Improving in vitro Pollen Germination of Five Species of Fruit Trees

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ABSTRACT

This study aims to test different media and olive oil to improve in vitro pollen germination of five fruit tree species (Olea europaea, Citrus maxima, Citrus paradisi, Prunus persica and Prunus domestica). Immersion pollen of Olea europaea in olive oil before spreading them over media improved their germination significantly. The highest germination percentage was recorded for pollen that were immersed in olive oil and germinated on media containing 0.8% agar, 10% sucrose, and 50 ppm citric acid (55.8%). For Citrus spp., olive oil completely suppresses pollen germination, in addition, pollen germination percentage of Citrus maxima was significantly higher on the medium containing 0.8% agar and 20% sucrose than other media. Pollen of Citrus paradisi germinated significantly better on media containing 0.5% or 0.8% agar and 20% sucrose. On the other hand, immersion pollen of Prunus persica in olive oil improved their germination, and the highest germination percentage was recorded for pollen that were immersed in olive oil and germinated on media containing 0.8% agar, 10% sucrose, and 100 ppm boric acid or 50 ppm citric acid (43.3% and 43.1%, respectively). Finally, immersion pollen of Prunus domestica in olive oil suppress their germination, and the highest germination percentage was recorded for pollen that germinated on media containing 0.8% agar, 10% sucrose, and 50 ppm boric acid (48.7%).

KEYWORDS: agar; boric acid; citric acid; citrus; olive oil; olea; pollen; Prunus.

INTRODUCTION

In Jordan, the fruit trees represent about 34.2% of agricultural area. The species of Olea (olive), Citrus (grapefruit, lemon, mandarins, orange, and pummelo), and Prunus (almond, apricot, cherries, peach, and plum) are very important in Jordan and they represent about 85.8% of the area planted with fruit trees (Department of Statistics, 2002). Many researches are done to improve the production through improving fruit set, one of the important factors affecting fruit set is pollen viability.

In vitro pollen germination and tube growth studies are valuable in identifying the effect of environmental factors and cultural practices on pollen viability. In this respect, it is very important to develop an excellent germination media for each species to get the right benefit of the in vitro pollen germination test.

Media requirements for in vitro pollen germination have been reported for a large number of species, with considerable variation among and within species. The basic structure of the medium consists of agar and sucrose with different concentrations. In many cases, it is important to add some chemicals that improve this test. Pollen of Olea europaea has been studied by many researchers those have tested different ideas to improve the germination test such as using tetracycline (Pinney and Polito, 1990) the low pistil extract concentrations (Fernandez-Escobar et al., 1983), fusicoccin (Rodriguez-Rosales et al., 1989), and immersing pollen in olive oil before spreading them over the media (Ateyyeh et al., 2000). Butt et al. (1993) found that pollen of Citrus reticulate, C. sinensis, C. paradisi, and C. limettioides showed the highest germination percentage when placed in media containing 15% glucose and 0.07% bacto agar. Nyomora et al. (2000) used medium containing 1% agar, 15% sucrose with or without boron at 100mg/L to study the effect of foliar application of boron on in vitro pollen germination of almond.

The aim of this study is testing different media and using the olive oil to improve in vitro pollen germination...
of the following fruit tree species (*Olea europaea*, *Citrus maxima*, *Citrus paradisi*, *Prunus persica* and *Prunus domestica*).

**Materials and Methods**

Flowers of five fruit tree species (*Citrus maxima*, *Citrus paradisi*, *Olea europaea*, *Prunus domestica*, and *Prunus persica*) were collected separately one day after anthesis from healthy trees, at this time, it was very easy to yield the pollen. *Olea europaea*, *Prunus domestica*, and *Prunus persica* trees grow in the campus of the University of Jordan, while *Citrus maxima* and *Citrus paradisi* trees grow at The Agricultural Research Station in the Jordan Valley.

**Preliminary Experiment**

In this experiment, pollen of the five fruit tree species were tested on all combinations of germination media, which consist of agar, sucrose, boric acid and/or citric acid with different concentrations as in Table (1). Pollen germination of the five fruit tree species was zero in many combinations (Table 2). Pollen of *Olea europaea* did not germinate on media containing 0.5% agar, media contain 20% or 30% sucrose, and media without boric acid or citric acid, while pollen of *Citrus spp.* did not germinate on media containing boric acid or citric acid and media containing both of them, finally, pollen of *Prunus spp.* did not germinate on media containing boric acid or citric acid and media containing both of them. Though, all these combinations were excluded from the main experiment.

**The Main Experiment**

The media for each species (Tables 3, 4, and 5) were prepared in 5 cm diameter petridishes. Part of pollen of each species were immersed immediately in glass tubes with olive oil before spreading them over the media, while the other part was spread over the media with thin film of distilled water. Experiments were conducted in a split-plot arranged in a completely randomized design with combinations of germination media as the main plot and olive oil treatment as the subplot. Each subplot treatment was replicated 5 times. After 12 hours total number of pollen and number of germinated pollen were counted. A compound microscope was used to determine pollen germination.

The analysis of data was performed by using the mixed procedure and the differences of least squares means by the SAS program.

**Results**

**Olea europaea**

The results show significant differences between the germination media treatments, the use of olive oil, and the combinations of both factors. The pollen were germinated on all media containing 0.8% agar, 10% sucrose, and citric or boric acid or both of them at 50 or 100 ppm concentrations. Without oil treatment, the three following combinations (0.8% agar+10% sucrose+50 ppm citric acid, 0.8% agar+10% sucrose+50 ppm boric acid, and 0.8% agar+10% sucrose+50 ppm boric + 50 ppm citric) give significantly higher germination percentage than other combinations, in addition, the immersion of pollen in olive oil improved the germination percentage significantly for each treatment. The highest germination percentage was recorded for pollen that were immersed in olive oil and germinated on media containing 0.8% agar, 10% sucrose, and 50 ppm citric acid (55.8%), which was significantly higher than other treatments (Table 5).

**Citrus spp.**

The results show significant differences among the germination media treatments, the use of olive oil, and the combinations of both factors. In addition, olive oil completely suppresses pollen germination. Pollen of *Citrus maxima* germinated on all media contain 0.5% or 0.8% agar and 10% or 20% or 30% sucrose. The germination percentage on the medium containing 30% sucrose was significantly lower than those containing 10% or 20% sucrose. The germination percentage on the medium containing 0.8% agar and 20% sucrose was significantly higher than the other media. Pollen of *Citrus paradisi* germinated on all media containing 0.5% or 0.8% agar and 10% or 20% or 30% sucrose. The germination percentage on the media containing 0.5% or 0.8% agar and 20% sucrose was significantly higher than other media containing 10% or 30% sucrose.

**Prunus spp.**

The results show significant differences among the germination media treatments, the use of olive oil, and the combination of both factors.
Pollen of *Prunus persica* germinated on all media containing 0.8% agar, 10% sucrose, and citric acid or boric acid at 50 or 100 ppm concentration. The immersion of pollen in olive oil improved the pollen germination significantly on all media except the medium containing 0.8% agar, 10% sucrose, and 50 ppm boric acid (Table 7). The highest germination percentage was recorded for pollen that were immersed in olive oil and germinated on media containing 0.8% agar, 10% sucrose, and 100 ppm boric acid (43.3%), but it was not significantly higher than germination percentage for pollen that were immersed in olive oil and germinated on media containing 0.8% agar, 10% sucrose, and 50 ppm citric acid (43.1%) (Table 7).

Pollen of *Prunus domestica* germinated on all media containing 0.8% agar, 10% sucrose, and citric acid or boric acid at 50 or 100 ppm concentration. The immersion of pollen in olive oil decrease the pollen germination significantly on all media except the medium containing 0.8% agar, 10% sucrose, and 100 ppm citric acid. The highest germination percentage was recorded for pollen that germinated on media containing 0.8% agar, 10% sucrose, and 50 ppm boric acid (48.7%), which was significantly higher than the other treatments (Table 7).

**DISCUSSION**

Lipids, phenolic compounds, carbohydrates, amino acids and proteins are generally present in the stigma exudates (Raghavan, 1997). Ciampolini et al. (1996) found that the stigma exudates of *Vitis vinifera* contain polysaccharides, lipids, proteins, pectins and tannins. In *Corylus avellana*, the stigma exudates contain polysaccharides, lipids, proteins (Ciampolini et al., 1998).

This exudates composition considered as the optimum medium for pollen germination and pollen tube growth. Most of the researchers get the ideas from this exudates composition to improve the *in vitro* pollen germination test. So the major component of the germination media is carbohydrates, other substances could be added to improve this test.

In this study, it was also found that immersion pollen of *Olea europaea* in olive oil improved their germination, this approve the results of Ateyyeh et al. (2000) The highest germination percentage was recorded for pollen that were immersed in olive oil and germinated on media containing 0.8% agar, 10% sucrose, and 50 ppm citric acid (55.8%). Ciampolini et al. (1993) found that the stigmatic exudates of olive is more lipidic in nature, whereas polysaccharides and proteins occur in poor concentration, therefore, lipids may play an important role in pollen germination of olive.

The pollen germination percentage of *Citrus maxima* was significantly higher on the medium containing 0.8% agar and 20% sucrose than the other media, while the pollen of *Citrus paradisi* germinated significantly better on media containing 0.5% or 0.8% agar and 20%. In addition, olive oil completely suppresses pollen germination of *Citrus maxima* and *Citrus paradise*. Cresti et al. (1982) found that the stigmatic exudates of *Citrus limon* composed of lipids, polysaccharides, and proteins. The lipidic component of the exudates is produced in the basal papillae cells, while the polysaccharidic component of the exudates is produced and secreted by the tip cells of the papillae. It could be suggested that, the pollen germinate on the tip cells of the papillae that are rich in polysaccharidic component, then the pollen tube grow with the help of both polysaccharidic and lipidic component.

Immersion pollen of *Prunus persica* in olive oil improved their germination, and the highest germination percentage was recorded for pollen that were immersed in olive oil and germinated on medium containing 0.8% agar, 10% sucrose, and 100 ppm boric acid or 50 ppm citric acid (48.7%).

To ensure the inhibition effect of lipids on pollen germination of *Citrus maxima*, *Citrus paradisi* and *Prunus domestica*, other experiments should be done to test other oils than olive oil.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>0.5 %</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10 %</td>
</tr>
<tr>
<td></td>
<td>20 %</td>
</tr>
<tr>
<td></td>
<td>30 %</td>
</tr>
<tr>
<td>Boric acid</td>
<td>0 ppm</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0 ppm</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
</tr>
</tbody>
</table>
Table 2. Germination media containing the following components’ concentrations gave no results.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Olea europaea</th>
<th>Citrus spp.</th>
<th>Prunus spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar (%)</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Sucrose (%)</td>
<td>20 or 30</td>
<td>20 or 30</td>
<td></td>
</tr>
<tr>
<td>boric acid/citric acid (ppm)</td>
<td>0/0</td>
<td>All combinations</td>
<td>50/50, 50/100, 100/50, and 100/100</td>
</tr>
</tbody>
</table>

Table 3. Germination media for the studied fruit tree species.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Olea europaea</th>
<th>Citrus spp.</th>
<th>Prunus spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8% agar and 10% sucrose</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>0.8% agar and 20% sucrose</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>0.8% agar and 30% sucrose</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>0.5% agar and 10% sucrose</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>0.5% agar and 20% sucrose</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>0.5% agar and 30% sucrose</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>0.8% agar, 10% sucrose, and 50 ppm boric acid</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>0.8% agar, 10% sucrose, and 100 ppm boric acid</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>0.8% agar, 10% sucrose, and 50 ppm citric acid</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>0.8% agar, 10% sucrose, and 100 ppm citric acid</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>0.8% agar, 10% sucrose, 50 ppm boric acid, and 50 ppm citric acid</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8% agar, 10% sucrose, 50 ppm boric acid, and 100 ppm citric acid</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8% agar, 10% sucrose, 100 ppm boric acid, and 50 ppm citric acid</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8% agar, 10% sucrose, 100 ppm boric acid, and 100 ppm citric acid</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Significance of main and interaction effects for media combination and oil treatment.

<table>
<thead>
<tr>
<th></th>
<th>Olea europaea</th>
<th>Citrus maxima</th>
<th>Citrus paradisi</th>
<th>Prunus domestica</th>
<th>Prunus persica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media combination</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Oil treatment</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Interaction</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

** = highly significant, p < 0.01
Table 5. Pollen germination of *Olea europaea*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th><em>Olea europaea</em></th>
<th>Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Oil</td>
</tr>
<tr>
<td>10% suc. + 0.8% agar + 50 ppm boric</td>
<td>27</td>
<td>de</td>
</tr>
<tr>
<td>10% suc. + 0.8% agar + 100 ppm boric</td>
<td>17.4</td>
<td>g</td>
</tr>
<tr>
<td>10% suc. + 0.8% agar + 50 ppm citric</td>
<td>30.2</td>
<td>d</td>
</tr>
<tr>
<td>10% suc. + 0.8% agar + 100 ppm citric</td>
<td>20.4</td>
<td>f</td>
</tr>
<tr>
<td>10% suc. + 0.8% agar + 50 ppm boric + 50 ppm citric</td>
<td>28.8</td>
<td>d</td>
</tr>
<tr>
<td>10% suc. + 0.8% agar + 50 ppm boric + 100 ppm citric</td>
<td>15.6</td>
<td>g</td>
</tr>
<tr>
<td>10% suc. + 0.8% agar + 100 ppm boric + 50 ppm citric</td>
<td>16.8</td>
<td>g</td>
</tr>
<tr>
<td>10% suc. + 0.8% agar + 100 ppm boric + 100 ppm citric</td>
<td>10.2</td>
<td>h</td>
</tr>
</tbody>
</table>

Means in columns having the same letters are not significantly different at p=0.05.

Table 6. Pollen germination of *Citrus* spp.

<table>
<thead>
<tr>
<th>Treatments</th>
<th><em>Citrus paradisi</em></th>
<th><em>Citrus maxima</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Oil</td>
</tr>
<tr>
<td>30% sucrose + 0.8 %agar</td>
<td>14.6</td>
<td>b</td>
</tr>
<tr>
<td>20% sucrose + 0.8 %agar</td>
<td>51.7</td>
<td>a</td>
</tr>
<tr>
<td>10% sucrose + 0.8 %agar</td>
<td>5.5</td>
<td>c</td>
</tr>
<tr>
<td>30% sucrose + 0.5 %agar</td>
<td>4.4</td>
<td>c</td>
</tr>
<tr>
<td>20% sucrose + 0.5 %agar</td>
<td>52.3</td>
<td>a</td>
</tr>
<tr>
<td>10% sucrose + 0.5 %agar</td>
<td>0</td>
<td>d</td>
</tr>
</tbody>
</table>

Means in columns of the same species having the same letters are not significantly different at p=0.05.

Table 7. Pollen germination of *Prunus* spp.

<table>
<thead>
<tr>
<th>Treatments</th>
<th><em>Prunus persica</em></th>
<th><em>Prunus domestica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Oil</td>
</tr>
<tr>
<td>10% suc. + 0.8% agar + 50 ppm citric</td>
<td>23.5</td>
<td>c</td>
</tr>
<tr>
<td>10% suc. + 0.8% agar + 100 ppm citric</td>
<td>23.6</td>
<td>c</td>
</tr>
<tr>
<td>10% suc. + 0.8% agar + 50 ppm boric</td>
<td>22.8</td>
<td>c</td>
</tr>
<tr>
<td>10% suc. + 0.8% agar + 100 ppm boric</td>
<td>28.7</td>
<td>b</td>
</tr>
</tbody>
</table>

Means in columns of the same species having the same letters are not significantly different at p=0.05.

REFERENCES


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Fernandez-Escobar, R., Gomez-Valledor, G. and Rallo, L.


