Morphological Characterization of Pollen in Some Varieties of Walnut (*Juglans regia*)

Nihal Acarsoy Bilgin

Faculty of Agriculture, Department of Horticulture, Ege University, İzmir, Turkey

**ABSTRACT**

In this study carried out to determine the pollen characteristics of walnut, Bilecik, Chandler, Fernette, Fernor, Kaman 1, Pedro, Şebin, and Şen 1 varieties were used as material. As a result of scanning electron microscopy (SEM) examination it was stated that the pollen shapes were oblate spheroidal for all the walnut varieties. The pollen of the varieties exhibited diversity in pollen length (37.54–40.52 µm), width (41.10–45.02 µm), pori diameter (2.45–3.61 µm), distance between pori (11.28–14.41 µm) and spinules (0.54–0.73 µm), pore (16.40–23.00) and spinules (20.50–43.50) number, spine width (0.18–0.26 µm) and length (0.09–0.28 µm). Results showed that in particular, the Fernor had the largest pore diameter, so their number per unit area was low. In contrast, Bilecik had the smallest pore diameter. Distance between pori of this variety was the least. Therefore, a large number of pori was observed on the surface. In addition, spinules sizes were determined to be larger in Şen 1, but smaller in Chandler variety than the others. In particular, these morphological properties of pollen can be used for identification of different varieties. Also, the pollen shape and exine patterning of the varieties studied were illustrated by SEM images.

**KEYWORDS**

*Juglans regia*; pollen micromorphology; scanning electron microscopy; statistical analysis

**Introduction**

Walnut is one of the oldest nut fruit species cultivated in the world. Archeological excavations mention the existence of walnut shells in the Neolithic age, 8000 years ago (Şen, 2011). Walnut belongs to the *Juglandales* order of the *Juglandaceae* family and part of the *Juglans* genus. Species of the genus *Juglans* consists of 4 sections: *Juglans, Trachycaryon, Cardiocaryon* and *Rhysocaryon* (McGranahan and Leslie, 1991). *Juglans regia* L., which is superior in terms of fruit quality, is widely grown. The fatty acid, protein and mineral contents of walnuts are very high. Therefore, it is an important part of a healthy diet. In Anatolia, which is the gene center, a large number of domestic and foreign varieties are grown almost everywhere (Ağca et al., 2014).

The edible part of the walnut is the kernel. Although apomixis is rarely observed, pollen is essential for the fruit set. Walnuts have both male (catkin) and female (pistillate) flower parts on the same tree (monoecious), with wind–pollinated flowers. Each flower on the catkin has 13–18 anthers. There are approximately 900 and 1.8 million pollen grains per anther and catkin, respectively (Forde, 1975). A walnut tree can produce an average of 5000 catkins (Şen, 2011). The size of pollen grains is medium (26–50 µm) and can travel quite some distance by the wind (Bammer, 2014). Pollen has hereditary properties that determine the genotype. Therefore, in addition to morphological, physiological and molecular methods, identification is carried out according to the pollen properties (Gul et al., 2019; Sufyan et al., 2018). The morphology of pollen can be examined in detail via scanning electron microscopy. The morphological and palynological studies were carried out on the identification of the collected new species using microscopic techniques (Bahadur et al., 2019). Also, these techniques...
are used to define and distinguish pollen micromorphological features of trees, shrubs and annual plants so they can be used to determine pollen characteristics both qualitatively and quantitatively (Amina et al., 2020; Naz et al., 2019). Numerous studies have been conducted to distinguish species and varieties in fruits. In this context, the width, length, shape and surface structure of pollen, the number and size of pore on the exine were determined (Arzani et al., 2005; Chwil, 2015; Evrenosoglu and Mısırlı, 2009; Geraci et al., 2012; Mert, 2010; Milatovic et al., 2020; Radovic et al., 2016). In addition, a large number of species and genera of the Juglandaceae family were examined by SEM and the morphological structures of the pollen were revealed (Bos and Punt, 1991; Evrenosoglu and Mısırlı, 2009; Mert, 2010; Milatovic et al., 2020; Stone and Broome, 1971). Although there are many species in this family, the varieties described in this study are included in the J. regia species. Some varieties are partially included in previous studies (Evrenosoglu and Mısırlı, 2009; Mert, 2010). In addition, this study differs from other studies because the pattern of ornamentation on the exine was described. The morphological characteristics of pollen can contribute to the identification of the genotype. Pollen storage is one of the ex situ approaches to conservation genotypes in gene banks. Pollen is used to distinguish genotypes. These findings are valuable for plant breeders. As it is known, there are different identification methods. This method can be applied more easily than the others and its cost is low (Evrenosoglu and Mısırlı, 2009; Nikolić and Milatović, 2016, 2017).

The present study was carried out to distinguish micromorphological characters of pollen in walnut. Pollen has specific characteristics according to variety. Research into the micromorphological properties of pollen is of significance in taxonomy. This study, which was planned and conducted by considering these conditions, aimed to determine the pollen properties of some domestic and foreign walnut varieties which are economically important using the scanning electron microscopy (SEM) technique. These characters examined are very important for the identification and discrimination of the related varieties.

**Materials and Methods**

Eight walnut varieties Bilecik, Chandler, Fernette, Fernor, Kaman 1, Pedro, Şebin and Şen 1, were used as plant materials. These varieties were taken from the Menemen Research, Application and Production Farm of Ege University in Turkey. The trees were located in an orchard the mentioned farm. All the selected walnut varieties are widely grown in Turkey. The pollen in this study was gathered from current tree varieties located in an orchard on the farm. Pollen was examined using the SEM method (Evrenosoglu and Mısırlı, 2009; Milatovic et al., 2020. Nikolić and Milatović, 2016, 2017).

To obtain pollen, the catkins were collected immediately after pollen shedding commenced (Forde, 1975). Catkins were kept on paper at room temperature and then the anthers were split open after about 24 hours. The pollen was stored at room temperature until analysis was performed. Dry pollen grains were sifted onto double-sided tape. The sample on each stub was coated with gold and palladium at a film thickness of 10 nm in a vacuum evaporator under a specific pressure using a Leica model sputter coater (Evrenosoglu and Mısırlı, 2009; Mert, 2010; Sufyan et al., 2018). Pollen grains were then observed by a scanning electron microscope (Thermo Scientific Apreo S model) and photographed at a magnification of 5000 × for whole grains and 50000 × for the exine patterning. The SEM was installed in Ege University in the Central Research Test and Analysis Laboratory Application and Research Center.

For each variety, 45 pollen grains were used to analyze the pollen length (polar axis), width (equatorial axis) and length/width ratio (Evrenosoglu and Mısırlı, 2009; Mert, 2010; Milatovic et al., 2020; Nikolić and Milatović, 2016, 2017). Also, pori diameter, distance between pori, distance between spinules, spinule width and length were measured in these samples. The number of pore was determined according to the method of Monoszon (1952). In addition, the spinules frequency (spinules per 10 µm²) was calculated as quantitatively. Based on the length/width ratio, the pollen
shape was identified as oblate spheroidal (0.88–1.14) (Erdman, 1943). The patterning ornamentation on the exine of the walnut varieties was described according to Punt et al. (2007). These samples were taken from 3 trees of each variety. All measurements were made by SEM.

The data were subjected to analysis of variance using the SPSS 20 statistical software program package. Significant differences between averages were defined by Duncan test at the $P \leq .05$ significant levels. The mean, minimum, maximum and standard deviation values of the properties examined were determined. The relationship between these values was revealed by conducting Pearson’s correlation analysis. Differences or similarities in the varieties were evaluated according to their analyzed properties by applying Principal Component Analysis (PCA) to the findings obtained. PCA is a very popular tool to summarize a dataset into a few factors which highlight the most important information. But the factors are sometimes difficult to interpret. A varimax rotation is a change of coordinates. The factors remain orthogonal after the rotation, preserving an essential property of the PCA. By using the rotation methods such as varimax, their interpretations have been easier. Moreover, Cluster analysis (CA) was utilized to create a dendrogram showing the similarities and differences between genotypes. Clustering is the grouping a set of data in such a way that data in the same cluster are more similar to each other than to those in other clusters. The relationship between the original variables and the components is expressed by component loadings. The component loadings are therefore used to physically interpret the components. Hierarchical cluster analysis dendrogram was used with Ward’s clustering method.

**Results**

From the examinations made with SEM, the resulting data on the pollen grain size of the walnut varieties are determined (Table 1). The mean pollen length, width and length/width ratio between varieties differed in a statistically significant way. Based on the varieties, the highest pollen length was found in Kaman 1 (40.52 µm), whereas the lowest was observed in Chandler (37.54 µm). Other varieties were within these limit values. For pollen width, the highest values determined in the Kaman 1 (45.02 µm) and Şebin (44.90 µm) varieties. For pollen width, the maximum value is Kaman 1 (45.02 µm) and the minimum value is Fernor (41.10 µm). Also, the length/width ratio ranged from 0.89 to 0.95. Pollen shapes of all varieties exhibited oblate spheroidal shapes (0.88–1.14) (Erdman, 1943). The data on the exine ornamentation patterning were determined (Table 2). Depending on the varieties there was a statistical difference in terms of pore diameter, distance between pori and the pore number. The pore diameter varied among the varieties as the highest average pore diameter were observed in Fernor (3.61 µm) whereas the lowest pori diameter were found in Bilecik (2.45 µm). The largest variation (standard deviations of 0.76) occurred in the Şebin variety (1.93–4.16 µm). The average distance between pores reached the highest value in Fernette with 14.41 µm. The lowest value was observed in Bilecik with 11.28 µm. The variation range for pore number was determined as 16.40 (Fernette and Fernor) – 23.00 (Bilecik). Among the varieties examined, Fernor had the largest pore

| Varieties | Pollen length (µm) | Pollen width (µm) | Length/width ratio |
|-----------|--------------------|------------------|-------------------|
|           | Min                | Max              | Mean ± SD         | Min                | Max              | Mean ± SD         | Min                | Max              | Mean ± SD         |
| Bilecik   | 36.75              | 42.10            | 40.12 ab ±1.86    | 38.52              | 45.20            | 41.96 cd ±1.91    | 0.91              | 0.99              | 0.95 a ±0.02      |
| Chandler  | 32.75              | 43.84            | 37.54 cd ±3.65    | 40.30              | 44.61            | 41.96 cd ±1.53    | 0.75              | 0.99              | 0.89 b ±0.07      |
| Fernette  | 34.64              | 44.12            | 38.96ab ±3.05     | 41.67              | 45.34            | 42.22 b ±1.21     | 0.78              | 0.99              | 0.90 b ±0.07      |
| Fernor    | 32.48              | 42.05            | 38.63 bc ±2.86    | 37.20              | 45.39            | 41.10 d ±2.68     | 0.82              | 1.00              | 0.94 a ±0.06      |
| Kaman 1   | 35.85              | 45.03            | 40.52 a ±3.39     | 42.44              | 50.25            | 45.02 a ±2.22     | 0.82              | 0.99              | 0.89 b ±0.06      |
| Pedro     | 34.65              | 45.51            | 40.49 a ±2.73     | 39.88              | 49.33            | 43.55 b ±2.69     | 0.82              | 0.99              | 0.93 ab ±0.06     |
| Şebin     | 34.76              | 45.15            | 40.19 ab ±3.31    | 40.36              | 49.34            | 44.90 a ±2.45     | 0.70              | 0.99              | 0.89 b ±0.09      |
| Şen 1     | 33.60              | 43.65            | 39.80 ab ±3.11    | 39.92              | 46.36            | 42.90 bc ±1.76    | 0.80              | 1.00              | 0.92 ab ±0.06     |

Abbreviations: Min: minimum values; Max: maximum values; SD: standard deviations. Mean values followed by different lower case letters are different significantly by Duncan’s multiple range test at $P \leq 0.05$. 

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diameter, so their number per unit area was low. In contrast, Bilecik had the smallest pore diameter. Distance between pori of this variety was the least. Therefore, a large number of pori was observed on the surface.

The values of the spinules on the pollen surface of walnut varieties are shown in (Table 3). These properties examined differed depending on the varieties (P ≤ .05). For spine length and length, Şen 1 (0.26 and 0.28 µm) was in the highest in the lowest statistical group. The lowest spine length and length values were found in Chandler and Pedro with 0.18 µm, and Fernette with 0.09 µm, respectively. The varieties formed different statistical groups in terms of distance between the spinules. According to this measurement, the Pedro variety was in the first group. This was followed by the Bilecik, Şen 1, Chandler, Şebin, Fernette, Kaman 1 varieties, and the Fernor variety was in the last group. Further, it was observed that the density of spinules per unit area was higher in Bilecik variety and less in Chandler variety.

The pollen shape and exine patterning of the studied varieties are shown in SEM images (Figure 1). The pollen has circular apertures on the exine surface, they are called pori (Figure 1(a,c,e,g,i,k,m,o)). These are uniformly distributed. Scanning electron microscopy analysis showed that pollen grains of all examined walnut genotypes are isopolar and pantoporate. The pollen shape is circular in polar view. Pollen is symmetrical with respect to equatorial diameter (Erdtman, 1943). Pollen ornamentation patternings are seen on the exine surface. There are pointed structures on the surface of pollen which are classified as spinules (Figure 1(b,d,f,h,j,l,n,p)).

The correlation coefficients of the features were found (Table 4). Accordingly, the highest positive correlation was determined between the length/width ratio and the pollen width (r = 0.742; p < .01). Similarly, depending on pollen length, an increase in width (r = 0.392) was found. By contrast, a negative correlation occurred between the length/width ratio and the pollen length value (r = −0.323; p < .01). Furthermore, the pore diameter had a negative correlation to pollen width (r = −0.292; p < .01) and length (r = −0.226; p < .05). Correlation was found between spine length

| Varieties | Pori diameter (µm) | Distance between pori (µm) | Pore number |
|-----------|-------------------|--------------------------|-------------|
|           | Min | Max | Mean | SD  | Min | Max | Mean | SD  | Min | Max | Mean | SD  |
| Bilecik   | 1.64| 3.21| 2.45 e | ±0.065| 9.63| 12.79| 11.28 b | ±1.07| 20.00| 28.00| 23.00 a | ±2.71|
| Chandler  | 2.72| 4.01| 3.25 b | ±0.39| 6.56| 17.09| 12.44 b | ±3.12| 16.00| 22.00| 18.40 bc | ±2.46|
| Fernette  | 2.13| 4.83| 3.09 bc | ±0.68| 9.13| 21.78| 14.41 a | ±4.58| 14.00| 18.00| 16.40 c | ±1.58|
| Fernor    | 2.81| 4.75| 3.61 a | ±0.58| 8.51| 17.66| 12.99 ab | ±2.77| 14.00| 18.00| 16.40 c | ±1.58|
| Kaman 1   | 1.93| 3.84| 2.75 de | ±0.55| 7.24| 17.21| 12.07 b | ±3.00| 16.00| 22.00| 18.40 bc | ±2.07|
| Pedro     | 2.41| 3.38| 2.86 cd | ±0.39| 10.18| 17.26| 12.47 b | ±2.13| 16.00| 20.00| 17.20 bc | ±1.69|
| Şebin     | 1.93| 4.16| 2.72 de | ±0.76| 6.40| 18.98| 12.31 b | ±4.03| 16.00| 22.00| 18.40 bc | ±1.84|
| Şen 1     | 1.84| 3.62| 2.54 e | ±0.52| 8.77| 21.07| 13.10 ab | ±3.86| 16.00| 22.00| 18.80 b | ±1.69|

Abbreviations: Min: minimum values; Max: maximum values; SD: standard deviations. Mean values followed by different lower case letters are different significantly by Duncan’s multiple range test at P ≤ 0.05.

| Varieties | Spine width (µm) | Spine length (µm) | Distance between spinules (µm) | Spinules number |
|-----------|-----------------|------------------|-------------------------------|---------------|
|           | Min | Max | Mean | SD  | Min | Max | Mean | SD  | Min | Max | Mean | SD  |
| Bilecik   | 0.20| 0.25| 0.23 b | ±0.02| 0.10| 0.20| 0.15 cd | ±0.04| 0.50| 0.99| 0.70 ab | ±0.14| 35.00| 50.00| 43.50 a | ±4.74|
| Chandler  | 0.15| 0.20| 0.18 d | ±0.02| 0.07| 0.12| 0.10 f | ±0.01| 0.20| 0.88| 0.64 abc | ±0.18| 15.00| 25.00| 20.50 d | ±3.69|
| Fernette  | 0.20| 0.25| 0.20 cd | ±0.02| 0.07| 0.12| 0.09 f | ±0.02| 0.49| 0.78| 0.62bc | ±0.10| 20.00| 40.00| 30.50 b | ±6.65|
| Fernor    | 0.20| 0.25| 0.22 bc | ±0.02| 0.10| 0.18| 0.13 de | ±0.02| 0.44| 0.77| 0.54 d | ±0.08| 25.00| 45.00| 34.00 b | ±6.15|
| Kaman 1   | 0.20| 0.26| 0.24 ab | ±0.02| 0.13| 0.20| 0.17 c | ±0.04| 0.49| 0.74| 0.62 c | ±0.08| 20.00| 30.00| 25.50 c | ±3.69|
| Pedro     | 0.10| 0.20| 0.18 d | ±0.03| 0.10| 0.14| 0.12 ef | ±0.02| 0.55| 0.99| 0.73 a | ±0.13| 20.00| 35.00| 25.50 c | ±4.38|
| Şebin     | 0.20| 0.30| 0.24 ab | ±0.03| 0.18| 0.25| 0.20 b | ±0.03| 0.39| 0.86| 0.63bc | ±0.15| 35.00| 45.00| 41.00 a | ±4.59|
| Şen 1     | 0.20| 0.30| 0.26 a | ±0.04| 0.25| 0.35| 0.28 a | ±0.04| 0.35| 0.93| 0.66 abc | ±0.15| 15.00| 35.00| 22.50 cd | ±5.89|

Abbreviations: Min: minimum values; Max: maximum values; SD: standard deviations. Mean values followed by different lower case letters are different significantly by Duncan’s multiple range test at P ≤ 0.05.
and width \( r = 0.678; \ p < .01 \) and pori diameter \( r = -0.335; \ p < .01 \). A strong correlation was found between spinules number and pore number, whereas a weak correlation was determined with spinule width.

As a result of Principle Component Analysis (PCA) performed in the eight walnut varieties, 4 principal components were revealed and they defined 87.23% of the varieties (Table 5). According to this analysis, distance between spinules, pore diameter, pore number and pollen width values made the highest positive contribution to PC1, constituting 26.23% of the total variance, which is the most important component. The spinule width and length contributed to PC2, accounting for 23.51% of the total variance. PC3 explains the spinules number and distance between pore explained 19.72% of the total variance. The pollen length and length/width ratio contributed to PC4, accounting for 17.76% of the total variance.

Clustering analysis was used to determine the degree of similarity between the walnut varieties, and is shown as dendograms (Figure 2). Consequently, the varieties were categorized under two main groups. Pedro, Kaman 1, Chandler and Şen 1, were included in the first group, and the Fernor and Fernette varieties were also subsequently added to these. The Şebin and Bilecik varieties were put together in the second group. The varieties in the groups showed similarities in terms of the features studied. The similarities or differences among the walnut varieties examined with CA showed a correlation with those examined by PCA in terms of the characteristics examined.

**Discussion**

There were differences in pollen sizes in the varieties studied. In the Şebin, Bilecik, Kaman 1 and Pedro varieties, the pollen length was 36.80 µm, 37.40 µm, 34.25 µm and 35.00 µm, respectively, while the pollen width was 42.45 µm, 43.15 µm, 40.65 µm and 41.10 µm (Mert, 2010). Also, according to other research, the length and width of the Şebin variety was reported to be 28.30 µm and 37.20 µm (Evrenosoglu and Mısırlı, 2009). No difference was found between the two native varieties examined in terms of pollen size and shape properties. In these findings, the values for these varieties were found to be higher than the other two studies. Accordingly, this study differs from the previous mentioned studies in terms of the other selected varieties and the characteristics examined such as exine properties. Şebin, Bilecik, Kaman 1 and Pedro varieties by Mert (2010) and Şebin variety by Evrenosoglu and Mısırlı (2009) were studied. However, the pollen morphology of Chandler, Fernette, Fernor, Kaman 1 and Şen 1 was examined for the first time in this study. Thus, differences among the varieties were determined in terms of all the characteristics studied. Other nut fruit species, such as chestnut (Beyhan and Serdar, 2009) and pistachio (Belhadj et al., 2007) have previously been studied for pollen size. Furthermore, it was observed that the width, length and pollen ratios of different fruit species have been examined by SEM (Chwil, 2015; Radovic et al., 2016; Soliman and Al–Obed, 2013). The length/width ratio varied between 0.89 and 0.96 in the varieties examined (Erdtman, 1943). Considering this ratio, pollen shapes for all varieties were defined as oblate spheroidal. Differing from the present study, other researchers described the pollen differently (Evrenosoglu and Mısırlı, 2009; Mert, 2010).

Pori and spinules were photographed on the pollen surface for the walnut varieties. The difference in their distribution according to varieties was noteworthy. In this respect, the data were significant. Regarding the pollen surface morphology for the dimensions of the colpus and mesocolpium different fruit species, and exine patterning in quince varieties, was expressed by Radovic et al. (2016). Similarly, pollen morphology on traditional varieties of Prunus species in Italy and in Serbia has been investigated (Geraci et al., 2012; Nikolić and Milatović, 2016, 2017). Pollen is used to distinguish genotypes. The morphology characteristics of pollen can contribute to the identification of the genotype. Pollen storage is one of the ex situ approaches to conservation genotypes in gene banks. These findings are valuable for plant breeders. As it is known, there are different identification methods. This method can be applied more easily than the others and its cost is low (Evrenosoglu and Mısırlı, 2009; Nikolić and Milatović, 2016, 2017).
Figure 1. Pollen shape (a, c, e, g, i, k, m, o) and exine patterns (b, d, f, h, j, l, n, p) of walnut varieties. Chandler (a, b), Fernette (c, d), Fernor (e, f), Pedro (g, h), Bilecik (i, j), Kaman 1 (k, l), Şebin (m, n), Şen 1 (o, p). Scale bars – 10 µm (a, c, e, g, i, k, m, o), 1 µm (b, d, f, h, j, l, n, p).
Figure 1. (Continued).
Table 4. Pearson correlation coefficients among traits in selected varieties.

|       | PL   | PW   | LWR  | PD   | DBP  | PN   | DBS  | SL   | SW   |
|-------|------|------|------|------|------|------|------|------|------|
| PW    | 0.392** |      |      |      |      |      |      |      |      |
| LWR   | -0.323** | 0.742** |      |      |      |      |      |      |      |
| PD    | -0.226*  | -0.292** | -0.131 |      |      |      |      |      |      |
| DBP   | 0.099    | 0.183 | 0.115 | -0.026 |      |      |      |      |      |
| PN    | -0.016   | -0.009 | 0.004 | -0.199 | -0.059 |      |      |      |      |
| DBS   | 0.138    | 0.031 | -0.058 | -0.122 | -0.179 | 0.140 |      |      |      |
| SL    | 0.091    | 0.185 | 0.119 | -0.335** | -0.021 | 0.163 | 0.015 |      |      |
| SW    | -0.025   | 0.128 | 0.156 | -0.201 | 0.015 | 0.164 | -0.057 | 0.678** |      |
| SN    | -0.058   | 0.086 | 0.152 | -0.104 | 0.008 | 0.300** | -0.013 | 0.049 | 0.229* |

Abbreviations: PL: pollen length; PW: pollen width; LWR: length/width ratio; PD: pori diameter; DBP: distance between pori; PN: pori number; DBS: distance between spinules; SL: spinules length; SW: spinules width; SN: spinules number. * Significant at P < 0.05. ** Significant at P < 0.01.

Table 5. Component loading in principle component analysis (PCA).

| Traits | PC1   | PC2   | PC3   | PC4   |
|--------|-------|-------|-------|-------|
| DBS    | 0.980 | -0.052 | -0.014 | 0.012 |
| PD     | -0.796 | -0.503 | -0.215 | 0.148 |
| PN     | 0.637 | 0.199 | 0.566 | 0.271 |
| PW     | 0.501 | 0.406 | 0.320 | -0.420 |
| SW     | -0.033 | 0.958 | 0.244 | -0.018 |
| SL     | 0.230 | 0.930 | -0.081 | -0.037 |
| SN     | -0.089 | 0.121 | 0.888 | 0.180 |
| DBP    | -0.434 | 0.001 | -0.740 | 0.116 |
| PL     | 0.220 | 0.229 | 0.038 | -0.940 |
| LWR    | 0.273 | 0.200 | 0.315 | 0.758 |
| Eigenvalue | 2.623 | 2.351 | 1.972 | 1.777 |
| Proportion (%) | 26.233 | 23.510 | 19.722 | 17.768 |
| Cumulative (%) | 26.233 | 49.743 | 69.465 | 87.233 |

Abbreviations: DBS: distance between spinules; PD: pori diameter; PN: pori number; PW: pollen width; SW: spinules width; SL: spinules length; SN: spinules number; DBP: distance between pori; PL: pollen length; LWR: length/width ratio. Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.

Figure 2. Dendrogram of hierarchical cluster analysis obtained by Ward’s clustering method.
In this study, the first four components defined 87.23% of the varieties while, in Prunus species and varieties, the first two components explained 64.32% of the total variation and consisted of the polar axis and the length of the equatorial diameter by Geraci et al. (2012). These results are partly consistent with the study in question. Similarly, a dendrogram was generated by clustering analysis on pollen morphology in different Prunus species by Geraci et al. (2012).

**Conclusions**

The pollen shapes were oblate spheroidal for all the selected walnut varieties. The pore and spinules were investigated in detail. The varieties showed statistical differences in terms of these characteristics. In particular, the Fernor variety had the smallest pollen width compared to other varieties. This variety had the largest pore diameter, so their number per unit area was low. On the other hand, Bilecik had the smallest pore diameter. Distance between pore of this variety was the least. Therefore, a large number of pore was observed on the surface. In addition, spinules sizes were determined to be larger in Şen 1, but smaller in Chandler variety than the others. As it is known, there are many identification methods. It is important to express the difference between genotypes by identifying pollen. In this study, pollen dimension can be used for identification of varieties by plant breeders.

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