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Molybdenum and Boron Affect Pollen Germination of Strawberry and Fertile and Infertile Flowers of Pomegranate

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ABSTRACT

In the present research the effects of molybdenum (Mo) and boron (B) on pollen germination in fertile and infertile flowers of pomegranate (*Punica granatum* L. cv. 'Rabab') and strawberry (*Fragaria* × *ananassa* Duch. cv. 'Pajaro') were investigated. After collecting unbloomed flowers of both plants, pollen grains were gathered then cultured on medium containing 12.5% sucrose + 0.8% agar + boric acid (0, 2.5 or 5 mg l^{-1}) + molybdic acid (0, 2 or 4 mg l^{-1} for pomegranate and 0, 2, 4 or 6 mg l^{-1} for strawberry). B increased pollen germination but Mo at higher concentrations decreased it. Pollen germination was increased when 2.5 and 5 mg l^{-1} B was added but without Mo in pomegranate fertile flowers compared to the control (without B and Mo) by 46.89, 60.07 and 34.88%, respectively and in infertile flowers this increase was 40.59, 45.73 and 26.77%, respectively. Moreover, Mo at 2 mg l^{-1} increased pollen germination, but at higher levels decreased it. Highest pollen germination in strawberry occurred with B at 5 mg l^{-1} + Mo at 2 mg l^{-1} and lowest germination on medium containing 2.5 mg l^{-1} B + 6 mg l^{-1} Mo.

Keywords: boric acid, *Fragaria* × *ananassa*, germination medium, molybdic acid, pollen, *Punica granatum*

INTRODUCTION

The capacity of pollen to germinate is related to the variety, nutritional conditions, and environmental factors. There is great variation in optimum germination conditions of pollen among plant species and varieties. Pollen viability levels, environmental conditions, and compatibility among varieties are important for the normal fruit set. Different nutrition and germination methods for many plant species and varieties have been established (Calzoni *et al.* 1979; Seilheimer and Stüsser 1982; Liu and Zhu 1985; Wang *et al.* 1993; Sutyemez and Kelen 1996; Eti *et al.* 1998; Kimura *et al.* 1998).

Although boron (B) seems to be involved in many processes, including sugar transport, cell wall synthesis and maintenance membrane integrity, RNA, protein synthesis, carbohydrate metabolism, respiration, indole-3-acetic acid (IAA) and phenol metabolism (Loomis and Durst 1992; Dordas and Brown 2000; Lee *et al.* 2009), nevertheless its precise role has not been elucidated. Flower retention, pollen formation, pollen tube growth or germination, nitrogen fixation and nitrate assimilation are also affected by B (Camacho-Cristobal *et al*. 2008; Hansch and Mendel 2009). B deficiency causes considerable morphological alteration in plants, such as the inhibition of root elongation (Neales 1960; Cohen *et al*. 1977).

Molybdenum (Mo) is a trace element found in the soil and is required for the growth of most biological organisms, including plants and animals (Williams and Frausto da Silva 2002). Only a handful of plant proteins are known to contain Mo. These proteins, however, are very important as they are involved in nitrogen assimilation, sulfur metabolism, phytohormone biosynthesis and stress reactions (Schwarz and Mendel 2006; Hansch and Mendel 2009). In reproductive tissues in maize, Mo deficiency could alter the phenotypes in developing flowers, including delayed emergence of tassels, small anthers, poorly developed stamens, and reduced pollen grain development. Also, pollen released from the anthers of Mo-deficient plants has been shown to be shriveled and had poor germination rates (Agarwala *et al*. 1978, 1979).

Fig. 1 Fertile (left) and infertile (right) flowers of pomegranate.

Molybdoenzymes are also involved in the synthesis of the phytohormones abscissic acid (ABA) and IAA. The Mo co-dependent aldehyde oxidase (AO) catalyses the final steps in the conversion of indole-3-acetaldehyde to IAA, and the oxidation of abscisic aldehyde to ABA (Marin and Marion-Poll 1997; Sagi *et al*. 2002; Hesberg *et al*. 2004).

Pomegranate (*Punica granatum* L.) is an important deciduous fruit tree grown in different parts of Iran and other countries. Iran is one of the biggest producers and exporters of this product. Pomegranate flowers develop into one of two types of flowers normally produced by pomegranates: hermaphrodite flowers (vase-shape) and male flowers (bell-shaped). Both types have several hundred stamens. Bell-shaped flowers have a poorly developed or no pistil and atrophied ovaries containing few ovules and are infertile. Therefore, bell-shaped flowers are referred to as male flowers and will senesce without fruit set. Vase-shaped flowers are fertile with a normal ovary capable of developing fruit (Holland *et al.* 200) (**Fig. 1**).

Strawberry (*Fragaria* × *ananassa* Duch.) has fruit with high health and nutritional value. Since the development of

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Short Communication

strawberry fruit is controlled by the number and proper distribution of fertilized achenes on the receptacle (Nitsch 1950), the viability of pollen grains and conditions required for germination need to be established.

Therefore, the objectives of this study were to evaluate pollen germination of strawberry flowers and fertile and infertile pomegranate flowers, and to assess the effects of B and Mo on this process.

MATERIALS AND METHODS

Pollen

Fertile and infertile flowers of pomegranate cv. 'Rabab' and strawberry cv. 'Pajaro' flowers that had not yet bloomed were collected at 8 a.m. from the Eram Botanicl Garden and a strawberry commercial greenhouse, respectively. These were transferred to the laboratory of the Agricultural College of Shiraz University. After sterilizing the required equipment with 70% alcohol, anthers were detached with a sharp blade and collected into a Petri dish. When anthers dehisced 2 days later, pollen grains were gathered and stored in Petri dishes at 4°C over crystalline $CaCl_2$.

Pollen culture

Pollen grains were cultured on media containing 12.5% sucrose + 0.8% agar + boric acid (0, 2.5, 5 mg l^{-1}) + molybdic acid (0, 2 and 4 mg l^{-1} for pomegranate and 0, 2, 4 and 6 mgL^{-1} for strawberry). All chemicals in this experiment were purchased from Merck Co., Germany. The pH of culture media was adjusted to 5.7 with 0.1 M HCl or 0.1 M NaOH. Pollen grains were scattered with sterilized cotton over the medium.

Determination of pollen germination percentage

Pollen germination was determined using a light microscope (Leitz GMBH Wetzlar, Germany). The experiment was designed in a completely randomized design with 4 replications (4 Petri dishes). Germinated and ungeminated pollen were counted on 8 scopes in each Petri dishes (about 100 pollen grains) and then percentage pollen germination was calculated.

Statistical analysis

All data were subjected to analysis of variance using SPSS v. 16. Means were separated by ANOVA followed by Tukey's test at α = 0.01.

RESULTS

Pollen germination of fertile and infertile flowers in pomegranate

Pollen germination in fertile and infertile flowers of pomegranate in *in vitro* culture in different media ranged from 34.88 to 60.07 and from 26.27 to 45.73%, respectively. Medium containing 5 mg l^{-1} boric acid without molybdic acid had the highest pollen germination percentage in fertile (60.07%) and infertile (45.73%) flowers. As the boric acid concentration increase the in medium, so too did pollen germination increase; however, if the concentration of MoO_4 exceeded 2 mg l^{-1} then germination decreased. Pollen germination of fertile and infertile flowers in medium containing 5 mg l^{-1} boric acid + 4 mg l^{-1} molybdic acid was 39.02 and 24.09%, respectively (**Fig. 2**).

Pollen germination percentage of strawberry

The germination of strawberry pollen in medium containing 5 mg l^{-1} B + 2 mg l^{-1} Mo and 5 mg l^{-1} B without Mo was significantly higher than in control medium without B or Mo (61.64, 64.97 and 39.52%, respectively; **Fig. 3**). The highest and lowest pollen germination occurred on medium containing 5 mg l^{-1} B + 2 mg l^{-1} Mo and 2.5 mg l^{-1} B + 6 mg l^{-1} Mo, respectively.

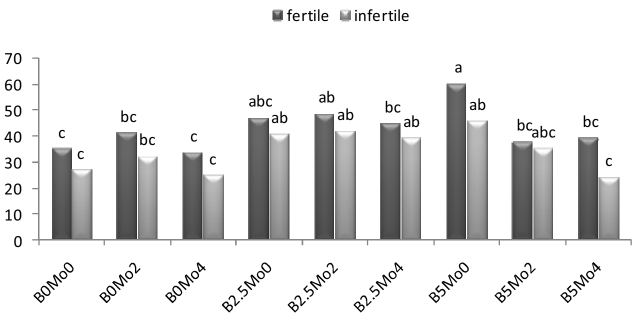

Fig. 2 Effects of boric acid (0, 2.5, 5 mg l^{-1}) + molybdic acid (0, 2, 4 mg l^{-1}) on pollen germination percentage in fertile and infertile flowers of pomegranate.

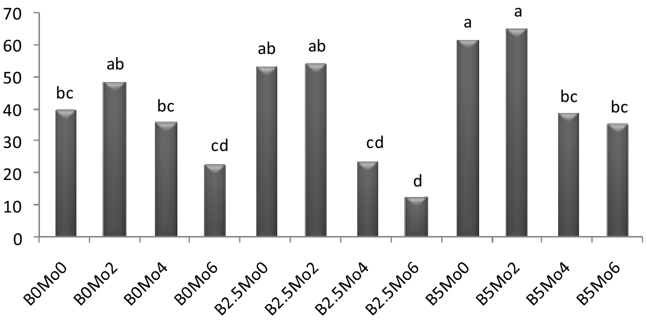

Fig. 3 Effects of boric acid (0, 2.5, 5) mg l^{-1} + molybdic acid (0, 2, 4 and 6 mg l^{-1}) on pollen germination percentage in strawberry flowers.

DISCUSSION

Eti (1991) claimed that medium type, temperature, moisture, pH, and nutrition levels are the most important factors in pollen germination in some fruit species and varieties.

B is an essential microelement required for growth and development of vascular plants. In many plants, B also plays a critical role in sexual reproduction. B has been shown to enhance pollen germination and pollen tube growth *in vitro* and may play a role in the control of secretary activities in pollen tubes by maintaining cell wall integrity of the growing pollen tubes, probably through its role as stabilizer of the cell wall pectic network (Brown *et al.* 2002), and by acting as a chemotactic agent for pollen tube growth, through reproductive tissues (Vaknin *et al.* 2008).

B performs crucial functions in the synthesis and/or structure of the plant cell wall via its binding with rhamnogalacturonan II chains, resulting in the formation of the B-polysaccharide complex. The B-complexing capability promotes the migration of B and polysaccharides across biological membranes to pollen tube wall synthesis sites (Kaneko *et al*. 1997; Lee *et al*. 2009). B might promote pollen germination by affecting H^+-ATPase activity, which initiates pollen germination and tube growth (Feijó *et al*. 1995; Obermeyer and Blatt 1995).

The results of our study indicate that with increasing boric acid in germination medium pollen germination percentage increased in both fertile and infertile pomegranate flowers and strawberry pollen. Wang *et al.* (2003) also reported that the pollen germination rate increased when the amount of B was increased in germination medium for seedless grapes. Biologically, Mo is a trace element, i.e. organisms only require it in minute amounts and plants' requirement for Mo is very low (Gupta 1997; Ralf 2007). Mo occurs in more than 50 enzymes catalyzing diverse redox reactions, including AO, which catalyses the last step in the biosynthesis of ABA. Presumably, extreme Mo in the medium results in an increase in ABA and then decreased pollen germination (Ralf and Hanschi 2002; Ralf 2007).

In general, B increased pollen germination in both plants; however, Mo at a low concentration promoted pollen germination in strawberry.

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