Improve the Medium for Germination of Almond Pollen in-Vitro and Germination Capacity of Stored Pollen

S. Piri¹, Sh. Mehri² and A. Imani³

Abstract— Almond is important nut crops which mostly for fruit set needs the pollination of flowers and followed by pistil fertilization. Therefore, pollen viability and its germination capability are essential. To optimize the pollen medium of almond and determination best medium, study was carried out with 48 types of culture medium containing different material and concentrations of boric acid (0 and 100mg/l), calcium nitrate (0, 150 and 300mg/l), sulphate magnesium (0 and 200mg/l), potassium nitrate (0 and 100 mg/l), sucrose (10 and 15 %) and agar (1 %) in the In-vitro using random complete design with three replications. Also, after selecting the best medium, to determine the best temperature for pollen germination, pollens of 3 almond cultivars were cultured in optimized pollen medium and located at 3 temperatures 15°C, 24°C and 30°C. The viability of pollens of 3 almond cultivars, three months after maintenance at 3 temperatures (4°C, -20°C and -80°C) was assessed in optimized pollen medium in terms of germination percentage. The results showed that maximum germination was in combination medium B2M2K2C2S (100 mg/l boric acid, 100mg/l sulphate magnesium, 0.0 mg/l potassium nitrate, 0.0 mg/l calcium nitrate, 15 % sucrose and 1 % agar) with 98% and lowest germination medium in combination B1K1M2C3S1 (0.0mg/l boric acid, 0.0 mg/l potassium nitrate, 100mg/l sulphate magnesium, 150mg/l calcium nitrate, 10 % sucrose and 1 % agar) with 27.00% occurred. It was found that pollen culture at 15°C and 24°C showed better germination percentage than 30°C. The viability of pollen of 3 almond cultivars, three months after maintenance at 3 temperatures (4°C, -20°C and -80°C) showed that maximum germination was in Rabie Pollen stored at -80°C with 90.66 % and the lowest germination (36.66%) in Touno pollen stored at +4°C.

Keywords— In-vitro, pollen germination, almond.

I. INTRODUCTION

ALMOND is important nut crops which mostly for fruit set needs the pollination of flowers and followed by pistil fertilization (Kester et al., 1991; Martines-Gomes et al., 2002). Therefore, pollen viability and its germination capability are essential. The biological review indicated that the pollen grains in the especial environment have the good growth and germination (Boavida and McCormick, 2007). On the other hand, the basic components of medium pollen contain calcium, boracic acid, magnesium, potassium and sucrose. In general, compounds in the pollen medium at different concentrations are found (Linskens, 1964). In addition, these elements, pH and temperature growth medium are two important factors that germination and growth are affected (Boavida and McCormick, 2007; Chebli and Geitmann, 2007). Among the elements of the primary role B in the development of pollen has been cleared so that B as a proposed structure prerequisite in the development of cell walls of pollen participate (Matoh et al., 1996; Fleischer et al., 1998).

It also has been known that B for pollen tube growth is essential and can form complex sugar - Borat participate and absorb, transport and metabolism of sugars in pollen increase pectin synthesis and also may contribute to the formation of cell wall active growing pollen tube is important (Chene et al. 1998). B necessary in experiments on pollen germination in the in-vivo and in-vitro has been proven (Nyomora et al., 2000; Jayaprakash and Saria, 2001; Wang et al., 2003). It is specified to apply B for germination of pollen grains strategy is effective in fruit trees (Hanson, 1991; Picchioni and Weinbaum, 1995; Nyomora et al., 1997; Nyomora et al., 1999; Hanson et al., 1985). To apply B on almond trees (Nyomora et al., 2000) and pear trees (Wojcik and Wojcik, 2003) resulting in an increase in pollen germination and pollen tube growth.

Role of calcium in pollen tube growth in recent years also has been reported (Malho et al., 1994; Malho and Trewevas, 1996; Malho et al., 2000). Pollen viability and germination capability of commercial almond cultivars in-vitro showed that the best germination results was in the medium temperature of 15°C to 24°C and including 10% sucrose, 100 ppm of H3BO3 and 2% Agar (Kester et al., 1991; Martines-Gomes et al., 2002). On the other hand reported during the processes necessary for fruit set, pollen production, pollen germination and pollen tube growth into the style are sensitive to high temperatures (Iwahori and Takahashi, 1964; Iwahori, 1965; Abdalla and Verkerk, 1968; Herrero and Johnson, 1980) and low temperatures (Weinbaum, 1984). To available the pollen viability suitable for making controlled hybridization out of season and maintain it in good condition can be important. Therefore, preserving viability of pollen in order to eliminate the problem in time and place of artificial pollination, more attention has been (Khosh-khui et al., 1976). Preserve the ability of pollen germination depends on the storage conditions like humidity, temperature, and air pressure (Linskens, 1964;

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Viability pollen is determinate by deferent methods including culture on a drop through the sucrose solution (2.5 to 20%) (Amma and Kulkarni, 1979), staining with Acitocarman (Ganeshan and Alexander, 1991; Alexander, 1996). Concentration of 10% sucrose, 5% agar and 10ppm Boric acid at 20°C for germination and pollen tube growth as effective medium were reported (Stanley and Linskens, 1974). It was fond that pollen germination in culture media containing sucrose, Boric acid and calcium nitrate, calcium plays an important role (Brewbacker and Kwack, 1963), despite the fact that the different effects of various culture media on pollen germination of some cultivars and species than has been reported (Mehan and Malik, 1975; Brewbacker and Kwack, 1963; Khan and Perveen, 2006a). Objectives of present research were optimization the pollen culture medium and the viability pollen of almond, after short times maintenance under different temperatures.

II. MATERIALS AND METHODS

Branches with unopened flowers were pruned from trees of 3 Almond cultivars (Rabie, Ferragnes and Tuono) growing in commercial orchards. Pollen was collected in large quantity from the cuttings, after 24 h, freshly opened blossoms. To optimize the pollen medium of almond and determination best medium, Pollen culture media were prepared with 48 types of culture medium containing different concentrations of boric acid (0 and 100mg/l), calcium nitrate (0,150and300mg/l), sulphate magnesium (0 and200mg/l), potassium nitrate (0 and100 mg/l), sucrose (10 and15 %) and agar (1%) in the In-vitro at temperature 24°C using random complete design with three replications. Also, after selecting the best medium, to determine the best temperature for pollen germination, pollens of 3 almond cultivars were cultured in B2M2K1C2S (100 mg/l boric acid, 100mg/l sulphate magnesium, 0.0 mg/l potassium nitrate, 0.0 mg/l calcium nitrate, 15 % sucrose and 1% agar and located at 3 temperatures 15°C,24°C and 30°C.

The viability of pollen of 3 almond cultivars, three months after maintenance at 3 temperatures (4°C,-20°C and -80°C) was assessed in optimized pollen medium in terms of germination percentage. Light microscopy was carried out under Nikon type-2 microscope.

III. RESULTS

The results from 48 types of medium composition on pollen germination of 2 almond cultivars in Table1 showed that maximum germination for almond cultivar Rabie was in combination medium B2M2K1C2S (100 mg/l boric acid, 100mg/l sulphate magnesium, 0.0 mg/l potassium nitrate, 0.0 mg/l calcium nitrate ,15 % sucrose and 1% agar) with 99.80%, and the lowest germination medium in combination B1M2K2C1S (0.0mg/l boric acid, 100 mg/l potassium nitrate, 200mg/l sulphate magnesium, 0mg/l calcium nitrate , 15 % sucrose and 1%agar) with 38.30%. Significant difference between cultivars in the ability for germination and pollen tube growth was not observed.

The viability of pollen of 3 almond cultivars, 3 months after maintenance at 3 temperatures(4°C,-20°C and -80°C) showed that maximum germination was in Rabie Pollen stored at -80°C with 88.56% and the lowest germination (63.44%) in Feragnness pollen stored at +4°C (Table 2).

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<th>Medium</th>
<th>Ferragnes</th>
<th>Rabie</th>
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<td>B2M2K2C1S</td>
<td>99.80%</td>
<td>38.30%</td>
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MKBCS:Soleplate magnesium = M • Potassium nitrate = K • Boric acid = B • Calcium nitrate = C•Sucrose=S;
M1=0mg/l • K1=0mg/l • C1=Omg/l • B1=Omg/l • M2=200mg/l •
K2=100mg/l • C2=300mg/l • B2=100mg/l • S=15%

*1Mean with the same letter are not in each row significantly

The viability of pollen of 3 almond cultivars, 3 months after maintenance at 3 temperatures(4°C,-20°C and -80°C) showed that maximum germination was in Rabie Pollen stored at -80°C with 88.56% and the lowest germination (63.44%) in Feragnness pollen stored at +4°C (Table 2).
The proportion of viable pollen exceeded 90% for all cultivars evaluated before storage. Average of pollen germination of Rabie, Tuono and Ferragness 3 months after maintenance at 4°C, 20°C and -80°C was 80.02 %, 73.92% and 66.33% respectively (Table 2).

Result from Table 2 showed that cultivars differed significantly in their viable pollen storage. As in Table 2 was observed mean of pollen germination following 3 months of storage decreased to 63. 44 %, 75. 10% and 81. 74% for 4°C, 20°C and -80°C respectively. Differences in pollen germination following storage at 4, -20 and -80 °C were significant but there was no significant difference between pollen germination following storage at -20 and -80 °C.

### TABLE II: MEAN COMPARISON OF POLLEN GERMINATION OF 3 ALMOND CULTIVARS, 3 MONTHS AFTER MAINTENANCE AT TEMPERATURES

| Cultivars | Mean of pollen germination (%) | Fresh at temperatures(°C) | Optimum medium
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<tr>
<td>Rabie</td>
<td>70.16</td>
<td>80.58</td>
<td>80.02</td>
</tr>
<tr>
<td>Tuono</td>
<td>64.36</td>
<td>74.38</td>
<td>82.66</td>
</tr>
<tr>
<td>Ferragness</td>
<td>55.00</td>
<td>70.00</td>
<td>40.00</td>
</tr>
<tr>
<td>Average</td>
<td>65.44</td>
<td>75.10</td>
<td>81.74</td>
</tr>
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1. Optimized medium: 100 mg/l boric acid, 0.05 mg/l sulphate magnesium, 0.0 mg/l potassium nitrate, 300 mg/l calcium nitrate. % success and 1% agar at 24°C

*Means with similar letters there are no significant difference by Duncan test (P<0.05).

IV. DISCUSSION

Results from Table 1 showed that we can say with certainty medium optimized for two cultivars of Rabie and Ferragnes about the same and can be used to test viability of other cultivars of almond, although the ability to compare germination between cultivars of different species have been reported deferent (Weinbaum et al., 1984).

The pollen both cultivars of almond Rabie and Ferragnes was determined lowest germination in most media without boric acid compared to media containing boracic acid found (Table 1). Because such elements are reportedly on the medium to promote pollen tube growth is essential and can form complex sugar to participate and absorption, transport and metabolism of sugars in the pollen and the increase in pectin synthesis may contribute to the pollen tube cell wall formation in developing active is important (Chene et al., 1998).

On the other hand the results of this study, it was found that germination in culture media containing Boric acid 100 mg/l compared with 0 mg/l and calcium nitrate 300 compared to low concentration of calcium nitrate was more effective. According to the report of Brewbaker and Kwack (1963) the presence of calcium in the pollen culture medium with appropriate concentration plays an important role in pollen germination, but if not used with optimal concentration of inhibitory effects of different and sometimes to cause toxicity in the medium that occur in this study. Pollen germination of some cultivars and species have been reported (Brewbaker and Kwack, 1963; Mehan and Malik, 1975; Khan and Perveen, 2006a).

The viability of pollen of 3 almond cultivars, three months after maintenance at 3 temperatures (4°C, -20°C and -80°C) in Table 2 showed that between of the viability of pollen of 3 almond cultivars was significant different. So Pollen germination of almond cultivars in early was high but germination further decreased. These results agree with report of preserve the ability of pollen germination in the different conditions (Linskens, 1964; Ranahwa et al., 1962; Snope and Ellisson, 1963).

Result from Table 2 showed that differences in pollen germination of cultivars following storage at 4, -20 and -80 °C were significant. The most important factors for successful pollen conservation are storage temperature, lowering of temperature tends to increase the period of viability. So, pollen germination of almond cultivars, 3 months after maintenance at 4°C, -20°C and -80°C was 63. 44%, 75. 10% and 81. 74 % respectively (Table 4).

These findings with the results of germination capacity of stored pollen of *Abelmoschus esculentus* L (Khan and Perveen, 2006a), germination capacity of stored pollen of *Solanum melongena* (Khan and Perveen, 2006b), low temperature storage of almond pollen (Martinez-Gomez et al., 2001), olive pollen storage and In vitro germination (Pinney and Polito, 1990), increasing germination capacity of strawberry pollen in low temperature (Aslantus and Pirlak, 2002) also concur with those of (Stanley and Linskens, 1974; Amma and Kulkarni, 1979) where pollen stored at low temperature presented better germination capacity than high temperature. Pollen stored at low temperature i.e., in -80°C showed better germination percentage in -20°C and 4°C. This condition seems to have more potential to maintain viability as compared to other conditions. Also, Pollen culture at 15°C and 24°C showed better germination percentage in 30°C.

It be hoped the results of this research is used to pollination management and hybridization programs of almond.

**REFERENCES**


