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MICROMORPHOLOGY OF POLLEN GRAINS FROM BISEXUAL AND FUNCTIONAL MALE FLOWERS OF POMEGRANATE

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ABSTRACT

Modern taxonomy uses pollen grain morphology as an important tool due to its specific and diverse features. Pomegranate is an andromonoecious species having two type flowers on the same plant, in other words, hermaphrodite flowers (bisexual) and male flowers (functional male flowers) that develop on the same plant. The length of polar axis (*P*) and the equatorial diameter (*E*) as well as *P/E* ratio of pollen grains, collected from both bisexual and functionally male flowers of the cultivar 'Caner I', were analyzed using scaning electron microscopy (SEM). The length of polar axis (*P*), the equatorial diameter (*E*), and *P/E* ratio was compared between the two grain. The sculpturing pattern of the exine of both type flowers was striate, with more parallel longitudinal ridges Pollen from both bisexual and functional male flower types is similar in size (≈21 μm). In contrast to the divergent pistil development observed between bisexual and functional male pomegranate flowers, no differences in pollen morphology were detected. The pollens had prolate shape (P\E=1.65 vs 1.59) in both types of flowers.

Keywords: *Punica granatum, Pollen Grains, Morphology, SEM.*

INTRODUCTION

Due to the fact that pollen grains have a definite shape, size, color, and structure for each species, genus, and family, research into the morphological characteristics of pollen is of great significance in taxonomy, phylogeny, and paleobotany. Electron microscopy is a very powerful technique for testing the morphological characteristics of pollen in the various species of plants (Evrenosoğlu and Mısırlı, 2009). Sotonyi et al. (2000) stated that dimensions of a pollen grain are to a large extent genetically determined and they can be of great importance for cultivar characterization. Morphological characteristics such as differences in size and surface structure of pollen grain are a useful tool for distinguishing species and cultivars of fruit trees (Moore et al., 1991; Geraci et.al., 2012). Research showed that exine surface, aperturation, shape and size of the grain are all useful features to distinguish rosaceous pollens (Hebda et al., 1988; Mert, 2009; Nagy, 2011). Pollen morphology in apples, peaches, cherries, pears and quince demonstrated species-

specific variation in size and exine sculpturing (Chung et al., 2010; Radovic et al., 2016).

Pomegranate (*Punica granatum* L.) has two types of flowers, a condition referred to as andromonoecy. In other words, hermaphrodite flowers (bisexual) and male flowers (functional male flowers) develop on the same plant. Sometimes, the same pomegranate tree can carry three types of flowers, namely hermaphrodite, male and intermediate forms (Engin et al., 2015). Wetzstein et al. (2011) characterized two types of flowers on the same tree: hermaphroditic bisexual flowers and functionally male flowers. Male flowers are smaller, with a campanulate calyx and a rudimentary ovary. Bisexual flowers (long-styled perfect flowers) are larger, have larger ovaries, and set more fruit than short style types. Information on the morphological characteristics of pollen grain to two types of flowers of pomegranate can be very advantageous to identify cultivars, which is of great benefit for the certification of germplasm and breeding purposes. In addition, the form of pollen grains and protuberances on them play a very important role in the pollination of fruit trees, i.e. pollen transfer (either entomophilous or anemophilous) (Dokic, 1988).

The present work aims to describe the morphology and anatomy of bisexual and functionally male flower types in pomegranate to better understand developmental differences between the flower types. In addition, histological evaluations of pollen grains from bisexual and functional male flowers were conducted using SEM to assess structure and size and shape of the pollen grain.

MATERIAL AND METHOD

The orchard site was located at the Çanakkale Onsekiz Mart University's Horticulture Experimental Farm, 5 m above sea level. 'Caner I' cultivar of pomegranate was selected for the research. Flowers at the same stage (before at the open petal stage) of both functional male and bisexual flowers were collected from 13-year-old trees, planted at distance of 3x5 m and taken to the laboratory. Bisexual and functional male flowers were separated based on the size of the pistil, which in male flowers is expressed as a shortened style. Morphological differences of both bisexual and male flowers were photographed digitally. Their anthers were detached slowly using a forceps and then put on a paper at room temperature of 22°C for about 12-18 hours to waste some of their moisture, split and release pollen. The pollen was further dried up at room temperature for 10-12 hours, then put into brown glass vials. Vials were stored in a refrigerator at +4 °C until examined.

Bisexual and functional male flowers were dissected and prepared using methods described by Engin and Unal (2007) for scanning electron microscopy (SEM). For the SEM study, samples were mounted directly on metallic stubs using double-sided adhesive tape and coated with gold in a sputtering chamber (BAL-TEC SCD 005 Sputter Coater). Observation of the prepared samples was carried out with a scanning electron microscope (SEM) JEOL JSM-7100F (Tokyo, Japan) at 15 kV. Sixty pollen grains from

both bisexual and functional male flowers from the 'Caner I' cultivar were examined for morphological characters; shape, length of the polar axis (*P*), length of the equatorial diameter (*E*) and ratio of polar axis to equatorial axis (*P/E*).

The experiment was carried out in three replicates. In each replicate, ten pollen grains were analyzed. The statistical analysis was performed using MINITAB software (Minitab Inc., ver.16) to determine differences in *P*, *E*, and *P/E* ratio. Significant differences between the mean values were determined using Duncan's multiple range test for significance at $p \leq 0.05$.

RESULTS AND DISCUSSION

Under field conditions, bisexual and functional male flowers ratios in pomegranate can impact crop productivity and yield. Functional male flowers drop and generally fail to set thus, fruits develop exclusively from bisexual flowers. The percentage of flowers that are male in pomegranate can be significant and more than 60% to 70% depending on variety and season (Engin et al., 2015; Mars, 2000).

'Caner I' cultivar of pomegranate characterized two types of flowers on the same tree: hermaphroditic bisexual flowers and functionally male flowers. Male flowers are smaller, with a campanulate calyx and a rudimentary ovary (Fig. 1, left). Bisexual flowers (long-styled perfect flowers) are larger, have a discoid stigma covered with copious exudates, elongated stigmatic papillae, a single elongated style and numerous stamens inserted on the inner walls of the calyx tube (Fig. 1, right).

Based on the analysis conducted by SEM, pollen from both bisexual and functional male flower types is similar. In contrast to the divergent pistil development observed between bisexual and functional male pomegranate flowers, no differences in pollen morphology were detected (Fig. 2). Pollen grains can be characterized as spheriodal with a smooth exine surface. In view of the number, position and type of the apertures, the grains are prolate.

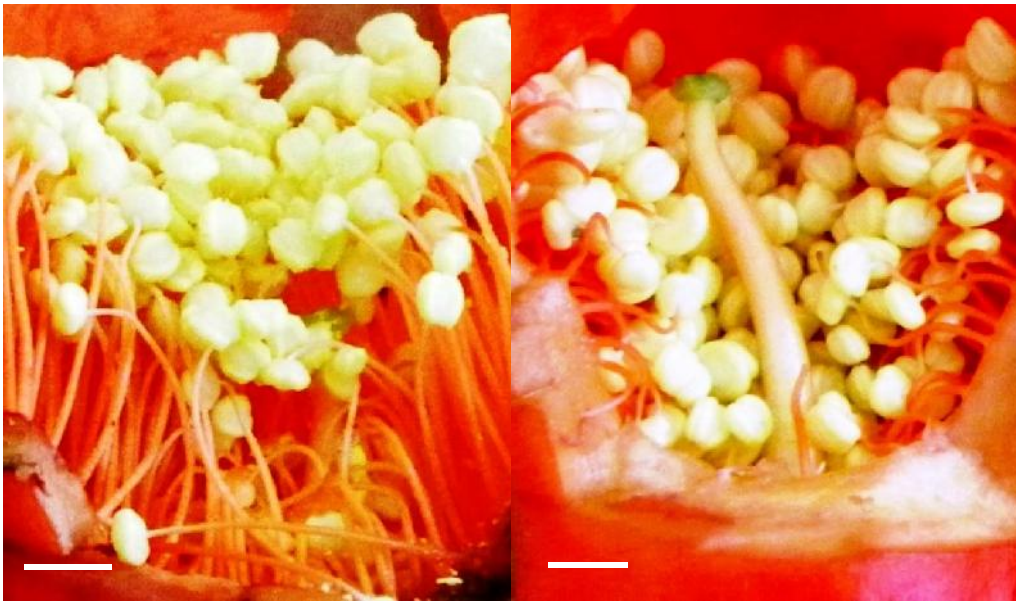

Figure 1. Close-up view of generative organs of pomegranate (*Punica granatum* L.) cultivar 'Caner I' (left: functional male, right=bisexual) Scale bar: 5.0 mm

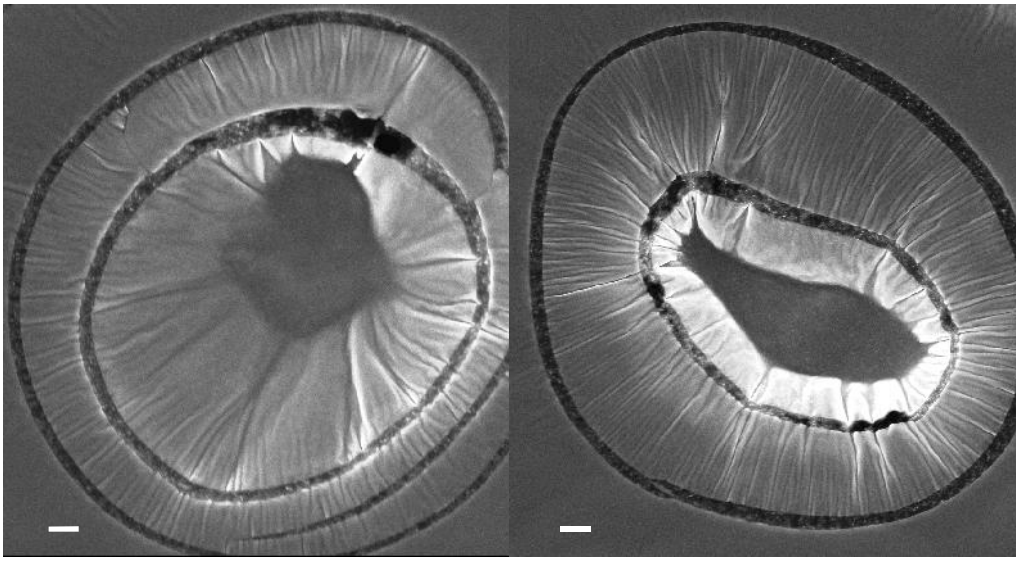

Figure 2. Scanning electron microscopy (SEM) of pollen grain (equatorial view) of pomegranate cultivar 'Caner I' (left: functional male flower and right: bisexual flower). Bars: 1 µm.

Length of the polar axis (*P*), length of the equatorial diameter (*E*) and ratio of polar axis to equatorial diameter (*P/E*) of pollen grains from bisexual and functional male flowers were demonstrated in table 1.

Table 1. Morphological characteristics of pollen grains from functional male and bisexual flowers of pomegranate (*Punica granatum* L.) cultivar 'Caner I' (Mean±SE).

	Polar axis (P) µm		Equatorial axis (E) µm		P/E ratio	shape
	Variation range	Mean value	Variation range	Mean value		
Functional male	27.1-28.2	27.70±1.02 a	15.8-17.2	16.70±1.32 a	1.65±0.07a	prolate
Bisexual	26.6-28.2	27.44±1.08 a	15.4-17.7	17.16 ±1.04 a	1.59±0.09 a	prolate
Pollen (Mean)		27.57		16.93		

*Means within a column (comparing pollens from bisexual versus male flowers) followed by different letters are significantly different at $P \leq 0.05$ using Duncan's multiple range test.

The investigated pollen grains in bisexual and functional male flowers of 'Caner I' cultivar did not differ in their size and shape. Polar length was maximum (28.2 µm) in both sexual morphs and minimum (26.6 µm) in the bisexual flower (Table 1). The width of pollen grains ranged from 15.4 µm to 17.7 µm in both types. The pollen is about the same size (about 21 µm). In relation to the results of Wetzstein et al (2011), pollen size of 'Wonderful' pomegranate cultivar is about 20 µm. This result show that pomegranate pollen is small sized. Shangshang et al. (2015) reported that P/E ratio of 55 indigenous pomegranate cultivars ranged between 1.54 and 2.05. The investigated pollen grain of pomegranate cultivar in bisexual and functional male flowers was classified as prolate, according to the classification of Erdtman (1969). Varasteh and Arzani (2009) characterized the shape of the pollen grains of 14 Iranian pomegranate cultivars as prolate based on *P*/*E* ratio. Evrenosoğlu and Mısırlı (2009) pointed out that in the species of the Mediterranean fruits, length of pollen grains varies from 26.2 to 54.2 µm and width from 15.2 to 29.6 µm. The length of the pollen grain in apple ranged from 40.1 to 43,8 µm and width from 20.9 to 23.2 µm (Currie et al., 1997). In Japanese pear the length was between 43.4 and 45.1 µm and the width between 21.8 and 22.9 µm (Matsuta et al., 1982). Pollen size generally ranges from 15 to 100 µm (Robertson, 2008). Bisexual and functional male flowers of 'Caner I' cultivar had small pollen (15.4-28.2 µm) with grooves on the surface. The sculpturing pattern of the exine is striate, with more parallel longitudinal ridges.

CONCLUSION

The analysis of morphological characteristics of pollen did not show significant differences among bisexual and functional male pomegranate flowers.

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