

VIABILITY, GERMINATION AND AMOUNT OF POLLEN IN SELECTED CAPRIFIG TYPES

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Abstract

Pollen quality of 5 caprifig genotypes selected in the East Mediterranean Region was determined. Triphenyltetrazolium chloride (TTC) and fluorescent diacetat (FDA) test procedures were used in evaluating pollen viability. Germination of pollen was determined by using various concentrations of sucrose supplemented with H_3BO_3 , KNO_3 or GA_3 in agar medium. Pollen production status of caprifig flowers was determined. The percentage of viable pollen in caprifig types ranged from 76.04 to 83.34% by TTC test and from 75.60 to 86.73% by FDA test. The germinations were higher on the media containing 20% sucrose and increased up to 74 % with the addition of H_3BO_3 or KNO_3 , but not GA_3 . The number of pollen per flower ranged from 4355 to 7169 grains. The selected caprifigs appeared to be satisfactory pollinators in respect to criteria investigated.

Introduction

Fig (*Ficus carica*) probably originated in Western Asia and spread to the Mediterranean Basin. About 30% of the world crop is now produced by Turkey (Anon., 2003). Turkey dominates the world market due to lower production cost, and the farmers in various regions are likely to benefit the good price of fig fruit. However, the premium quality of fig fruits is strongly affected by the climatic conditions (Nalbant *et al.*, 1998). The typical fig-producing regions have mild winters and hot dry summers. In this respect, East Mediterranean Region of Turkey with its increasing irrigation infrastructure seems to have a good potential for fig growing. Well-known Turkish fig cultivars such as 'Sari Lop' and 'Bursa Siyahi' for export are grown in the favorable climates of Marmara and Aegean Regions of the country (Vidaud, 1996). These cultivars are believed to reduce quality under the more arid climatic conditions. Since East Mediterranean Region has natural fig population, which is appreciated by local markets and horticulturist, we have initiated a selection study to determine the best table figs among the local genotypes (Ilgin & Kuden, 1997).

There are two types of commercial figs: the common fig that bears fruits without pollination and the Smyrna fig that requires pollination by a fig wasp (*Blastophaga spp.*). The selected female figs out of the natural population required caprification (Ilgin & Kuden, 1997). Thus, we have selected 5 caprifig genotypes among the natural population, which had matured pollen accordingly to the occurrence of fig wasp in June (Ilgin, 1995). The viability of pollen and/or germination percentage as well as pollen amount affects the yield of cross-pollinated fruit trees. Therefore, selecting suitable caprifigs is of utmost importance for growers. Various test procedures have been used to determine pollen viability in fruit trees (Norton, 1966; Heslop-Harrison & Heslop-Harrison, 1970; Parfitt

& Ganeshan, 1989), since they are faster and easier than pollen germination tests. But, in some cases viability tests gave inconsistent results with the germination status of pollen (Parfitt & Ganeshan, 1989). Germination tests can be considered as more reliable way to determine the exact amount of viable pollen (Bolat & Pirlak, 1999). Pollen production i.e., amount of pollen per flower may also be important to a better pollination (Eti, 1990). In the present study, we examined the viability, germination and amount of pollen in selected caprifig genotypes.

Materials and Methods

In June, fresh pollen were collected from 5 selected caprifig types (46 EI 01, 46 EI 02, 46 EI 03, 46 EI 04, 46 EI 05) in Kahramanmaraş Province of Turkey, located in 37° 56' north latitude and 36° 56' east longitude.

***In vitro* pollen viability tests:** Triphenyltetrazolium chloride (TTC) and fluorescent diacetat (FDA) test procedures were used in determining pollen viability. In TTC test, 0.2 g triphenyltetrazolium chloride and 12 g sucrose were dissolved in 20 ml distilled water ((Norton, 1966). Petri dishes containing the TTC solution were evenly dusted with fresh pollen and kept at room temperature for 2 h under daylight, and then pollen grains were examined using a light microscope (x 100). The viability of pollen was scored according to staining level: pollen with bold red color as viable, with light red color as semi-viable and with yellowish-green color or colorless as non-viable. In FDA test, 2 mg fluorescent diacetat and 1.71 g sucrose were dissolved in 10 ml distilled water (Heslop-Harrison & Heslop-Harrison, 1970) and the pollen were dusted. All pollen grains, which fluoresced brightly in a fluorescence microscope were scored as viable. Viability percentages were determined, using four replicates of about 100 grains each.

***In vitro* pollen germination tests:** Pollen germination was conducted in Petri dishes on basal medium with 1 % agar and various concentrations of sucrose (5, 10, 15, 20, 25, 30 %). To improve pollen germination, based on the result of the first experiment, a second test was conducted with 20 % sucrose supplemented with H_3BO_3 (0.025, 0.050, 0.01 %), KNO_3 (0.025, 0.050, 0.01 %) or GA_3 (0.025, 0.050 %).

In both germination experiments, fresh pollen were evenly dusted onto 0.01 ml of germination solutions and incubated at 20°C constant temperature for 8 h. A pollen grain was considered to be germinated when the length of the pollen tube was equal to or longer than the diameter of the grain. Germination was scored by a light microscope (x 100) in four random fields (about 50 grains / field) of two Petri dishes for each caprifig type.

Determination of pollen production: The anthers of 20 flowers for each caprifig type were counted and after pollen shedding, the number of pollen was determined based on hemacytometric method (Eti, 1990). Pollen production per flower was calculated by multiplying the number of anthers per flower with number of pollen per anther (Eti & Stösser, 1988).

All data in the experiment were subjected to analysis of variance and the mean separation was done by Turkey's MRT at $P \leq 0.01$.

Results and Discussion

The percentage of viable pollen in caprifig types ranged from 76.04 to 83.34% by TTC test and from 75.60 to 86.73% by FDA test (Table 1). In general, TTC and FDA tests did not differ much in determining the viability of pollen. The higher percentage of non-viable pollen by FDA test indicated that semi viable pollen by TTC should be considered as non-viable. This was possibly because some non-living organelles in the cells could be stained to some extent by TTC.

The highest percentages of viable pollen were found in type 46 EI 04 and 46 EI 05 by either TTC or FDA; but the remaining types had also good amounts of viable pollen (over 75%) with both the tests. Therefore, TTC and FDA may be equally accepted in determining the viability of caprifig pollen; however, the choice can be affected by cost or ease of the method. This finding was in accordance with previous reports, indicating that both viability tests give similar results (Seilheimer & Stöser, 1982; Eti, 1990). The high percentage of pollen viability found in caprifig types may indicate a good pollen germination rate in a suitable *in vitro* condition.

The caprifig pollen did not germinate at all on a medium without sucrose (Table 2). Increasing sucrose concentrations up to 20% improved pollen germination percentages. However, germinations decreased with higher sucrose concentrations (25 or 30%). This finding showed the negative effect of higher sucrose concentrations on pollen germination as reported in some stone fruits (Bolat & Pirlak, 1999). In all caprifig types tested, the highest pollen germinations (between 60.08 and 68.48 %) were achieved on the media containing 20% sucrose. To improve germination further, a second experiment was set up and several concentrations of H_3BO_3 , KNO_3 and GA_3 were added on the media containing 20% sucrose. The germination percentages of the some caprifig pollen were highest (over 70%) with the addition of 0.050% H_3BO_3 , followed by 0.025% KNO_3 (Table 1). Germination percentages of the caprifig pollen were higher with the addition of these chemicals than the 20% sucrose alone. The germination percentage found in this study was in accordance with the results of Awamura *et al.*, (1995) in which caprifig pollen germination increased to above 70% by adding stigmatic exudates from long styled pistillate flowers to the culture medium. Contradictory to the germination studies mentioned above, it was reported that better pollen germination in caprifigs was obtained with 5% sucrose concentration (Zeybekoglu *et al.*, 1997). But, in our study, pollen germinations were as low as 8-10% with this sucrose concentration.

In this study, pollen viability was higher than pollen germination. This result was in accordance with previous studies (Pearson & Harney, 1984; Bolat & Pirlak, 1999). Various germination test and media as well as culture conditions may affect the germination results of a given cultivar (Stanley & Linskens, 1974). Although intact (viable) pollen might be expected to have a good germination capacity, lower germination percentages are often obtained, possibly due to insufficient *in vitro* procedures. Unfortunately, the correlation between *in vitro* and *in vivo* germination of pollen in caprifigs has not been investigated. In our study, both viability tests indicated the existence of high percentage of viable pollen in caprifig types.

Table 1. Percentage of pollen viability in caprifig by TTC and FDA tests*.

Caprifig types	TTC			FDA	
	Viable	Semi-viable	Non-viable	Viable	Non-viable
46 EI 01	76.04 b**	4.54 b	19.42 a	75.60 d	24.40 a
46 EI 02	80.56 ab	3.40 b	16.04 ab	82.86 bc	17.14 bc
46 EI 03	78.39 ab	4.50 ab	17.12 a	81.06 c	18.94 b
46 EI 04	82.11 a	5.70 a	12.20 bc	85.03 ab	14.97 bc
46 EI 05	83.34 a	6.06 a	10.60 c	86.73 a	13.27 c

* Mean of four replicates (about 100 grains each)

** Mean separation by Turkey's MRT at $P \leq 0.01$.**Table 2. Percentages of caprifig pollen germination on 1 % agar medium supplemented with various concentration of sucrose and chemicals*.**

Sucrose concentration	Caprifig types				
	46 EI 01	46 EI 02	46 EI 03	46 EI 04	46 EI 05
0 %	0.00 f **	0.00 f	0.00 f	0.00 f	0.00 e
5 %	9.49 e	10.20 e	8.57 f	10.51 e	11.44 d
10 %	30.30 d	36.44 d	29.80 e	37.95 d	38.98 c
15 %	21.18 c	53.91 c	46.71 d	56.52 bc	58.87 b
20 %	60.08 a	65.50 a	62.71 a	67.44 a	68.48 a
25 %	54.83 b	58.09 b	56.08 b	59.13 b	58.95 b
30 %	49.38 c	52.05 c	50.25 c	54.19 c	55.82 b
20 % sucrose plus					
0.025 % H ₃ BO ₃	60.89 bc	66.05 b	63.75 c	66.98 c	69.63 bc
0.050 % H ₃ BO ₃	68.32 a	70.87 a	69.05 a	72.78 a	74.08 a
0.01 % H ₃ BO ₃	60.99 bc	63.41 bc	62.42 c	66.41 c	67.09 cd
0.025 % KNO ₃	58.70 c	60.71 c	59.58 d	63.07 d	65.29 de
0.050 % KNO ₃	60.96 bc	64.13 b	61.87 c	65.99 c	67.69 cd
0.025 % KNO ₃	64.38 b	69.53 a	66.80 b	70.93 b	71.75 ab
0.025 % GA ₃	50.10 d	57.31 d	54.69 e	59.13 e	62.90 e
0.01 % GA ₃	48.64 d	52.38 e	50.73 f	55.29 f	56.85 f

*Germinations were scored in four random areas of two Petri dishes.

**Mean separation by Turkey's MRT at $P \leq 0.01$.

The number of anthers per caprifig flower was about 4 (Table 3) Similarly, in another study this number was reported to be 5 for caprifigs (Tanriver *et al.*, 1996). The number of pollen per anther differed significantly among the caprifig types. The highest number of pollen per anther was achieved in 46 EI 03 (1748 grains), followed by 46 EI 02 (1568 grains). In other types, the number of pollen per anther ranged from 1043 to 1306 grains. The calculated numbers of pollen per flower were significantly higher in 46 EI 03 and 46 EI 02 caprifig types (above 7000 grains). There does not appear to be any report regarding to pollen production data in figs. Galil & Meiri (1981) reported that the fig wasp (*Blastophaga spp.*) became passively coated with pollen on their exit from the fig. In this respect, the high amount of pollen found in the caprifigs may play an important role in the transfer of pollen by this vector.

Table 3. Pollen production in caprifig types*.

Caprifig types	Number of anthers / flower	Number of pollen /anther	Total number of pollen / flower
46 EI 01	4.2	1043 c	4355 b
46 EI 02	4.6	1568 ab	7132 a
46 EI 03	4.1	1748 a	7169 a
46 EI 04	4.3	1306 bc	5617 ab
46 EI 05	4.3	1388 b	5883 ab
	N.S**	$P \leq 0.01$	$P \leq 0.01$

*Means of twenty flowers.

**N.S. Non-significant.

Conclusion

In determining the pollen quality, viability tests are often considered to be faster and easier methods than the germination tests, since the effects of external factors such as temperature, humidity, and germinating media are minimized. The results of our study strongly supported this approach. Either TTC or better FDA could be used in determining the pollen viability and indicate germination status in caprifigs; the choice might be affected by cost or ease of the method.

The five caprifig genotypes selected in the East Mediterranean Region appear to have sufficient pollen viability and/or germination to be used in fig pollination; however, this needs to be tested by *in vivo* pollinations for yield. The high amount of pollen found in the caprifig flowers may play an important role in the transfer of pollen by fig wasp.

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