Preliminary Pollen Grain Characterization of Several Apple and Plum Varieties

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RESEARCH ARTICLE

Abstract
Palynology is one of the most used methods to determine the species of different plants. It acts like a fingerprint, being unique to each plant species. The aim of this paper was to observe differences both at morphological level and from the pollen viability point of view of 21 apple (Malus domestica Borkh.) and 19 plum (Prunus domestica L.) varieties, differences that can be correlated with genotype variation in further genotyping research, with the final goal of using these results as a basis for genotype-assisted breeding programs. Pollen grain (PG) morphology was assessed using a Scanning Electron Microscope and pollen viability was analyzed using in vitro germination. This study demonstrated significant differences in both the size of PGs and the percentage of viable pollen through in vitro germination. Among the apple varieties, Belle de Boskoop presented the largest PGs, and among the plum varieties Timpurii de Țurlești. Aura apple variety had the lowest viability percentage.

Keywords: Malus domestica; Prunus domestica; pollen germination; pollen morphology

INTRODUCTION
Agricultural crop production and the growth of plants are being jeopardized by different factors, mainly climate change. This greatly affects the genetic resources of different areas of the world (Barak et al., 2021). Agriculture can be improved by increasing regulating ecosystem services, while reducing the intensive agriculture practices (Carisio et al., 2020). The Prunus genus is very common in different areas of the world (Barak et al., 2021), being a very adaptable plant that can grow in vast climate conditions, from the Siberian zone down to the Mediterranean region. Along with genetically controlled mechanisms, the production stability of different plum varieties depends on plenty of factors which are needed for the reproductive process, such as pollen viability and pollen tube growth (Cerovic et al., 2021). Apple (Malus domestica) is the most common fruit tree and has been cultivated since antiquity in Europe and Asia (Ramirez and Davenport, 2013). An important biologic factor in apple growing is pollination, and pollen viability and germination are two fundamental factors that indicate the
productivity potential of a cultivar (Petrisor et al., 2012). Moreover, differences in PGs size and the unique surface ultrastructure are very useful for distinguishing species and varieties of fruit trees (Evrenosoglu and Misirli, 2009; Geraci et al., 2012). Studying pollen characteristics from specific genotypes and varieties is very important for breeding programs (Calic et al., 2013). PGs have various physiological and structural adaptations, like heterogeneous sculpture ornamentation (Chwil, 2015).

Pollination is a very important step in plant reproduction, stimulating the growth and development of ovaries. It is represented by the transfer of pollen from anthers to stigmas within a plant species and it is necessary for the ovule fertilization and for the development of fruits and seeds (Glisic et al., 2017), being directly related to the crop quality and quantity of fruits (Pal et al., 2015). Successful pollination is critical for dependable fruit production and for apple diversification in different areas of the world. Pollen germination is the first step in a series leading to ovule fertilization, fruit growth and development. In vitro studies are usually conducted to assess pollen viability (Calzoni et al., 1979; Imani et al., 2011; Bolat et al., 1999; Sharafi, 2011). The typical carbon source used for pollen germination is sucrose, in concentrations up to 25% (Ramirez and Davenport, 2013). The present study aims to characterize PGs from different varieties of *Prunus domestica* and *Malus domestica*.

**MATERIALS AND METHODS**

For this study, PGs were harvested from 19 plum varieties (Anna Spath, Black Diamond, Cacanska Lepotica, Dara, Early Rivers, Gras Ameliorat, Gras Românesc CL 205, Haganta, Jojo, Renclod Althan, Renclod Violet, Record, Romanta, Stanley, Timpurii de Țurlești, Topen, Vânăt Românesc; Vinete Românești CL 300, Zamfira) and 21 apple varieties (Aura, Belle de Boskoop, Crețesc Auriu, Crețesc, Delicios de Voinesti, Domnesc, Florina, Generos, Golden Delicios, Granny Smith, Idared, Jonathan, Jonagold, *Malus floribunda*, Parmain auriu, Pionier, Rebra, Romus 3, Romus 5, Rustic, Wagener Premiat), from ICDP Pitești-Mărăcineni Research Institute, Mărăcineni, Romania. The anthers were harvested before anthesis occurred and they were left to dry in Petri dishes, for PG release. The dried pollen was stored at 3 °C prior to analysis.

**Pollen viability through in-vitro germination**

One of the methods used for pollen viability determination is *in-vitro* germination (Dafni and Firmage, 2000; Lankinen et al., 2018). PGs from each variety were transferred into Petri dishes containing a solution of 10% sucrose (Sulusoglu and Cavusoglu, 2014; Yoder et al., 2009). They were kept at room temperature for 48 hours (Moisescu et al., 2020), after which they were analyzed using optical microscopy. PGs were considered viable if after 48 hours they presented morphological changes at the germination apertures.

**SEM Imaging**

SEM images were acquired with a FEI Inspect S50 Scanning Electron Microscope. The PGs were placed on double coated carbon conductive tape and mounted into the microscope. Images were acquired in Low Vacuum mode at a pressure of 200 Pa and a voltage of 5kV in order to avoid charging.

**Optical Imaging**

Optical images were acquired using a Leica DM 1000 LED optical microscope coupled with a Leica DFC 295 photo/video camera. The PG samples required no preparation and were left in the Petri dishes at the moment of imaging. Furthermore, PG polar axis (P) and equatorial axis (E) were measured and the shape index (P/E) was calculated. 50 PGs were measured for each variety.

**Statistical analysis**

Data was statistically analyzed by ANOVA and Duncan’s multiple range test (DMRT) using Microsoft Excel 2016 and IBM SPSS Statistics (version 27) software. In all tests, it was considered the significance level of P < 0.05.

**RESULTS AND DISCUSSIONS**

**Pollen viability**

Plum pollen had very high viability percentage (over 85%), with 9 varieties reaching 100% (Figure 1). The lowest percentage value was recorded for the RC Violet variety with 85.7%. The Black Diamond variety did not present any PGs at the moment of analysis. The results obtained are in conformity with the ones obtained by Bal and Nandan (2013) according to which the pollen viability ranged between 77.50% and 97.82% in different plum varieties.
Figure 1. Plum (*Prunus domestica*) varieties pollen viability. Error bars represent standard deviation. Bars with the same letters are not significantly different at P<0.05 according to Duncan’s multiple range test.

Figure 2. Apple (*Malus domestica*) varieties pollen viability. Error bars represent standard deviation. Bars with the same letters are not significantly different at P<0.05 according to Duncan’s multiple range test.

Apple varieties had lower overall pollen viability percentages (Figure 2), compared with the plum pollen. Three varieties (Cretesc, Parmain Auriu, Wagener Premiat) had 100% viable pollen, while the Aura variety had the lowest percentage at 32.9% (Figure 3). Similar results were obtained by Muradoglu et al. (2017) when analyzing pollen from different apple varieties, with viability ranging between 70.67% and 81.32%.

Figure 3. PG viability of plum (a) and apple (b) varieties observed under optical microscope, at 10x magnification.
Pollen grain morphology

![Graph showing pollen grain morphology for Plum varieties.](image)

**Figure 4.** Plum (*Prunus Domestica*) varieties Polar axis (P) (a) and equatorial axis (E) (b). Error bars represent standard deviation. Bars with the same letters are not significantly different at P<0.05 according to Duncan’s multiple range test.

Regarding the morphology characteristics (Figure 4) of plum PG, Dara variety had the highest P value (55.82 µm), while Black Diamond variety had the lowest P value (36.11 µm). Stanley variety had the highest E value (33.44 µm), while Black Diamond variety had the lowest E value (19.9 µm). According to the ANOVA single factor test, there are significant differences between the varieties.

Apple PG morphology (Figure 5) showed that Granny Smith variety had the highest P value (average of 48.1 µm), while Golden Delicios variety had the lowest P value averaging at 35.5 µm. Belle de Boskoop variety had the highest E value (27 µm), while Delicios de Voinesti variety had the lowest E value (21.24 µm). According to the ANOVA single factor test, there are significant differences between the varieties.
Figure 5. Apple (*Malus Domestica*) varieties Polar axis (P) (a) and Equatorial axis (E) (b). Error bars represent standard deviation. Bars with the same letters are not significantly different at P<0.05 according to Duncan’s multiple range test.

The shape index of both plum and apple varieties PGs is presented in Table 1.

<table>
<thead>
<tr>
<th>Plum variety</th>
<th>Shape index P/E</th>
<th>Apple variety</th>
<th>Shape index P/E</th>
</tr>
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<tbody>
<tr>
<td>Anna Spath</td>
<td>1.78</td>
<td>Aura</td>
<td>1.72</td>
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<td>Black Diamond</td>
<td>1.81</td>
<td>Belle de Boskoop</td>
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<td>Cacanska Lepotica</td>
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<td>Dara</td>
<td>1.76</td>
<td>Cretesc</td>
<td>1.78</td>
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<tr>
<td>Early Rivers</td>
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<td>Delicios de Voinesti</td>
<td>2.06</td>
</tr>
<tr>
<td>Gras Ameliorat</td>
<td>1.64</td>
<td>Domnesc</td>
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<tr>
<td>Gras Romanesc CL 205</td>
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<tr>
<td>Haganta</td>
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<td>Generos</td>
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</tr>
<tr>
<td>Jojo</td>
<td>1.92</td>
<td>Golden Delicios</td>
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<td>Renclod Althan</td>
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<td>Renclod Violet</td>
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<td>Idared</td>
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<td>Record</td>
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<td>Jonathan</td>
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<td>Jonagold</td>
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<td>Timpurii de Turlesti</td>
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<td>Parmain Auriu</td>
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<tr>
<td>Vinete Romanesti CL 300</td>
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<td></td>
<td></td>
<td>Rustic</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Wagener Premiat</td>
<td>2.00</td>
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</tbody>
</table>
Figure 6. The morphology of PGs from analyzed plum (a) and apple (b) varieties observed under a scanning electron microscope.

The shape of normally developed PGs of both plum and apple varieties was elliptical (Figure 6). The PGs presented narrow germinal furrows which did not unite in the polar area. The apple PGs exine showed clear longitudinal and parallel striations, observation also made by Xiaoxin et al. (2015) and Dar et al. (2019). The exine of plum PG presented also clear longitudinal striations, except some of the varieties (Dara, Haganta, Jojo, Stanley) where the striations were presented in a swirl pattern (Figure 7).

Figure 7. Swirl pattern striations on plum PG.
CONCLUSIONS
This study demonstrated significant differences in both the size of PGs and the percentage of viable pollen through in vitro germination. Among the apple varieties, Belle de Boskoop presented the largest PGs, and among the plum varieties Timpurii de Turlesti. Aura apple variety and Renclo Violet plum variety presented the lowest viability percentage. Although the appearance of the PG exine present striations for both plum and apple varieties, the pattern is specific to each variety. This study could help further genotyping research, with the final goal of using these results as a basis for genotype-assisted breeding programs.

Author Contributions: V.I.P and A.A.U. conceived and designed the experiment; M.I. and V.I.P. collected the data; A.A.U. and L.B supervised the project; V.I.P. performed the analysis and wrote the paper.

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Conflicts of Interest
The authors declare that they do not have any conflict of interest.

REFERENCES