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An Investigation on Pollen Viability, Germination and Tube Growth in Some Stone Fruits

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Abstract: In this study, the viability, germination and tube growth of pollen from apricot (*Prunus armeniaca* L.) (in five cultivars), sweet cherry (*Prunus avium* L). (in four cultivars) and sour cherry (*Prunus cerasus* L.) (in one cultivar) were investigated. Three stain tests (TTC, IKI and safranin) and two *in vitro* germination tests (hanging drop and agar–plate) were used to estimate pollen viability and germination in these species. The viability, germination and tube growth of pollens varied significantly according to species, cultivars and tests. However, pollen viability in the safranin test was generally higher than in the others. The highest pollen germination was obtained with the 15% sucrose solution in the hanging drop and agar plate tests in all cultivars except Şekerpare. The pollen germination rates in this solution in hanging drop and agar–plate tests varied between 49.77–72.90% and 57.83–84.42% in apricot; 47.92–57.38% and 52.40–66.60% in sweet cherry and 49.16% and 53.82% in sour cherry, respectively. The pollen germination in the agar–plate method was higher than in the hanging drop method. The IKI stain test and pollen germination in apricot and sweet cherry were positively and significantly correlated ($r=0.686^{**}$ and $r=0.704^{*}$). The highest length of pollen tube in sweet cherry and sour cherry was obtained in the agar plate test at 15% sucrose solution but it varied in apricot cultivars.

Key Words: Apricot, sweet and sour cherry, pollen viability, germination, tube growth.

Bazı Sert Çekirdekli Meyve Türlerinde Çiçek Tozu Canlılık, Çimlenme Gücü ve Çim Borusu Gelişiminin Belirlenmesi

Özet: Bu çalışmada 5 kayısı (*Prunus armeniaca* L.), 4 kiraz (*Prunus arivum* L.) ve 1 vişne (*Prunus cerasus* L.) çeşidinde çiçek tozu canlılık, çimlenme oranları ve çim borusu gelişimi incelenmiştir. Bu türlerde çiçek tozu canlılık ve çimlenme düzeylerinin belirlenmesinde üç canlılık (TTC, IKI ve Safranin) ve iki çimlenme testi (asılı damla ve petride agar) kullanılmıştır. Çiçek tozu canlılık ve çimlenme düzeyleri ile çim borusu gelişimi tür, çeşit ve terslere göre önemli farklılıklar göstermiştir. En yüksek çiçek tozu canlılık oranları safranin boyama yönteminde elde edilmiştir. Asılı damla ve petride agar yöntemlerinde en yüksek çiçek tozu çimlenme oranı %15 sakkaroz çözeltisinde elde edilmiştir (asılı damlada kayısıda %49.77–72.90, kirazda %49.08–57.38 ve vişnede %49.16; petride–agar yönteminde kayısıda %57.83–84.42, kirazda %52.40–66.60 ve vişnede %53.82). Petride agar yönteminde çiçek tozu çimlenme oranı asılı damla yönteminden daha yüksek bulunmuştur. Kayısı ve kirazda IKI canlılık testi ile çiçek tozu çimlenme oranı arasında pozitif korelasyonlar bulunmuştur ($r=0.686^{**}$, $r=0.704^{*}$). Kiraz ve vişnede en fazla çiçek tozu çim borusu uzunluğu petride agar yönteminde %15 sakkaroz solusyonunda elde edilirken, kayısıda bu bakımdan çeşitler arasında farklılık belirlenmiştir.

Anahtar Sözcükler: Kayısı, kiraz, vişne, çiçek tozu canlılığı, çiçek tozu çimlenmesi, çiçek tozu çim borusu gelişimi.

Introduction

The viability, tube growth and morphological homogenity raleted to pollen quality are the most important properties in fruit trees. These properties are useful for plant breeders, geneticists, and growers. However, an easy method for determining pollen viability is required to increase the efficiency of the breeding program and the selection of a suitable pollenizer while the orchard is being establihed.

Many stin tests have been used such as acetocarmin, propione carmin, anilin blue, Alexander's stain, IKI (iodine+potassium iodide), FDA (fluorescein diactate), NBT (*p*–nitro blue tetrazolium), MTT (2,5–diphenyl tetrazolium bromide) and TTC (2, 2, 5–triphenyl tetrazolium chloride) to determine the pollen viability of fruit trees (1, 2, 3, 4, 5, 6, 7). Stain tests have advantages as indicators of pollen viability because they are faster and easier than pollen germination. But, in some cases, different results may be obtained in stain tests on some fruit species or cultivars. Therefore, to determine the actual amount of viable pollen, germination tests are necessary. The hanging drop and agar–plate tests are generally used to determine the rate of pollen germination. While fruit tress generally require only

water or a sugar source for pollen germination, other species need a more complete medium (5). The rate of pollen germination of some fruit species and cultivars varies depending on the medium or chemical concentration. For this reason, the suitable pollen germination medium should be obtained for each fruit species and cultivar. Some studies have been carried out on the relationships between viability and pollen germination. Oberle and Watson (1) found no relationship between the two characteristics, but Werner and Chang (2), Pearson and Harney (4), Parfitt and Ganeshan (7) and Norton (8), reported a positive correlation between them. The objectives of this study were to optimize the viability, germination and tube growth of some Prunus pollen, and to determine the interrelationships between studied properties.

Material and Methods

In this research, pollen was collected from mature apricot (Hasanbey, Şalak, Mahmudun Eriği, Karacabey and Şekerpare cvs), swet cherry (Salihli, Sapıkısa, Kırdar and Napoleon cvs) and sour cherry (Kütahya cv.) trees grown in the Çoruh Valley–Turkey in 1996. The balloon stage flowers of all cultivars were collected and their anthers were stored at 23°C for 24 h.

TTC (2, 3, 5–triphenly tetrazolium chloride), IKI (iodine+potassium iodide) and safranin stain tests were used to determine pollen viability. Pollen grains were sowed at 1%TTC solution and studied after two hours (8). The grains of pollen were counted to determine viability after a couple of minutes in the IKI medium (1g KI and 0.5g I dissolved in 100ml distilled water) (9). The pollen viability was obtained one hour following sowing in the safranin medium [(1g safranin dissolved in 95% alcohol (40 ml) and the final volume was made up to 100 ml. One part safranin was mixed with two parts gliserol and one part distilled water (1:2:1)] (10). To determine viability, about three hundred pollen grains of each replicate from four different areas were counted under a light microscope. During this experiment, pollen morphological homogenity was also investigated (6).

Six different sucrose concentrations (0, 5, 10, 15, 20 and 25%) were used in hanging drop tests to determine pollen germination. On the other hand, in the agar–plate tests the sucrose solutions varied from 5% to 20% and were added to 1.5%. Difco agar. In both tests, the pollen was incubated at 22°C for 24 h under dark conditions. The six slides and plates were scanned for each pollen source. In the germination tests, 100 grains from each replication in six microscopic areas were counted randomly. All observations of slides and plates were carried out at X 100 magnification using a light microscope. In addition, the lengths of one hundred pollen tubes in each replication in six areas of agar–plate medium were measured using an ocular micrometer on a light microscope at the end of the 24 h incubation period. The values for viability, germination and morphologicaly homogenity of pollens were subjected to arcsin square–root transformation before statistical analysis. The experimental model was completely randomized block design with five replicates. A Tukey test was used to compare the means. Correlation analysis was performed to determine the relationships between pollen viability, germination and other properties (11).

Results and Discussion

The rate of pollen viability in apricot and cherry was found to be significantly different in only the IKI medium (Table 1). The highest pollen viability of apricot was obtained in the safranin stain test (88.08% and 90.65%) except in Mahmudun Eriği cv. The sweet cherry pollen viability exhibited differences depending on the stain tests. For the Salihli cultivar, the highest rate of pollen viability was 90.25% in the safranin stain test. It was 86.55% in IKI and 94.27% in the safranin tests for the Napoleon cv. Viability of sour cherry varied from 66.78% (IKI stain) to 85.23% (safranin stain). Results showed that the pollen viability differed with respect to stain tests and fruit species. Similar results have been reported by Oberle and Watson (1), Parfitt and Ganeshan (7), and Eti (9).

The pollen morphological homogenity did not vary between apricot cultivars, but it was significanty different at the 1% leve for cherry cultivars (Table 1). The pollen homogenity of apricot cultivars was higher than that of sweet cherry and sour cherry. The lowest morphological homogenity of pollen among these species was obtained in sour cherry. In other words, the highest levels of abnormal pollen were determined to be in sour cherry. Similar results have also been reported by Anvari (12) for Köröser sour cherry cv.

Pollen germination rates of apricot cvs. in hanging drop tests exhibited significant differences in 15%, 20% and 25% sucrose solutions and the rates of sweet cherry cvs. were also significant in 5%, 10%, 20% and 25% sucrose solutions. On the other hand, the differences in pollen germination rates between cultivars were significant in all the sucrose concentrations in agar–plate tests except 5% sucrose solution in sweet cherry. The sucrose concentrations in the hanging drop and

Table 1. Viability in stain tests and morphological homogenity of pollens in some Prunus species.

Cultivars	Viability (%) TTC	IKI	Safranin	Morphological Homogenity (%)
		APRICOT		
Hasanbey	85.73	78.38abc	88.55	96.66
Şalak	83.40	72.28c	87.18	96.82
Mahmudun Eriği	79.50	87.74a	85.55	94.04
Karacabey	82.39	84.56ab	90.65	99.57
Şekerpare	75.67	77.00b	88.08	96.37
$D_{\%5}$	–	7.58	–	–
		SWEET CHERRY		
Salihli	80.95	82.73a	90.25	91.55a
Napoleon	72.60	86.55a	94.27	89.56a
Sapıkısa	67.35	83.50a	90.70	86.16a
Kırdar	74.15	71.75b	89.35	78.30b
$D_{\%1}$	–	3.90	–	5.50
		SOUR CHERRY		
Kütahya	70.95	66.78	85.23	75.81

agar–plate medhods were found to have different effects on pollen germination of all cultivars (Table 2, 3). The optimum sucrose concentration in the hanging drop method for pollen germination of all cultivars (except Salihli cv) was 15%. Pollens were even germinated in 0% sucrose, but pollen germination was enhanced by increasing the sucrose concentration up to 15%. The sucrose concentrations of 20–25% had an inhibitory effect on pollen germination. The highest germination percentage was 72.90% for apricot (Hasanbey cv), 57.38% for sweet cherry (Kırdar cv) and 49.16% for sour cherry (Kütahya cv) in 15% sucrose concentration in hanging drop tests (Table 2). On the other hand, the highest pollen germination was obtained in Mahmudun Eriği cv. for apricot (84.42%), Napoleon cv. for sweet cherry (66.60%) and sour cherry (84.42%) in 15% sucrose solution in agar plate tests (Table 3). The rate of pollen germination of sour cherry was found to be generally lower than that of apricot and sweet cherry cvs. Anvari (12) and Eti (9) have reported that the rate of

Table 2. The pollen germination rates of some Prunus species in hanging drop tests at different sucrose concentrations.

Cultivars	Pollen Germination (%) Sucrose Concentrations (%) 0	5	10	15	20	25
			APRICOT			
Hasanbey	8.22	19.66	45.60	72.90a	51.70a	49.28a
Şalak	16.27	21.10	41.87	51.03c	38.56ab	26.45b
Mah. Eriği	12.00	25.00	42.92	68.47a	51.05a	19.63c
Karacabey	13.61	19.75	39.63	63.27b	33.88b	23.33b
Şekerpare	11.16	15.68	35.91	49.77c	34.30b	19.91bc
$D_{\%5}$	–	–	–	5.20	6.52	3.40
			SWEET CHERRY			
Salihli	23.70	34.50a	55.10a	54.68	51.80a	31.15a
Napoleon	12.77	29.71b	40.85c	49.08	38.55c	27.52a
Sapıkısa	13.57	22.27c	47.32b	47.92	37.17c	28.67a
Kırdar	21.12	34.32a	43.80bc	57.38	44.50b	19.01b
$D_{\%5}$	–	3.00	3.87	–	3.12	4.65
			SOUR CHERRY			
Kütahya	23.82	29.35	45.88	49.16	44.17	25.06b

Table 3. The pollen germination rates of some Prunus species in agar–plate tests at different sucrose concentrations.

Cultivars	Pollen Germination (%) Sucrose Concentrations (%) 5	10	15	20
		APRICOT		
Hasanbey	45.58b	56.82b	67.62b	51.02c
Şalak	51.87a	48.63c	59.36c	44.92d
Mahmudun Eriği	47.40ab	78.41a	84.42a	81.85a
Karacabey	32.40c	51.98c	69.72b	53.88c
Şekerpare	42.21b	60.40b	57.83c	68.30b
$D_{\%5}$	4.60	3.62	4.24	5.10
		SWEET CHERRY		
Salihli	44.47	61.32a	66.32a	47.90ab
Napoleon	49.52	56.50ab	66.60a	52.92a
Sapıkısa	43.47	61.02a	65.57a	45.70b
Kırdar	36.75	50.83b	52.40b	36.17c
$D_{\%5}$	–	4.67	3.98	5.40
		SOUR CHERRY		
Kütahya	29.57	45.15	53.82	51.21

pollen viability and germination of the Köröser sour cherry are low due to the high level of abnormally shaped pollens. The results obtained in pollen germination in fruit species in 15% sucrose solution were similar to the results of other studies. In the literature, the germination percentage has been determined to be 66% for peach (2), 92% for almond, 55% for apricot, 61% for peach and 44% for plum in 15% sucrose solution (13). In addition, Eti (9), Parfitt and Almedhi (13) and Seilheimer and Stösser (14) have indicated that germination percentages vary significantly according to fruit species or cultivars. For example, the germination percentages of twenty–three apple cultivars were determined to range from 3.7% to 88.6% in 0.7% agar+ 10% sucrose medium (14). On the other hand, the pollen germination in agar–plate tests was higher than in hanging drop tests. Lee et al. (5) have stated that the agar concentration of the medium strongly influences the pollen germination of jojoba. Stanley and Liskens (15) have also reported that there are some advantages in using agar germination tests such as: the ease of taking carbohydrate, creating stable relative humidity and providing aerobic conditions.

The pollen viability of all cultivars was higher than the pollen germination. These data were consistent with the results of Pearson and Harney (4) in Rosa pollens and Parfitt and Ganeshan (7) in Prunus pollens.

Sucrose concentrations affected the pollen tube growth of sweet and sour cherries. The highest pollen tube measurements of sweet and sour cherry were obtained at 15% sucrose concentration, but it varied in apricot cultivars. Pollen tube lengths ranged from 198 to 357 µm, 209 to 335 µm and 174 to 188 µm in apricot, sweet cherry and sour cherry respectively (Table 4).

The correlations between pollen viability, morphological homogenity and germination tests were also determined. The relationship between pollen homogenity and viability tests was significantly different for apricot in TTC and for cherry in IKI tests (Table 5). The correlation coefficient between TTC and the morphological homogenity of apricot was 0.557*. The relationship between IKI and morphological homogenity was significant at the 5% level (r=0.682*) for cherry. The relationships between germination percentage of agar–plate tests and IKI stain tests on apricot and sweet cherry were r=0.686** and r=0.704*, respectively. However the relationships between agar–plate and TTC and safranin tests were not significantly different at the 5% level (Table 5). Studies to determine the relationship between pollen viability and germination tests in fruit species have had different results. For example, viability as determined by 2, 3, 5–triphenyl tetrazolium chloride stain testing of apple, pear, peach and grape pollens was not well correlated with the actual germination rate (1). However, Werner and Chang (2) have found that the correlation between the MTT stain test and pollen germination of peach was significant but it was not significant in IKI, propione carmine, anilin blue and NBT tests. On the other hand, Parfitt and Ganeshan (7) have

Table 4. The pollen tube length in agar plate tests at different sucrose concentrations of some Prunus species.

Cultivars	Pollen Tube Length (µm) Sucrose Concentrations (%) 5	10	15	20
		APRICOT		
Hasanbey	274	295	282a	301
Şalak	267	306	219b	275
Mahmudun Eriği	357	290	224b	261
Karacabey	290	251	198b	239
Şekerpare	283	268	244ab	262
$D_{\%5}$	–	–	48	–
		SWEET CHERRY		
Salihli	216	218	292	236
Napoleon	233	235	335	223
Sapıkısa	209	213	254	225
Kırdar	233	239	293	249
$D_{\%5}$	–	–	–	–
		SOUR CHERRY		
Kütahya	174	177	188	174

Table 5. Correlations between stain and germination tests, tube length and homogenity of apricot and sweet cherry pollens.

Characteristics	IKI	Safranin	Homogenity	Hanging drop	Agar–plate	Tube length
APRICOT						
TTC	–0.010	0.223	0.557*	0.039	–0.225	–0.340
IKI		0.307	0.129	0.385	0.686**	0.425
Safranin			0.401	0.039	0.009	0.002
Homogenity				–0.317	–0.128	–0.435
Hanging Drop					0.494	0.356
Agar–plate						0.466
SWEET CHERRY						
TTC	–0.096	0.073	0.243	0.019	–0.152	0.070
IKI		0.478	0.682*	0.066	0.704*	0.082
Safranin			0.350	0.204	0.234	0.305
Homogenity				–0.006	0.742*	0.163
Hanging Drop					–0.159	0.835**
Agar–plate						–0.271

** : Significant at 1% level. *: Significant at 5% level.

determined that the pollen stain tests (asetocamine, Alexander Stain's, TTC, MTT and NBT) are not reliable or consistent and are not positively correlated with *in vitro* germination tests. The inconsistency between our data and the results given above may have been due to differences in the research material.

As a result, these stain tests may be used to determine pollen viability in these species to provide only a rough estimate of viability. However, the exact amount of viable pollen may be determined *in vitro* by pollen germination. The main focus of this study was to investigate the relation between pollen viability and germination in terms of cultivars rather than species.

References

1. Oberle, G.D. and Watson, R., The use of 2, 3, 5–triphenyl tetrazolium chloride in viability tests of fruit pollens. Proc. Amer. Soc. Hort. Sci., 61, 299–303, 1953.
2. Werner, D.J. and Chang, S., Stain testing viability in stored peach pollen. HortScience, 16 (4): 522–523, 1981.
3. Widrlechner, M.P., Pellett, H.M., Ascher, P.D. and Fuhrman, S.C., In vivo pollen germination and vital staining in decidious azaleas. HortScience, 18 (1): 86–88, 1983.
4. Pearson, H.M. and Harney, P.M., Pollen viability in rosa. HortScience, 19 (5): 710–711, 1984.
5. Lee, C.W., Thomas, J.C. and Buchmann, S.L., Factors affecting in vitro germination and storage of jojoba pollen. J. Amer. Soc. Hort. Sci., 110 (5): 671–676, 1985.
6. Eti, S. and Stösser, R., Fruchtbarkeit der Mandarinensorte 'Clementine' (*Citrus reticulata* Blanco) I. Pollenqualitat und Pollenwachstum. Gartenbauwissenschaft 53 (4): 160–166, 1988.
7. Parfitt, D.E. and Ganeshan, S., Comparison of procedures for estimating viability of Prunus pollen. HortScience, 24 (2): 354–356, 1989.
8. Norton, J.D., Testing of plum pollen viability with tetrazolium salts. Proc. Amer. Soc. Hort. Sci., 89, 132–134, 1966.
9. Eti, S., Bazı meyve tür ve çeşitlerinde değişik in vitro testler yardımıyla çiçek tozu canlılık ve çimlenme yeteneklerinin belirlenmesi. J. Çuk. Univ., Agr. Fac., 6 (1): 69–81, 1991.
10. Elçi, Ş., Sitogenetikte Araştırma Yöntemleri ve Gözlemler. 100. Yıl Univ. Publication, Van, 1994.
11. Dowdy, S. and Wearden, S., *Statistics for Research*. John Wiley and Sons Inc. New York, 1983.
12. Anvari, S.F., Untersuchungen über das Pollenschlauchwachstum and die Entwicklung der Samenanlangen in Beziehung zum Fruchtansatz bei Sauerkirschen (Prunus cerasus L.). Dissertation Universitat Hohenheim, 1977.
13. Parfitt, D.E. and Almehdi, A.A., Liquid nitrogen storage of pollen from five cultivated Prunus species. HortScience, 19 (1): 69–70, 1984.
14. Seilheimer, M. and Stösser, R., Die Eignung verschiedener Apfelsorten als Pollenspender. Erwerbsobstbau, 24, 62–65, 1982.
15. Stanley, R. G. an Linskens, H. F., Pollen: Biology, Biochemistry, Management. Springer–Verlag, New York, 1974.