Influence of boric acid, sucrose and temperature on the germination of *Leonurus cardiaca* L., pollen

Aydin Shekari¹, Vahideh Nazeri¹*, Majid Shokrpour¹

1. Former MSc student, Associate Professor and Assistant Professor of Horticultural Sciences, Faculty of Agriculture, University of Tehran, Karaj, Iran.

**Corresponding author:** Vahideh Nazeri

**ABSTRACT:** *Leonurus cardiaca* L., commonly known as motherwort, is a member of the Lamiaceae family. It has been used to cure cardiovascular problems, stress and nervous irritability. In vitro pollen germination is a very convenient and effective technique to study many basic and applied aspects of pollen biology. Review in the literature revealed that there is no report on the pollen biology of this species. Therefore, this experiment was designed to investigate influence of culture media with different concentrations of boric acid (75, 100 and 125 mg/l) and sucrose (10 and 15 %) in liquid and solid cultures on pollen germination and effect of temperature (10, 25 and 35 °C) on in vitro pollen germination of *L. cardiaca*. Results show that the pollen germination is highest in solid medium and a combination of 15 % sucrose, 100 mg/l boric acid and 1 % agar (82.96 %). Maximum pollen germination occurred at temperature of 25 °C.

**Keywords:** Motherwort, pollen germination, temperature

**INTRODUCTION**

*Leonurus cardiaca* (Lamiaceae) commonly known as motherwort is an important medicinal plant growing in many regions of Iran. It has been used to cure cardiovascular problems, stress, anxiety, and nervous irritability (Mil Kowska-Leyck *et al.*, 2002). It is a perennial herb widespread in Europe, East Asia to the Himalayas, West Asia, Northern Africa, and North America usually found in country areas throughout the hills and plains (Wojtyniak *et al.*, 2013). Chemical compounds such as alkaloids, iridoids, flavonoids, saponins, cardenolids like glycosides and diterpenoids have been detected and isolated from the leaves and flowers. The healing of heart diseases is mainly connected with flavonoids (Mockute *et al.*, 2005).

Information on pollen characteristics is essential for any successful plant breeding programs and crop improvement. The case study of pollen germination in some varieties and species have been identified (Aslantus and Pirlak, 2002). Pollen viability can be evaluated by: (1) staining techniques (tetrazolium, aniline blue and fluorescein diacetate); (2) in vitro and in vivo germination tests; or (3) analyzing final seed set (Abdelgadir *et al.*, 2012). The choice of the method depends on crop or species (Dafni and Firmage, 2000). Staining method might not give a reliable evaluation of viability. The most reliable method is to assess final seed set but it is not useful because it takes so much time to obtain proper information. *In vitro* pollen germination is a very convenient and effective technique to study many basic and applied aspects of pollen biology (Kristen and Kappler, 1990). Medium combination used for pollen germination varies from species to species. Temperature is one of the most important environmental factors that could affect pollen performance during the progamic phase (Hedhly *et al.*, 2005). It has been shown that temperature affects pollen germination and pollen tube kinetics in the style (Shivanna *et al.*, 1991).

This experiment was designed to investigate the selection of suitable medium combination of sucrose and boric acid for in vitro germination and the effect of temperature on *in vitro* germination of *L. cardiaca* pollen.
Materials and Methods

Study site and plant materials

Seeds of one Leonurus cardiaca populations were collected from Khansar in Esfahan province. Plants were grown during April to September, 2014, in the greenhouse of the Horticulture Department of University of Tehran, Iran. Flowers were collected at anthesis stage, during 8.00 to 10.00 am, and transferred to the laboratory for further experiments.

In vitro germination test

In order to optimize the pollen culture medium of L. cardiaca, 8 different solid and liquid culture media with different compositions were prepared. Solid media were composed of different levels of boric acid (75, 100 and 125 mg/l), sucrose (10 and 15 %) and agar (1%). The sucrose of 10 and 15 % with boric acid of 100 mg/l were considered for liquid media. Anthers were shaked on the media and incubated at 25 °C in darkness for 24 h for germination. Pollen grains were considered as germinated when the pollen tube length was greater than the diameter of the pollen grain. A minimum of 100-150 pollens were counted per petri dishes with 3 replicates. Germination percentage was determined by dividing the number of germinated pollen grains by the total number of pollen per field of view. A light microscope (KF2, Zeiss, Germany) were used to determine the pollen germination.

Effect of temperature on pollen germination

After determination of the best pollen germination medium, effect of different temperatures on in vitro pollen germination were studied. The petri dishes (pollen cultures) were placed in incubators at 10, 25 and 35 °C for 24 h. After the incubation, the petri dishes were taken out from incubator and were observed under light microscope and the pollen germination percentage were determined.

Statistical analysis

Experimental design was completely randomized design (CRD) with three replications for each treatment. Data were analyzed with a one way ANOVA model (SPSS version 22) and the means were compared using a Duncan Multiple Range Test (P < 0.05).

RESULTS AND DISCUSSION

In vitro pollen germination test

Effect of the different culture media containing sucrose-boric acid combination on pollen germination is shown in Fig 1. Maximum percentage of pollen germination were observed on the solid media containing 15 % sucrose and two concentrations, 100 and 125 mg/l, of boric acid with means of 82.96 and 82.7 %, respectively. Sucrose had a significant effect on pollen germination. Significant differences were observed among concentrations of 10 % and 15 % sucrose in both solid and liquid media with the highest percentage of pollen germination in 15% sucrose (P < 0.01). The most effective concentrations of boric acid on germination were 100 and 125 mg/l. The highest percentage of pollen germination in liquid medium was 31.24 % belonged to combination of sucrose 15% and boric acid 100 mg/l. However, optimum medium were identified as a combination of 15%, sucrose 100 mg/l boric acid and 1 % agar. Sucrose has a main role in maintaining osmotic pressure and also is a substrate for the metabolism of the pollen (Shivanna and Johri, 1985). Previous studies have shown that the optimum sucrose concentration for pollen germination ranges from 1 to 40% (Luza and Polito, 1985). The optimum sugar concentration for pollen germination differs considerably among different plants, Areca catechu, 40 % (Liyun Liu et al., 2013) and pepper, 5-10% (Mercado et al., 1994). The role of boric acid in pollen germination and pollen tube growth of vascular plants have been documented (Sidhu and Malik 1986). Boron is directly involved in the synthesis of pectin and pollen tube development. Sucrose in combination with boric acid increase pollen germination and pollen tube development, because boron makes a complex with sugar and this sugar borate complex is known to be capable of better translocation than non-borate, non-ionized sugar molecules (Sidhu and Malik, 1986). However, in this study we have demonstrated that the use of a medium containing 15% sucrose was better than that containing 10% sucrose for in vitro pollen germination. Our results indicated that 100 mg/l boric acid could be a suitable concentration. If the optimal concentration of boric acid is not used, perhaps have an inhibitor effects and may cause toxicity in medium. This study revealed that application of liquid medium compared to solid medium considerably decreases pollen germination. Probably, due to the accumulation of water in the cells under liquid condition, some complications may...
be occurred and if continued, it will lead to cell death. Adding agar to culture medium reduces the occurrence of bursting and improves both germination and pollen tube growth (Luza and Vito, 1985).

**Fig.1.** Pollen germination in different culture media in *Leonurus cardiaca* (mean ± S.E), means were compared by a Duncan's Multiple Range Test (P < 0.05)

<table>
<thead>
<tr>
<th>Series</th>
<th>Germination (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>61.51</td>
</tr>
<tr>
<td>2</td>
<td>65.69</td>
</tr>
<tr>
<td>3</td>
<td>66.48</td>
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<tr>
<td>4</td>
<td>75.28</td>
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<td>5</td>
<td>82.96</td>
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<td>6</td>
<td>82.7</td>
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<tr>
<td>7</td>
<td>31.25</td>
</tr>
<tr>
<td>8</td>
<td>18.07</td>
</tr>
</tbody>
</table>

**Effect of temperature on in vitro pollen germination**

The effect of three temperatures, 10, 25 and 35 °C on the in vitro pollen germination of *Leonurus cardiaca* was evaluated and results showed in Fig 2. Maximum pollen germination occurred at 25 and 35°C temperatures, while the minimum observed at 10°C. The results showed no significant difference among 25 and 35°C temperatures (P < 0.01). Effects of different environmental factors on pollen germination and pollen tube growth have been widely described in the literature (Dafni and Firmage, 2000). Among environmental factors, temperature has an essential effect on pollen germination and rate of germination (Young et al., 2004). The current study, which examined pollen germination in response to three temperatures, showed that the optimum value for pollen germination and pollen tube length was 25 °C and while both parameters reduced under low temperature (10 °C). This is consistent with results obtained for Papaya (Cohen et al., 1989) and Pistacia (Acar and Kakani, 2010).
Conclusion
Sucrose imposes significant effects on *Leonurus cardiaca* pollen germination. The effects are most obvious at a sucrose concentration of 15 %. Boric acid can significantly promote the germination of *L. cardiaca* pollen. Germination promotion is most notable at a boric acid concentration of 100 mg/l and 125 mg/l. The solid culture medium with agar presents better effects than does the liquid culture medium without agar. The optimum culture medium for developing *L. cardiaca* pollen comprises 15 % sucrose, 100 mg/l boric acid and 1 % agar.

The optimum temperature required for in vitro pollen germination of *L. cardiaca* was 25 °C. He obtained results constitute the first contribution on the pollen biology of the *L. cardiaca* generating new questions for future research.

REFERENCES


