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Research Article

Investigation of Pollen Viability and Germination Characteristics of Some Caprifigs Selected from the Eastern Mediterranean Region of Türkiye

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ABSTRACT

Caprifigs (*Ficus carica* var. *caprificus*) are essential for caprification and hybridization studies in cultivated figs, a gynodioecious species. Rising summer temperatures due to global climate change increase the need to evaluate the pollinator properties of caprifigs, especially in regions experiencing sudden temperature fluctuations, such as the Mediterranean region of Türkiye. This study assessed the pollen viability and germination status of selected caprifig genotypes from the Eastern Mediterranean Region of Türkiye. In the study, 20 caprifig genotypes from Adana, Kahramanmaraş, Mersin, and Osmaniye provinces were compared with standard caprifig cultivars (Ak İlek, Armut İlek, Elma İlek, Hamza, Küçük Konkur, and Taşlık). Pollen viability of the caprifig genotypes was evaluated using TTC and FDA tests. Pollen germination was studied in a medium containing 1% agar + 3% sucrose + 100 ppm H_3BO_3 + 300 ppm $Ca(NO_3)_2$ + 200 ppm $MgSO_4$ + 100 ppm KNO_3. Pollen viability values ranged from 67.05% to 98.60% in the TTC test and from 70.75% to 94.32% in the FDA test. Pollen germination values ranged from 30.22% to 88.90%. As a result, the Kahramanmaraş07, Mersin01, and Osmaniye08 genotypes selected from the natural population of the Eastern Mediterranean Region of Türkiye were as valuable as standard cultivars in terms of pollen viability and germination properties.

Keywords: Caprifig, TTC, FDA, pollen germination

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INTRODUCTION

The fig (*Ficus carica* L.) has a functionally gynodioecious reproductive system, with caprifig male and female fruits produced on separate trees. Female trees produce the edible figs, while male trees (caprifigs) are essential for pollination and support the life cycle of the obligate fig wasp, *Blastophaga psenes* L. (Çalışkan and Bayazıt, 2012; Flaishman, 2022).

Pollination in fig trees occurs through a highly specialized mutualistic relationship known as 'caprification', in which fig wasps transfer pollen from caprifig trees to the flowers of edible fig trees. Caprifigs are not only essential pollen donors but also provide the ecological conditions necessary for the development of the pollinator wasp (Caliskan et al., 2017; Caliskan et al., 2023). The quality of pollen produced by caprifigs, particularly its viability and germination potential, is an important factor influencing the success of this natural pollination mechanism. Pollen viability reflects the physiological integrity and survival of pollen grains, while germination rate indicates the pollen's ability to form a tube and achieve fertilization (Bolat and Pirlak, 1999). These traits are also critical for selecting effective pollinator individuals for breeding and orchard management programs. In addition, effective caprification using compatible pollens of caprifig can improve fruit size, enhance skin and pulp color, and increase levels of total soluble solids, sugars, organic acids, aroma components, and phytochemicals (Gaaliche et al., 2011; Trad et al., 2012; Yaman and Çalışkan, 2014; Rosianski et al., 2016).

Key characteristics that determine the effectiveness of caprifig trees as pollinators include high pollen viability, strong germination capacity, synchronized flowering with edible fig trees, and the ability to support the life cycle of the fig wasp (Ilgın et al., 2017; Yaman and Çalışkan, 2016a). Therefore, it is important to select suitable caprifig genotypes not only for effective pollination but also to improve the nutritional and commercial quality of fig fruits.

Pollen germination in caprifigs is influenced by genotype (Yaman and Çalışkan, 2016b), light or darkness, sugar composition (glucose, fructose, and sucrose), nutrient medium composition, pH of the medium (Caliskan et al., 2016), and pollen pore number (Caliskan et al., 2021). In addition, several staining methods, including FDA (fluorescein diacetate) and TTC (triphenyl tetrazolium chloride), have been widely used to assess pollen viability in caprifigs (Ilgın et al., 2017; Gaaliche et al., 2013) and other fruit species (Bayazit et al, 2011; Yıldız and Kaplankıran, 2014).

Türkiye is recognized as the native land of figs. It is one of the richest regions in the world in terms of genetic diversity, particularly for caprifig genotypes, which play a crucial role in natural pollination and breeding programs (Aksoy et al., 2003; Simsek et al. 2020; Çalışkan and Dalkılıç, 2022). However, studies on caprifigs are limited. Evaluating pollen viability and germination in caprifig genotypes is important for selecting superior pollinator sources and increasing the success rate of caprification, especially in commercial fig production systems. In this study, the viability and in vitro germination capacity of pollen from different caprifig individuals were determined to better understand fig pollination ability and contribute to future breeding efforts.

MATERIALS AND METHODS

This study was conducted on 20 caprifig genotypes selected from the provinces of Adana (Adana01, Adana02, Adana03, Adana10, Adana12), Kahramanmaraş (Kahramanmaraş07, Kahramanmaraş08, Kahramanmaraş09, Kahramanmaraş10, Kahramanmaraş12), Mersin (Mersin01, Mersin08, Mersin12, Mersin13, Mersin19), and Osmaniye (Osmaniye01, Osmaniye06, Osmaniye08, Osmaninye09, Osmaniye10) in the Eastern Mediterranean Region of Türkiye. In addition, standard caprifig cultivars, including 'Ak İlek', 'Armut İlek', 'Elma İlek', 'Hamza', 'Küçük Konkur', and 'Taşlık', obtained from the Fig Research Station (Aydın) were used as reference plant materials.

For the pollen viability test, 15 fully ripened fruits (at the onset of wasp emergence and pollen dispersal) were collected from each genotype and cultivar. The ostiole end of each caprifig fruit was cut and spread onto aluminum foil. The fruits were left at room temperature for approximately two days until completely dried,

allowing the anthers to fully dehisce. The released anthers were then collected and stored in glass vials. The pollen grains obtained from these anthers were kept at +4°C until viability and germination tests were performed (Caliskan et al. 2016).

Pollen viability was assessed using two staining techniques: TTC (2,3,5-Triphenyl Tetrazolium Chloride) and FDA (Fluorescein Diacetate). A 1% TTC solution was used for the viability test, following the procedure described by Eti (1991). On each microscope slide, one drop of either TTC or FDA solution was placed in separate spots using a pipette. Pollen grains were gently dusted onto each droplet with a sable brush, and the droplets were then covered with cover slips.

For each genotype and cultivar, two slides were prepared, and pollen viability was evaluated by counting in three randomly selected microscopic areas per slide. In the TTC test, observations were made under a light microscope two hours after pollen application. In the FDA test, evaluations were conducted five minutes after application using a fluorescence microscope.

In the TTC test, pollen grains that appeared dark red under the microscope were considered 'viable', those with light red staining were classified as 'partially viable', and unstained or pale yellow–cream colored grains were considered 'non-viable'. However, due to incomplete staining observed in caprifig pollen, only fully stained grains were considered 'viable', and unstained ones were categorized as 'non-viable' in this study. In the FDA test, viability was determined based on fluorescence: pollen grains emitting bright green fluorescence were classified as 'viable', while those displaying dull or faint green fluorescence were considered 'non-viable'.

To determine the pollen germination levels of caprifig genotypes, in vitro germination percentages were evaluated using the petri dish agar method. The germination medium was prepared based on the optimal formulation identified by Yaman and Caliskan (2016b) for caprifig pollen germination and included 1% agar+3% sucrose+100 ppm H_3BO_3+300 ppm $Ca(NO_3)_2$+200 ppm $MgSO_4$+100 ppm KNO_3. The pH of the medium was 5.0 (Caliskan et al., 2016). After pollen grains were evenly sown onto the surface of the medium, the petri dishes were incubated in the dark at 25°C for 24 hours. For each genotype and cultivar, three petri dishes were prepared, and observations were made under a light microscope in three randomly selected fields per dish. Pollen grains were counted as germinated when the length of the pollen tube equaled or exceeded the diameter of the pollen grain (Stanley and Linskens, 1974).

The data were analyzed using the SAS package program (SAS, 2005) to determine the mean, minimum, maximum, standard error, and coefficient of variation.

RESULTS AND DISCUSSION

The results on pollen viability in caprifig genotypes and cultivars, as determined by TTC and FDA tests, were presented in Table 1. Among the caprifig genotypes, Kahramanmaraş07 had the highest pollen viability (96.01%), while Osmaniye06 had the lowest viability (67.05%). Pollen grains not stained by TTC were considered non-viable, with the proportion of non-viable pollen ranged from 3.99% (Kahramanmaraş07) to 33.95% (Osmaniye06). In caprifig cultivars, pollen viability ranged from 81.61% (Küçük Konkur) to 98.60% (Ak İlek). The highest proportion of non-viable pollen grains based on TTC staining was found in Taşlık (18.39±3.25%), while the lowest was recorded in Ak İlek (1.40±1.63%). The overall mean pollen viability across all genotypes and cultivars was 87.46%. When caprifig genotypes were compared with cultivars, similar TTC viability results were observed. The coefficient of variation (CV) for the TTC viability test was 7.95%.

In the FDA viability test, pollen viability among the genotypes ranged from 72.60% in Adana10 to 94.32% in Kahramanmaraş10. Pollen grains showing dull green fluorescence were considered non-viable, with the highest non-viability observed in Adana10 at 27.40%. Among the cultivars, the highest pollen viability was recorded in Elma İlek (81.83%), while the lowest was observed in Ak İlek (70.75%). The percentage of non-viable pollen in caprifig cultivars ranged from 18.17% in Elma İlek to 29.25% in Ak İlek. The overall average pollen viability determined by the FDA test across all genotypes and cultivars was 79.73%, while the average non-viability rate was 20.27%. When comparing caprifig genotypes with cultivars, genotypes generally showed higher pollen viability. The coefficient of variation (CV) for the FDA viability test was 7.10%.

These results showed significant variation in pollen viability among caprifig genotypes and cultivars when assessed using TTC and FDA staining techniques. The relatively high viability rates observed in several genotypes suggest that caprifigs have considerable potential as effective pollinators. In particular, Kahramanmaraş07 and Kahramanmaraş10 may be promising candidates for fruit set in edible figs and for breeding programs aimed at enhancing pollination efficiency.

When comparing genotypes with cultivars, genotypes generally exhibited higher viability in both TTC and FDA tests. This suggests that wild or regionally adapted genotypes may have superior pollen performance compared to commonly cultivated cultivars. The TTC and FDA tests were largely consistent in classifying high- and low-viability genotypes, although some discrepancies were observed-likely due to the different biochemical mechanisms each test detects (dehydrogenase activity versus membrane integrity and esterase activity). The coefficients of variation for the TTC (7.95%) and FDA (7.10%) tests were relatively low, indicating reliable measurements and stable trait expression among the tested materials.

Table 1. TTC and FDA results of caprifig pollen

Genotype	TTC (%)		FDA (%)	
	Viable	Non-viable	Viable	Non-viable
Adana01	89.63±5.87	10.37±5.87	81.29±9.99	18.71±9.99
Adana02	87.91±8.08	12.09±8.08	79.82±7.18	20.18±7.18
Adana03	88.06±10.40	11.94±10.40	81.00±5.58	19.00±5.58
Adana10	84.52±2.77	15.48±2.77	72.60±10.33	27.40±10.33
Adana12	71.00±10.72	29.00±10.72	80.56±3.28	19.44±3.28
Kahramanmaraş07	96.01±2.00	3.99±2.00	81.19±6.12	18.81±6.12
Kahramanmaraş08	90.66±1.57	9.34±1.57	78.44±8.43	21.56±8.43
Kahramanmaraş09	86.71±5.40	13.29±5.40	78.25±5.72	21.75±5.72
Kahramanmaraş10	95.38±3.69	4.62±3.69	94.32±2.04	5.68±2.04
Kahramanmaraş12	86.75±7.57	13.25±7.57	93.35±6.31	6.65±6.31
Mersin01	88.71±3.05	11.29±3.05	73.25±6.73	26.75±6.73
Mersin08	72.25±18.03	27.75±18.03	84.77±6.91	15.23±6.91
Mersin12	93.22±4.73	6.78±4.73	77.09±9.36	22.91±9.36
Mersin13	92.97±4.39	7.03±4.39	86.74±8.50	13.26±8.50
Mersin19	73.30±9.70	26.70±9.70	82.55±9.60	17.45±9.60
Osmaniye01	89.08±5.84	10.92±5.84	78.69±2.26	21.31±2.26
Osmaniye06	67.05±12.59	32.95±12.59	82.85±4.29	17.15±4.29
Osmaniye08	90.47±5.59	9.53±5.59	79.80±7.03	20.20±7.03
Osmaniye09	94.74±2.14	5.26±2.14	78.09±2.76	21.91±2.76
Osmaniye10	91.83±2.95	8.17±2.95	75.04±9.24	24.96±9.24
Ak İlek	98.60±1.63	1.40±1.63	70.75±5.34	29.25±5.34
Armut İlek	90.01±5.45	9.99±5.45	75.44±6.91	24.56±6.91
Elma İlek	82.83±4.05	17.17±4.05	81.83±8.74	18.17±8.74
Hamza	85.14±16.42	14.86±16.42	73.02±2.27	26.98±2.27
Küçük Konkur	81.61±3.25	18.39±3.25	76.81±9.34	23.19±9.34
Taşlık	86.09±9.59	13.91±9.59	75.56±9.34	24.44±9.34
Mean	87.46	12.64	79.73	20.27
Minimum	67.05	1.40	72.60	27.40
Maximum	98.60	32.95	94.32	5.68
Variation of coefficient (%)	7.95	63.45	7.10	27.92

Table 2. Germination results of caprifig pollen (%)

Genotype	Germination ratio
Adana01	52.29±9.58
Adana02	55.21±1.50
Adana03	58.14±15.29
Adana10	51.27±8.13
Adana12	66.00±6.76
Kahramanmaraş07	85.55±4.76
Kahramanmaraş08	77.00±5.75
Kahramanmaraş09	78.89±1.78
Kahramanmaraş10	31.94±7.71
Kahramanmaraş12	71.57±7.15
Mersin01	88.90±3.95
Mersin08	42.61±1.57
Mersin12	46.67±7.08
Mersin13	61.87±8.79
Mersin19	38.75±3.31
Osmaniye01	64.57±9.45
Osmaniye06	37.76±14.34
Osmaniye08	80.10±2.19
Osmaniye09	63.45±13.09
Osmaniye10	55.69±6.59
Ak İlek	30.22±14.62
Armut İlek	74.84±5.90
Elma İlek	63.66±8.67
Hamza	62.76±0.27
Küçük Konkur	67.13±7.14
Taşlık	45.27±4.66
Mean	59.70
Minimum	30.22
Maximum	88.90
Variation of coefficient (%)	27.07

In previous studies on caprifigs, pollen viability determined by the TTC test was reported to vary by genotype and region, ranging from 76.04% to 83.34% in Kahramanmaraş (Ilgın et al., 2007), 45.7% to 84.0% in Tunisia (Gaaliche et al., 2013), and 74.71% to 98.81% in Hatay (Yaman and Çalışkan, 2016a). Similarly, Ilgın et al. (2007) reported that pollen viability in caprifigs from Kahramanmaraş ranged from 75.60% to 86.73% using the FDA test, while Yaman and Çalışkan (2016a) found viability values between 66.50% and 83.62% in genotypes selected from Hatay.

In viability tests, a pollen viability rate above 50% was considered an indicator of excellent quality pollen. All results confirm that the studied caprifigs exhibit sufficiently high pollen viability and that these genotypes have viable and potentially functional pollen suitable for effective caprification.

Among the caprifig genotypes, the highest pollen germination rate was observed in the Mersin01 genotype at 88.90%, while the lowest was found in the Kahramanmaraş10 genotype at 31.94%. Among caprifig cultivars, the highest pollen germination rate was recorded in the Armut İlek cultivar (74.84%), and the lowest in the Ak İlek cultivar (30.22%). The overall mean pollen germination rate across all genotypes and cultivars was 59.70%. When caprifig genotypes were compared with cultivars, the pollen germination results were found to be generally similar. The coefficient of variation for the pollen germination test was 27.07%, indicating considerable variability among the tested samples. However, all genotypes and cultivars tested exceeded the generally accepted minimum threshold for functional germination (>30%), as reported by Stanley and Linskens (1974) for viable pollen evaluation in fruit species.

Previous studies reported similar pollen germination values, ranging from 35% to 89% in genotypes from Turkey (Yaman and Çalışkan, 2016b) and from 40% to 86% in Tunisian caprifigs (Gaaliche et al., 2013). These findings are consistent with our results, suggesting that both genetic background and environmental conditions significantly influence pollen germination capacity (Caliskan et al., 2016; Yaman and Çalışkan, 2016b).

CONCLUSIONS

This study revealed significant variation in pollen viability among caprifig genotypes and cultivars using both TTC and FDA staining methods. Some caprifig genotypes, particularly Kahramanmaraş07, Kahramanmaraş09, and Mersin01, showed high germination levels, indicating strong potential as effective pollinizers. The higher viability observed in genotypes compared to cultivars suggests that wild or local caprifigs may offer valuable genetic traits for breeding and orchard management. The results highlight the need to consider pollen viability as a key selection criterion in fig breeding programs, especially given the critical role of caprifig trees in caprification and their potential impact on fruit quality in edible fig cultivars. Further research integrating pollen viability with fruit set rates and offspring performance could significantly contribute to understanding pollinator effectiveness in edible figs.

ETHICAL APPROVAL

The study, entitled " Investigation of Pollen Viability and Germination Characteristics of Some Caprifigs Selected from the Eastern Mediterranean Region of TürkiyeTitle of the Study ", was conducted in accordance with the relevant scientific, ethical and citation rules. No falsification was made of the collected data, and this study has not been sent to any other academic media for evaluation. As it does not require ethics committee approval, it can be considered to be in accordance with the relevant ethical standards.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

AUTHOR CONTRIBUTION

All authors contributed equally.

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