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In vitro Pollen Viability and Pollen Germination in Medlar (*Mespilus germanica* L.)

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Abstract

The study was carried out to determine in vitro pollen viability and pollen germination of 4 cultivar and 4 wild types of medlar (Mespilus germanica L.) plants. Two pollen viability tests (TTC and IKI) and one in vitro pollen germination test (Agar Plate) at four different germination media were used in the study. The viability varied significantly according to the genotypes of medlar and tests used. However pollen viability in the IKI test was generally higher (84.83-100 %) and stable than TTC test (11.78-91.66 %) in freshly obtained pollen. In the second step, pollen viabilities of six months stored at +4 ^{0}C and freshly obtained pollen were compared at IKI test. After 6 months storage at +4 C^0, pollen viability was highly maintained (72.22 %- 94.05 % for all genotypes). Therefore no significantly difference observed among storage time or plant types. The fresh pollen germination in agar plate tests varied between (16.4 %-66.67 %) for all germination media and plant types but any significantly difference was seen among the media or plant types.

Keywords: Medlar, *Mespilus germanica*, pollen viability, germination.

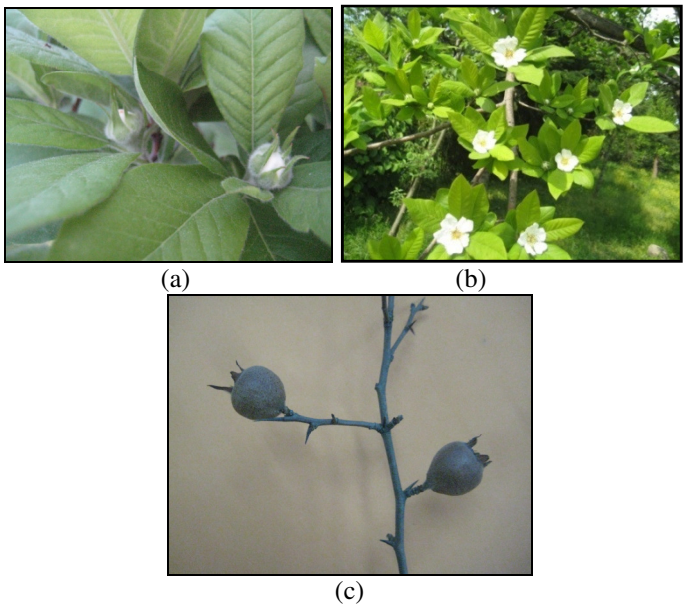
Introduction

Medlar (*Mespilus germanica* L.) belonging to the Rosaceae family and is native to Southeastern Europe, Anatolia, Crimea, Caucasia and the northern parts of Iraq and Iran[1]. The perennial fruit or the other plant part used in folk medicine[2-5]. The medlar fruit has been gaining commercial importance in recent years, attracting research on its chemical composition[6]. Some of the study on chemical composition have been performed by Dincer et. al.[7], Glew et. al.[8], Hacıseferoğulları et. al.[9], Gruz et. al.[6] and Rop et. al.[10]. Agricultural studies[11-13] have been carried out at the same time. To determine fruitful plant types via fertility level of new cultivars are also very important for scientists in medlar as being all fruity plants. Some researchers have been emphasized the relation between pollen viability and fertility or pollination capacity on fruit or seed productivity[14-18].

Long-term pollen storage is a tool for gene conservation and breeding. Most of the stored pollen at different cold or freeze condition showed that some of pollen can be storage for a while[19-23]. Knowledge of medlar pollen viability and germination capacity of the pollen are very limited. So far only one study[24] was reached about medlar pollen germination. The objectives of this scientific study were to determine the *in vitro* viability and *in vitro* germination rate of medlar pollen collected from natural habitat and orchards under Kocaeli city environmental condition in Turkey.

Material and Methods

In this research, pollen was collected from mature 4 wild (W1, W2, W3, W4) and 4 cultivated (C1, C2, C3, C4) trees (figure-1; a, b, c) grown in 8 different areas of Kocaeli city, North-western part of Turkey in 2012 spring season. The 50 flowers at close balloon stage of each plant type was collected and their filaments with anthers separated and left in a *Petri* dishes at room temperature for 24 hours under light condition. The obtained pollen was divided into two parts. One parts of the pollen namely "fresh pollen" were used at tests on the first day. Second part of the pollen namely "stored pollen" (stored at +4 C^0 for 6 months at refrigerator in erlenmayer flasks) used at sixth month of studies.

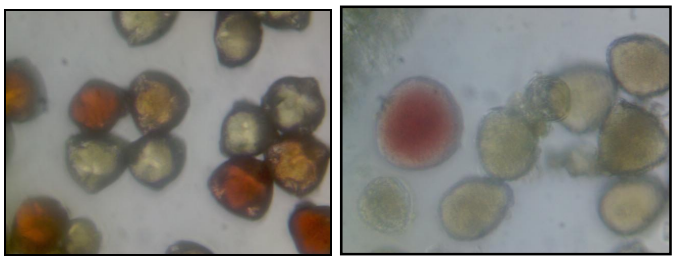


(a) (b)

(c)

Figure-1
Wild 1 plant type; a.b.Some flowering steps; c. Fruit setting

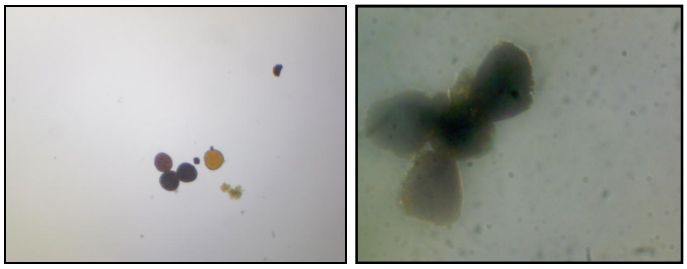
The pollen viability tests were tested individually for each plant type. TTC (2,3,5-triphenyl tetrazolium chloride)[25,26] and IKI (Iodine potassium iodide)[27] stain tests used for this purpose. A few drops of 1% TTC (0.2 g. TTC and 12 g. sucrose were dissolved in 20 ml distilled water) or IKI solution (1 g KI and 0.5 g I dissolved in 100 ml distilled water[28]) were dropped by Pasteur pipettes on microscope slides and pollen were shaked with a slim brush (each brush used only one plant type) covered with a coverslip. 3 different areas of each coverslip of used four microscope slides with three replication were counted within a few minutes for IKI and 2 hours for TTC tests. Viable pollens were dyed in red and light red; dead pollens were not dyed in TTC (figure-2; a, b) and viable pollens were dyed in dark brown and dead pollens were not dyed or pale yellow in IKI (figure-3; a, b). The second viability at IKI test in the same way was done in the 6th month with stored pollen at +4 C^0 (figure-4; a, b).



(a) **(b)**

Figure-2

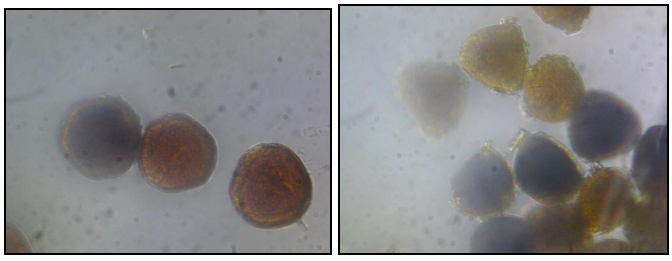
a. Wild 2 plant type; b. Cultivar 2 plant type; Images of viable and dead pollen grains of freshly obtained after staining with TTC. Photographed using light microscopy (x400)



(a) **(b)**

Figure-3

a.Wild 3 plant type; b. Cultivar 3 plant type; Images of viable and dead pollen grains freshly obtained after staining with IKI. Photographed using light microscopy (a:x100; b:x400)



(a) **(b)**

Figure-4

a.Wild 4 plant type; b. Cultivar 4 plant type; Images of viable and dead pollen grains after +4^{0}C storage, after staining with IKI. Photographed using light microscopy (x400)

Pollen germination tests were analyzed on 4 different media[29]. Contents of the medium 1: 2 g/l agar+200 g/l sucrose+100 mg/l H_3BO_3 pH was 5.27; medium 2:5 g/l agar+200 g/l sucrose+100 mg/l H_3BO_3 pH was 5.56; medium 3:2 g/l agar+200 g/l sucrose+100 mg/l H_3BO_3+300 g/l $Ca(NO_3)_2$. $4H_2O$+200 mg/l $MgSO_4.7H_2O$+100 mg/l KNO_3, pH was 5.22; medium 4: 5 g/l agar+200 g/l sucrose+100 mg/l H_3BO_3+300 g/l $Ca(NO_3)_2$. $4H_2O$+200 mg/l $MgSO_4.7H_2O$+100 mg/l KNO_3, pH was 5.33. The media were prepeared at 60^{0}C in a hot plate, acidity were left as found in prepeared media, poured to 6 cm in diameter three disposible *Petri* plates for each plant type at each medium. After cooling down the medium, pollen were again shaked with a slim brush and incubated six hours at room temperature in dark condition. Pollen grains were considered as germinated when the lenght of the pollen tube exceeded its diameter[19].

To evaluate pollen viability and germination, light microscope with 10x ocular with 10x and 40x objectives were used. The experiments were designed as completely randomized block design with 3 replication. Statistical analyses were performed using Minitab statistical program and means were compared with Duncan's Multiple Range Test[30] at $P \leq 0.05$ probability level. Although all original data presented in tables as percentages, before statistical analyze percentage data were transformed to arcsin square root transformation.

Results and Discussion

Fresh pollen viability rate of the eight plant genotypes tested with TTC and IKI are given in table-1. Among test types, pollen viability rates at IKI test and TTC test between 84.83-100% and 11.78-91.66% respectively. IKI test showed higher and more stable than TTC test results. Within plant types Wild 3 and Wild 4 (91.66 % and 86.79 %) at TTC test and Cultivar 3 (100%) type at IKI test showed statistically the highest viability rates. When compared the fresh pollen with 6 months stored at +4 C^0 pollen viability at IKI tests (table-2), there were no statistically differencies observed in plant types or storage periods, although a little decreased result occured numerically. Considered *Rosaceae* family especially pome fruits because scientific data on medlar pollen viability could not be reached, Petrisor et. al.[17] showed in ten apple cultivars pollen viability changed between 52.55-89.92 %. In another study Dalkiliç and Mestav[31] showed fresh pollen viability of seven cultivars of quince in 1% IKI were between 90.8-98.1%. Bhat et. al.[23] studied weekly with 3 different pear cultivar in 4 different pollen storage methods with 2% acetocarmine solution until 12th week. First day at room temperature, the pear pollen viability changed in 66-74.67% according to cultivar. After the 12th week stored at room temperature and stored at +4 C^0 pollen viability decreased at least 0-21.50% respectively. Mentioned study also showed pollen stored at low temperature gave better result than room temperature storage.

Fresh pollen germination for all plant types used, 16.4-51.98% in medium 1; 20.55-46.29% in medium 2; 30.02-48.74% in medium 3 and 35.07-66.67% in medium 4. According to the

data pollen germination rate did not exceed 66.67% in all plant type or media used (table-3) (figure-5; a, b and figure-6; a, b). According to the single study can be reached on medlar pollen germination[24], germination percentage varied between 36.4-80.1 % among five medlar genotypes using *in vitro* medium containing 17% sucrose, 10 ppm boric acid and 1.2 % agar. The results are close to our findings and some differencies in data can be commented with differencies in plant genotypes and used media.

Table-1
Pollen viability (%)* of *Mespilus germanica* in TTC and IKI tests at one day old fresh pollen

Test Types	Plant Types								
	C1	C2	C3	C4	W1	W2	W3	W4	Mean of each test
TTC	18.68 cde**B***	13.95 de B	11.78 e B	65.26 b B	40.67 bcd B	43.41 bc B	91.66 a A	86.79 a A	46.53
IKI	93.25 ab A	93.7 ab A	100 a A	94.79 ab A	98.54 ab A	98.37 ab A	96.29 ab A	84.83 b A	94.97
Mean of each plant type	55.97	53.83	55.89	80.03	69.61	70.89	62.5	85.81	

LSD=17.68 Sx= 6.137. *Data (%) were transformed to arcsin before statistically analyses, ** Lower case indicates plant type (within the lines) dissimilarities for each viability test, at p≤0.05 probability level, Duncan Tests, *** Capital letters indicate the viability tests (within the columns) dissimilarities for each plant type, at p≤0.05 probability level, Duncan Tests.

Table-2
Pollen viability (%)* of *Mespilus germenica* in IKI tests at one day old fresh pollen and 6 months old storaged at +4 ^{0}C

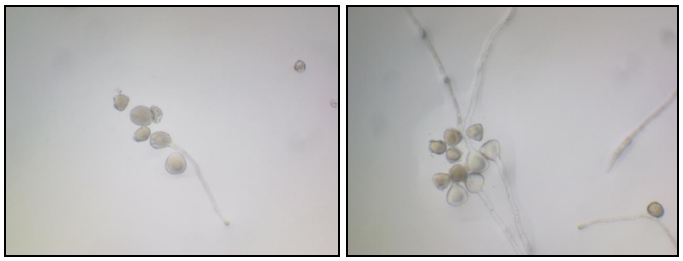
IKI Test	Plant Types								
	C1	C2	C3	C4	W1	W2	W3	W4	Mean of storage period
Fresh pollens	93.25 **	93.7	100	94.79	98.54	98.37	96.29	84.83	94.97
6 months stored pollens at +4 ^{0}C	86.14	80.71	93.27	90.71	94.05	87.11	72.22	83.85	86.01
Mean of each plant type	89.7	87.21	96.64	92.75	96.3	92.74	84.26	84.34	

LSD=20.44 Sx= 7.095. * Data (%) were transformed to arcsin before statistically analyses, ** There were no differences statistically at p≤0.05 probability level according to plant types or storage periods.

Table-3
Fresh pollen germination (%)* tests at four different germination media

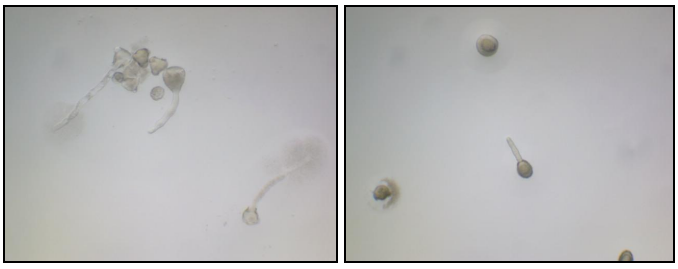
Germination Media	Plant Types								
	C1	C2	C3	C4	W1	W2	W3	W4	Mean of each medium
Medium 1	31.47**	33.49	46.06	34.64	29.97	51.98	25	16.4	33.63
Medium 2	46.29	26.03	36.43	26.98	28.09	43.89	20.55	33.33	32.7
Medium 3	43.89	38.89	48.74	37.32	46.82	44.93	33.33	30.02	40.5
Medium 4	62.18	35.07	44.84	40.83	54.24	51.67	66.67	43.33	49.85
Mean of each plant type	45.96	33.37	44.02	34.94	39.78	48.12	36.39	30.77	

LSD=13.68 Sx=4.843. *Data (%) were transformed to arcsin before statistically analysis, ** There were no statistical differences at p≤0.05 probability level according to plant types or germination media.



(a) **(b)**

Figure-5
a.Wild 1 plant type; b. Cultivar 4 plant type in Medium 3; Images of germinated pollen grains in medium 1, Photographed using light microscopy (x100)



(a) **(b)**

Figure-6
a. and b. Cultivar 2 plant type; Images of germinated pollen grains in medium 2 and medium 4 respectively, Photographed using light microscopy (x100)

According to another study on pome fruits; Petrisor et. al.[17] found pollen germination rates between 93.22-100 % for ten apple cultivars. Sharafi[24] showed that pollen germination rates found between 32.1-73.1% for five *Eriobotria japonica*; 26.4-61.2 % for five *Creatagus oxyacantha*; 47.2-70.6% for five *Malus pumila*; 43.2-87.3% for five *Pyrus communis* and 41.9-82.3% for five *Cydonia oblonga* in *in vitro* medium mentioned above. Bhat et. al.[23] found 61.17-66.12% for three *Pyrus* spp. pollen grains germination in 15% sucrose solution.

The present study indicates that medlar pollen germination performance of the examined eight genotypes can vary with medium types but this is not different statistically. According to personal observation; besides in Medium 3 and 4 pollen germination rates were higher than being Medium 1 and 2; in Medium 3 and 4 pollen tube lenghts were more longer and views were more clear and vivid than being Medium 1 and 2 for all plant types.

Conclusion

In determining the pollen viability, TTC and IKI tests; and determining germination rate; agar-plate methods with four different media were used. IKI test was found more reliable and stable than TTC test. Secondly controlled storage were found to be effective in prolonging pollen viability in medlar. Although there were no significantly difference among germination media, some additives as ions gave the best results. It is expected that the study, almost is one of the first studies on *in vitro* pollen viability and *in vitro* pollen germination of medlar, will be cause further study on observation and calculation of fruit set and breeding. The work also may be induced the different long term pollen storage techniques at different degrees and methods for medlar. In this way controlled pollination can be possible between cultivars that do not blooming at the same period and storage pollinizer of plant types.

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