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Assessment of pollen viability and germination in seven varieties of lemon

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ABSTRACT

We investigated the viability and *in vitro* germination of pollen in seven lemon varieties (*Citrus limon* (L.) Burm f.) ('BATEM Sarısı', 'BATEM Pınarı', 'Interdonato', 'Kütdiken', 'İtalyan Memeli', 'Meyer' and 'Lamas'). The viability was tested with TTC (2, 3, 5–triphenly tetrazolium chloride) and *in vitro* germination was tested with agar–plate to estimate pollen viability and germination in these varieties. Additionally, effect of different sucrose concentration levels (5%, 10%, 15%, 20% and 25%) for *in vitro* pollen germination was observed. The results indicated that pollen of 'Meyer' had the highest viability with 86.74%. The pollen of 'Lamas' and 'Meyer' varieties had the highest *in vitro* germination with 39.77% and 39.04%, respectively. This study results showed that 20% and 25% sucrose concentrations in the agar plates had the highest germination with 36.16% and 32.12%, respectively.

***Keywords*:** lemon, pollen, viability, germination, sucrose

Introduction

Citrus is the most widely produced fruit group in the world. There has been a rapid increase in the production both in the world and in Turkey. Approximately 759.711 tons of lemon fruits are produced in Turkey (FAO 2014). With total export of 374.734 tons lemons are ranking as the first within the citrus fruits in Turkey (AKIB 2014).

Information about the ability of pollen grains to germinate when they reach the stigmas of flowers of their own species is valuable both for horticultural purposes and general botanical research. Viability tests provide a means of assessing the potential of pollen to germinate on the stigma (Firmage and Dafni 2001). Several different methods have been suggested to determine the viability of pollen (Dafni 1992; Kearns and Inouye 1993; Marcucci et al. 1984; Demirkeser et al. 2001).

The objective of this study was to determine the pollen viability and *in vitro* germination percentage of different lemon cultivars.

Material and methods

Studies were carried out on pollen grains of seven cultivars of lemon 'BATEM Sarısı', 'BATEM Pınarı', 'Interdonato', 'İtalyan Memeli', 'Kütdiken' , 'Lamas' and 'Meyer'. Pollens collected during the spring of 2011 and 2012 were used in these experiments.

In order to determine the pollen viability and germination capacity, well-grown flower from each variety were picked about ten o'clock in the balloon stage flowers. They were put in paper bags and were brought to the laboratory. The anthers were left to dehisce for 24 h at room temperature at about 23°C, and the fresh pollen was immediately used for pollination (Distefano et al. 2009).

Effects of various sucrose concentrations on the pollen germination and pollen viability rates with Tetrazolium (TTC) test were determined. TTC (2, 3, 5–triphenlytetrazoliumchloride), stain tests were used to determine pollen viability. One or two drops of TTC solution was put on a clean micro slide and kept for 3-4 h at ambient conditions (Norton 1966). For this assay, two lamella for each cultivars and four regions of each lamella were investigated; viable, semi-viable and dead pollen numbers and their percentages were determined. Pollen viability was scored based on the staining level as pollen with red color viable, with

light red semi-viable and with colorless nonviable.

Five different sucrose concentrations (5, 10, 15, 20 and 25%) were used in the agar–plate tests to determine pollen germination and were added to 1% agar and 0.1% boric acid. In tests, the pollen was incubated at 21°C for 24 h under dark conditions. The two plates were scanned for each pollen source. In the germination test, five microscopic areas from each replication were counted randomly at the end of the 24 h incubation period. The values for viability and germination of pollens were subjected to square–root transformation for statistical analysis. The experiment carried out completely randomized plot design. Duncan's test was used to compare the means.

Results

The pollen viability in lemon varieties was found to be significantly different in the TTC solution (Table 1). The highest pollen viability was obtained from Meyer variety (86.74%) followed by Kütdiken (69.22%) in the TTC stain test.

Table 1.The rate of pollen viability in the TTC

Cultivars	The rate of viable pollen(%)
BATEM Pınarı	52.66 cd *
BATEM Sarısı	40.62 d
Interdonato	59.48 bc
İtalyan Memeli	54.57 c
Kütdiken	69.22 b
Lamas	52.29 cd
Meyer	**86.74 a**

*Different letters indicated significant differences (P<0.05) according to the Duncan test

The differences in pollen germination among cultivars were significant in all the sucrose concentrations in agar–plate test. The sucrose concentrations in the agar–plate methods were found to have different effects on pollen germination of all cultivars. The highest pollen germination was obtained in Lamas (39.77%) and Meyer (39.04%). The optimum sucrose concentrations in the agar-plate method for pollen germination of all cultivars were 20% and 25% (Table 2).

Table 2.The rate of pollen germination in the agar-plate test

Cultivars	Sucrose concentrations (SC)					Means of cultivars
	5%	10%	15%	20%	25%	
BATEM Pınarı	20.10	22.04	12.72	19.52	28.98	20.67 bc*
BATEM Sarısı	15.29	15.08	17.38	28.65	20.65	19.41 c
Interdonato	11.80	13.38	13.28	31.53	22.09	18.42 c
İtalyan Memeli	31.36	34.38	31.63	37.31	40.60	35.06 a
Kütdiken	25.51	23.34	18.93	23.30	41.54	26.52 b
Lamas	36.11	29.65	42.09	44.09	46.90	39.77 a
Meyer	23.89	38.19	40.32	40.47	52.34	39.04 a
Means of SC	23.44 B	25.15 B	25.19 B	32.12 A	36.16 A	

*Different letters indicate significant differences (P<0.05) according to the Duncan test

Discussion

Eti (1991); Parfitt and Almedhi (1984); Seilheimer and Stösser (1982) have indicated that germination percentage vary significantly according to fruit species or cultivars. Sucrose concentrations affected the pollen germination of lemon varieties. Our study, the optimum sucrose concentrations for pollen germination of all cultivars were 20% and 25%. Ateyyeh (2005) found that pollen of *Citrus maxima* showed the highest germination percentage when placed in media containing 20% sucrose. In the literature, sucrose concentration has been determined as % 10 (Seilheimer and Stösser 1982) and % 15 (Werner and Chang 1981; Parfitt and Almehdi 1984) in the pollen germination percentage of different fruit species. 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.

It is clear that no one test is suitable for testing viability in all species. It is also apparent that some of the stains that have been suggested most often over estimate viability.

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