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DETERMINATION OF POLLEN QUALITY AND QUANTITY IN MULBERRY (*MORUS ALBA* L.)

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Abstract

In this study, the pollen grains of eight different mulberry types in İspir and Pazaryolu districts of Erzurum were tested for the determination of viability, germination rates, pollen production levels and morphologically homogeneity. Viability of the pollens was determined by TTC (2, 3, 5-triphenly tetrazolium chlorid) and IKI (iodine + potassium iodide) tests. Pollen germination experiments were performed with 'Agar-plate (1%)' method in sucrose solutions of 0, 5, 10, 15, 20 and 25% concentrations. In addition, pollen production and morphologically homogeneity were determined by the 'Hemacytometric method'. The pollen viability of all types used in this study was obtained in high ratios. Pollen germination rates were the highest for 15% and 20% sucrose solutions. The highest pollen production level was obtained from the genotype 6. The morphologically homogeneity levels of pollens changed from 97.36 to 98.86% in types.

Key words: Pollen, Mulberry (*Morus alba* L.), Hemacytometric, Homogeneity.

Introduction

The mulberry belongs to the *Urticales* order, the genus *Morus* of the family Moraceae. Freeman (1978) classified the mulberry tree into 12 species, Huo (2002) reported 14, Koidzumi (1914) 24 species and 1 subspecies (Machii *et al*., 2001), Martin *et al*. (2002) more than 30, Datta (2002) reported 68 species. The mulberry tree is a hardy species, adapting itself to different climate and soil conditions (Zheng *et al*., 1988). These are widely spread throughout all regions from the tropics to the sub-arctic and from sea level to altitudes as high as 4000 m (Machii *et al*., 2000). The mulberry tree originated in different areas of the world: *Morus alba* L. differentiated in China, Japan, Thailand, Malaysia and Birmania; *Morus nigra* comes from Turkey, Iran, Arabia and other Middle East countries; *Morus rubra* from North America (Bellini *et al*., 2000; Roger, 2002). Many ancient and naturally distributed regions have been greatly changed because of human interference (Zheng *et al*., 1988). Since germplasm of mulberry such as a lot of fruit species and one of the oldest areas of cultivate in Turkey, it is grown each region of that (Özbek, 1977). The production of mulberry fruits in 2012 was 74.170 tonnes from 2.446.9027 bearing trees in Turkey (Anon., 2013). Mulberry growing area in Northeastern Anatolia is generally located around the river valleys. Particularly, Çoruh river valley has notable populations of mulberry, which have been cultivated for their delicious edible fruits.

In most mulberry growing countries, particularly in Asian countries like India and China, mulberry is grown for its foliage to feed the silkworm (*Bombyx mori* L.). However, in most of the European countries including Turkey and Greece, mulberry is grown for fruit production rather than foliage (Gerasopoulos & Stavroulakis, 1997; Ercisli, 2004). The mulberry tree in Turkey is a multi-purpose species, since it can be used as a forest tree, ornamental tree, fruit production, and the leaves production for silk industry. Fruit of this species is not only consumed fresh but also used to produce jam, dried, pekmez, pestil, syrup, köme, and vinegar.

Mulberries are monoic, hermaphrodite and generally dioecious. Inflorescence is catkin with pendent or drooping peduncle bearing unisexual flowers. Inflorescence is always auxiliary. Male catkins are usually longer than the female catkins. Male flowers are loosely arranged and after shedding the pollen, the inflorescence dries and falls off. Number of parianth lobes are 4. Number of stamens is 4 and implexed in bud (Erdoğan, 2003) (Fig. 1).

Fig. 1. Mulberry male flowers.

The principal purpose of fruit growing is to obtain yields with high quality and quantity. As similar to other fruit species, it is important for mulberry to improve its yield and quality. 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 includes pollen produced in a flower, pollen morphological homogeneity, pollen germination, pollen tube growth and pollen competition; it is an important component of fertilization success in fruit trees (Thompson, 2004; Janick & Moore, 1996). Information on fertilization biology of mulberry is limited. Pollens are essential for pollination and fertilization. Thus, pollen germination and growth of pollen tubes are important research materials for morphological, physiological, biotechnological, ecological, evolutional, biochemical and molecular biological studies (Eti, 1991; Dafni & Firmage, 2000; Ottavio, 1992; Dane *et al*., 2004). Low fertilization rate and fruit set are closely related to different properties of pollens (quantity, germination rate and morphologically homogeneity). Defining these properties and identifying the relationships between fertilization and these properties are very important for practical fruit growing (Stösser, 1984; Thompson, 2004).

Information on pollen properties of mulberry has been very limited in the literature. Therefore the objective of this study was to investigate the viability, germination and pollen production level of the mulberries.

Material and Methods

This work was carried out on a mulberry collection maintained at the Pazaryolu (Genotype 1, 2, 3, 4) and İspir (Genotype 5, 6, 7 and 8) Municipality Orchards located in Çoruh Valley, Turkey, in 2012-2013 years. The latitude of İspir and Pazaryolu is 40°29′ and 40°24′, longitude is 40°59′ and 40°46′, altitude is 1,180 m and 1010 m, respectively. In this laboratory study eight mulberry genotypes (2 male (Genotype 2 and 6) and 6 monoic) were used. The experiments were conducted during 2012 and 2013 years.

Pollen performance: Pollens were obtained from flowers of the genotypes 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. The amount of pollen production per anther, per flower and morphological homogeneity percentages of mulberry genotypes were assessed with the hemocytometer (Marien-feld, Germany) slide (Eti, 1990). The morphological homogeneity level of pollen was also investigated with the same method.

Pollen germination capability was determined by the in-vitro germination test agar–plate (1 % agar + 5, 10, 15, 20, 25 % sucrose and incubated at the constant temperature of 25°C under dark conditions. The percentage of pollen germination was determined after 24 h incubation period.

The pollen viability level was estimated by using TTC (2, 3, 5-triphenyl tetrazolium chloride) and IKI (Iodine-Potassium-Iodure) stains (Norton, 1966; Baker & Baker, 1979; Eti, 1991). Pollens were scattered onto TTC and IKI solutions, and stained pollens were counted after 2 hours. To determine the pollen viability, pollens of each genotype (of four different areas) were observed onto two slides under a light microscope (×100 magnification). Counting was divided into three groups based on staining density. Dark red stained pollens were referred as viable, light red as semi-viable, and unstained as non-viable (Eti & Stösser, 1988). The grains of pollen were counted to determine viability after a couple of minutes in the IKI medium (1 g KI and 0.5 g I dissolved in 100 ml distilled water) (Eti, 1991). Pollens stained in few minutes were counted under light microscope. Dark brown stained pollens were referred as viable, yellowish as semi-viable, and unstained as non-viable. To determine viability, about three hundred pollen grains of each replicate from four different areas were counted under a light microscope.

Statistical analyses: Statistical analyses were performed with the General Linear Model using SPSS (V.20; Statistical software, SPSS. Inc., USA). The percentage data were first transformed to arcsine square root transformation, and an analysis of variance was performed. The differences among means were analyzed using the Duncan's multiple range test at $p<0.05$ significance.

Results and Discussion

Pollen production: Besides the amount of pollen production in the flowers of a cultivar, the rate of morphologically normal pollen grains is also important (Derin & Eti, 2001). Genoytpe 6 ranked first as to the number of anthers, pollen production capacity (Table 1). Morphological homogeneity was generally high in investigated mulberry types. Average anther numbers of investigated mulberry types were found between 68,0 (Genotype 7) and 182,0 (Genotype 6). There were statistically significant differences in terms of pollen numbers among the types, the highest and lowest values were determined as 14850 at Genotype 6 and 5875 at Genotype 5, respectively. There were significant differences as regards of the number of pollen in an anther among the types. The highest amount of pollen per anther was found in Genotype 6 (3712,5) and the lowest in Genotype 5 (1468,75). Eti (1991) reported that the values of morphologically homogeneity were between 51.8-100.0% in different fruit species and cultivars. Dokuzoğuz (1964) and Ülkümen (1973) reported that high pollen production, morphological homogeneity and pollen viability are important for fertilization. In addition, these properties called as pollen quality criterion, pollen quantity in flowers should be high values (Eti, 1996).

Table 1. Some quantitative characteristics of pollen of mulberry genotypes (average values of 2 experimental years).

Genotypes	Number of anthers in a flower cluster	Pollen number in a flower cluster	Mean pollen number in an anther	Morphological homogeneity (%)
1 ♂♀	176 d	11862,5 c	2965,63 c	98,86 b
2 ♂	178 d	12537,5 c	3134,38 c	98,69 b
3 ♂♀	126 bc	7112,5 ab	1778,13 ab	98,18 ab
4 ♂♀	128 c	7075,0 ab	1768,75 ab	98,58 b
5 ♂♀	94 ab	5875,0 a	1468,75 a	98,12 ab
6 ♂	182 d	14850,0 d	3712,50 d	98,73 b
7 ♂♀	68 a	6787,5 ab	1696,88 ab	97,36 a
8 ♂♀	126 bc	8250,0 b	2062,50 b	98,13 ab

*Values within a row followed by different letters are significantly different (p<0.05)

Table 2. Pollen viability rates in mulberry types determined by TTC and IKI tests (%) (average values of 2 experimental years).

Genotype	Stains				
	TTC			IKI	
	Viable	Semi-viable	Non-viable	Viable	Non-viable
1 ♂♀	48,14 ± 4,62 abc	31,18 ± 4,31 c	25,68 ± 4,27 ab	82,57 ± 3,70 b	17,43 a
2 ♂	57,45 ± 2,36 c	26,18 ± 2,29 bc	16,36 ± 2,41 a	86,46 ± 1,84 b	13,54 a
3 ♂♀	52,33 ± 3,12 bc	23,14 ± 1,64 bc	24,52 ± 3,48 ab	85,02 ± 1,54 b	14,98 a
4 ♂♀	35,75 ± 4,18 a	27,87 ± 2,35 bc	36,38 ± 4,63 b	80,54 ± 2,75 ab	19,46 ab
5 ♂♀	40,52 ± 3,94 ab	20,94 ± 2,01 abc	38,53 ± 4,96 b	75,39 ± 2,41 a	24,61 b
6 ♂	64,81 ± 4,61 c	19,00 ± 2,93 a	16,19 ± 3,51 a	86,60 ± 1,73b	13,40 a
7 ♂♀	63,44 ± 4,95 c	11,88 ± 3,02 a	24,68 ± 4,21 ab	84,24 ± 2,57 b	15,76 a
8 ♂♀	49,57 ± 3,46 abc	26,63 ± 4,89 bc	24,55 ± 2,77 ab	85,86 ± 1,70 b	14,15 a

*Values within a row followed by different letters are significantly different (p<0.05)
**Mean ± standard error. Six replicates were used for each treatment

Table 3. The rates of pollen germination in "Agar-plate" method at different sucrose concentrations of mulberry genotypes (%). (average values of 2 experimental years).

Concentration (%)	Genotypes							
	1	2	3	4	5	6	7	8
1% Agar	9,05 a±0,95	13,48 a±1,30	10,57 a±2,72	7,98 a±1,09	9,77 a±0,69	17,95 a±1,44	9,29 a±0,90	12,83 a±0,91
1% Agar +5% Sucrose	15,13 ab±2,60	30,48 b±3,03	20,73 b±2,91	15,36 b±0,69	24,95b±1,49	30,03 b±1,41	22,53 b±1,71	28,12 b±1,51
1% Agar +10% Sucrose	21,27 bc±1,27	43,52 c±2,10	42,69 c±4,68	20,98 c±0,58	35,91 c±1,85	50,0 d±0,87	34,63 c±2,05	44,71 d±1,99
1% Agar +15% Sucrose	25,11 c±1,21	59,36 d±2,63	66,91 d±4,06	27,33 d±1,45	49,49 d±2,88	76,62 f±3,4	48,07 d±2,25	67,33 f±2,84
1% Agar +20% Sucrose	47,61 d±2,72	72,41 e±1,71	40,47 c±2,38	36,56 e±1,93	61,43e ±2,00	67,25 e±1,5	62,03 e±3,42	53,63 e±2,07
1% Agar +25% Sucrose	15,53 ab±2,44	59,65 f±2,17	26,78 b±2,02	19,09 bc±2,06	48,03 d±1,46	37,5 c±1,61	42,2 d±1,65	35,59 c±3,00

*Values within a row followed by different letters are significantly different (p<0.05)
**Mean ± standard error. Six replicates were used for each treatment

Staining tests: IKI and TTC staining tests were used for pollen viability. The pollen viability values of mulberry genotypes are given in Table 2. In the FDA test, In the TTC test, the highest percentage of viable pollen grains (stained dark red) was found to be 64,81% in Genotype 6 and the lowest was found to be 35, 75% in Genotype -4. The percentage of semi-viable pollen grains (stained light red) varied between 11, 88% (Genotype-7) and 31, 18% (Genotype-1). The lowest percentage of non-viable pollen grains was found to be 16, 19% in Genotype -6 and the highest was found 38, 53% in Genotype 5 types.

Viability rates obtained by IKI test were generally close to those of TTC test. There were statistically significant differences among the percentages of viable and non-viable pollens in IKI test. In the IKI test the highest viable pollen was found in Genotype 6 (86, 60%) and the lowest in Genotype 5 (75, 39%) as in TTC test.

Many stain tests have been used such as acetocarmin, propione carmin, anilin blue, Alexander's stain, IKI (iodine + potassium iodide), FDA (fluorescein diacetate), MTT (2, 5-diphenyl tetrazolium bromide) and TTC (2, 3, 5-triphenyl tetrazolium chloride) to determine the pollen viability of fruit species and other plants.

Since different results were obtained in these studies a standard staining concentration and method could not be able to offer. In other words method and stain matter were different for different fruit species and cultivars (Oberle & Watson, 1953; Quarta *et al*., 1985; Pearson & Harney, 1984; Eti & Stösser, 1988; Parfitt & Ganeshan, 1989; Eti, 1991; Stösser *et al*., 1996; Bolat & Pırlak, 1999; Bots & Mariani, 2005; Pırlak & Güleryüz, 2005; Koyuncu, 2006; Ercişli, 2007). Results of this study indicated that, TTC and IKI stain matters could be used successfully in mulberry, which no such study has been reported, for pollen viability test.

Pollen germination tests: The germination percentages of pollen grains of the mulberry types are given in Table 3. It was obtained that the effects of sucrose concentration on pollen germination were statistically significant. Increasing in sucrose concentration for species increased germination rate up to one (15-20%) level and decreased after that point. The highest percentages of pollen germination were observed from 15% sucrose concentration in Genotype 3, Genotype 6 and Genotype 8 and 20% sucrose concentration in Genotype 1, Genotype 2, Genotype 4, Genotype 5 and Genotype 7. The lowest percentages were observed from 0% (control) concentration in all types.

The *In vitro* pollen germination is affected by plant species from which the pollen was collected, time of collection, the season, mode of collection and conditions of its storage (Stanley & Liskens, 1974). In addition to these factors, *In vitro* pollen germination is also influenced by the density of the pollen sown, the composition of germination medium, pH value, etc., (Moore & Janick, 1983).

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