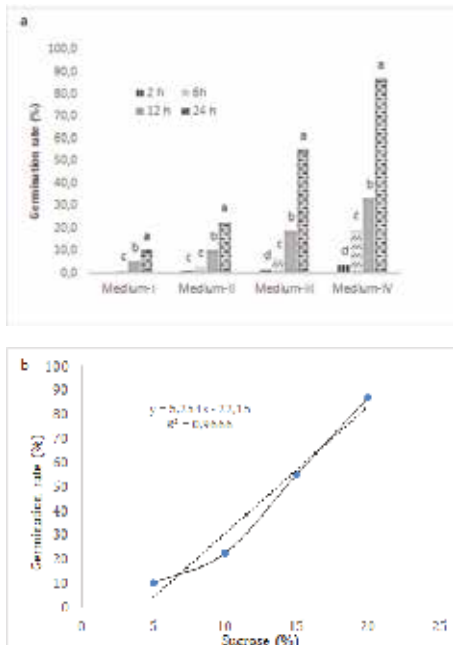


Figure 1. *In vitro* pollen germination percentages of medlar at 2 h, 6 h, 12 h and 24 hours ( $p < 0.05$ ) at different sucrose concentration (a); Regression curve between germination rate and sucrose concentration (b)

When different incubation periods are compared in the same germination medium, the difference between the values is statistically significant ( $p < 0.05$ ). Pollen germination raised as much as 3 fold between 6 h and 12 hours reached own maximum percentage at 24 hours (10.1; 22.3; 54.9; 86.8, respectively) for all mediums. Results of incubation duration experiments were similar to the findings of Yıldız and Yılmaz (2002), who reported that the germination pollen of strawberry cultivar ‘Tufts’ began within 1 h at 24°C. Tosun and Koyuncu (2007) also reported that the germination rates increased with incubation period in cherries.

Table 2. *In vitro* pollen germination percentages of medlar in different sucrose concentrations and incubation periods

Germination Medium	Incubation Period				Mean
	2h	6h	12h	24h	
Medium I	0.5c <sup>a</sup>	1.1c	3.2d	10.1d	1.57d
Medium II	0.8c	2.7c	9.9c	22.3c	7.25c
Medium III	1.5b	5.1b	18.7b	54.9b	16.82b
Medium IV	2.5a	19.9a	44.5a	86.8a	43.52a

\*Values within a column followed by different letters are significantly different ( $p < 0.05$ ).

When the data were evaluated, in a medium with different sucrose concentrations, a linear increase was observed between the rate of germination and the duration of incubation (Figure 2).

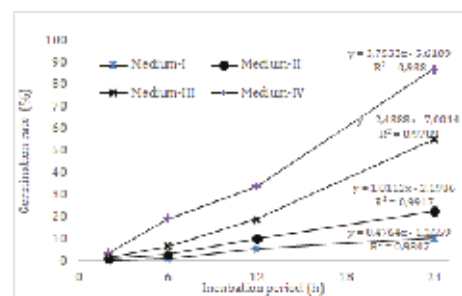


Figure 2. Linear regression curves between germination rate and incubation period

The *in vitro* elongation of pollen tubes was affected by different sucrose concentration (Table 3). The effect of different concentrations of sucrose on the pollen tube elongation was statistically significant ( $p < 0.05$ ). As well as the pollen germination rate, pollen tube growth

increased during the incubation period and measured at 24 hours later. As the sucrose concentration increased, the length of the pollen tube increased, reaching the highest value in Medium IV (164.2  $\mu\text{m}$ ). The shortest pollen tubes were measured at Medium I, the lowest sucrose concentration (16.8  $\mu\text{m}$ ).

Table 3. Pollen tube growth of medlar in different sucrose concentrations ( $\mu\text{m}$ ) 24 hours later

Germination medium	Pollen tube length ( $\mu\text{m}$ )
Medium I	16.8d*
Medium II	35.9c
Medium III	110.7b
Medium IV	164.2a

\*Values within same column followed by different letters are significantly different ( $p < 0.05$ ).

Koyuncu and Güçlü (2009) reported that the *in vitro* pollen germination and tube growth were clearly affected by incubation period. Sharafi (2011) found the *in vitro* medium containing 17% sucrose, 10 ppm acid boric and 1.2% agar. Cultured pollens were incubated in dark condition at 25°C for 24 h optimum medium for pollen germination and tube growth.

## CONCLUSIONS

The amount of pollen production and the morphological homogeneity level were determined for ‘İstanbul’ medlar variety. Pollen viability found high (98% for IKI). Pollen germination rates increased by increasing with incubation period. Medium IV (20% sucrose + 1% Agar-Agar + 5 ppm  $\text{H}_3\text{BO}_3$ ) and 24 hours incubation period was found optimum pollen germination and pollen tube growth condition. Fertilization biology studies should be continued at *in vivo* conditions. We hope these results will be useful for researchers and growers for future breeding studies.

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