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

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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/1), 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 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 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 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, boric 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° to 24C and including 10% sucrose, 100ppm 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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Ranhawa *et al.*,1962; Snope and Ellison, 1963).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/1), 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 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 38.30.00% occurred. While maximum pollen germination for almond cultivar Ferragnes was in combination medium B2M1K1C2S (100 mg/l boric acid, 0.0mg/l sulphate magnesium, 0.0 mg/l potassium nitrate, 300 mg/l calcium nitrate ,15 % sucrose and 1% agar) with 99.70%,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 58.30% .Significant difference between cultivars in the ability for germination and pollen tube growth was not observed.

TABLE I: Means comparisons of different culture media on pollen germination of 2 almond cultivars Ferragnes and Rabie

Medium	Ferragnes	Medium	Rabie
B1K1M1C1S1	03.00^{o}	B1K1M1C1S1	30.00^{o}
B1K1M1C2S1	26.70^{t}	B1K1M1C2S1	30.00^{o}
B1K1M1C3S1	31.70^{rst}	B1K1M1C3S1	31.70^{o}
B1K1M2C1S1	31.70^{rst}	B1K1M2C1S1	36.70^{no}
B1K1M2C2S1	33.30^{rst}	B1K1M2C2S1	40.00^{n}
B1K1M2C3S1	35.00^{qrst}	B1K1M2C3S1	48.30^{m}
B1K1M1C1S2	38.30^{pqrs}	B1K1M1C1S2	58.30^{l}
B1K1M1C2S2	38.30^{pqrs}	B1K1M1C2S2	63.30^{kl}
B1K1M1C3S2	41.70^{pqr}	B1K1M1C3S2	65.00^{kl}
B1K1M2C1S2	41.70^{pqr}	B1K1M2C1S2	68.30^{ijk}
B1K1M2C2S2	43.30^{pq}	B1K1M2C2S2	70.00^{ijk}
B1K1M2C3S2	45.00^{p}	B1K1M2C3S2	71.70^{ijk}
B2K1M1C1S1	45.00^{p}	B2K1M1C1S1	71.70^{ijk}
B2K1M1C2S1	58.30^{o}	B2K1M1C2S1	71.70^{ijk}
B2K1M1C3S1	61.70^{no}	B2K1M1C3S1	73.30^{hij}
B2K1M2C1S1	65.00^{mno}	B2K1M2C1S1	73.30^{hij}
B2K1M2C2S1	65.00^{mno}	B2K1M2C2S1	75.00^{ghi}
B2K1M2C3S1	68.30^{lmn}	B2K1M2C3S1	75.00^{ghi}
B2K1M1C1S2	68.30^{lmn}	B2K1M1C1S2	76.70^{fghi}
B2K1M1C2S2	68.30^{lmn}	B2K1M1C2S2	76.70^{fghi}
B2K1M1C3S2	71.70^{klm}	B2K1M1C3S2	81.70^{efgh}
B2K1M2C1S2	73.30^{jklm}	B2K1M2C1S2	81.70^{efgh}
B2K1M2C2S2	75.00^{ijkl}	B2K1M2C2S2	83.30^{defg}
B2K1M2C3S2	78.30^{hijl}	B2K1M2C3S2	85.00^{cdef}
B1K2M1C1S1	80.00^{ghijk}	B1K2M1C1S1	85.00^{cdef}
B1K2M1C2S1	80.00^{ghijk}	B1K2M1C2S1	85.00^{cdef}
B1K2M1C3S1	80.00^{ghijk}	B1K2M1C3S1	85.00^{cdef}
B1K2M2C1S1	81.70^{fghij}	B1K2M2C1S1	85.00^{cdef}
B1K2M2C2S1	81.70^{fghij}	B1K2M2C2S1	86.70^{cde}
B1K2M2C3S1	83.30^{efghi}	B1K2M2C3S1	86.70^{cde}
B1K2M1C1S2	83.30^{efghi}	B1K2M1C1S2	90.00^{bcde}
B1K2M1C2S2	86.70^{defgh}	B1K2M1C2S2	91.70^{abcd}
B1K2M1C3S2	86.70^{defgh}	B1K2M1C3S2	91.70^{abcd}
B1K2M2C1S2	88.30^{cdefg}	B1K2M2C1S2	91.70^{abcd}
B1K2M2C2S2	90.00^{bdcef}	B1K2M2C2S2	91.70^{abcd}
B1K2M2C3S2	91.70^{abcde}	B1K2M2C3S2	91.70^{abcd}
B2K2M1C1S1	91.70^{abcde}	B2K2M1C1S1	91.70^{abcd}
B2K2M1C2S1	91.70^{abcde}	B2K2M1C2S1	93.30^{abc}
B2K2M1C3S1	91.70^{abcde}	B2K2M1C3S1	93.30^{abc}
B2K2M2C1S1	91.70^{abcde}	B2K2M2C1S1	93.30^{abc}
B2K2M2C2S1	91.70^{abcde}	B2K2M2C2S1	93.30^{abc}
B2K2M2C3S1	93.30^{abcd}	B2K2M2C3S1	93.30^{abc}
B2K2M1C1S2	95.00^{abcd}	B2K2M1C1S2	93.30^{abc}
B2K2M1C2S2	96.30^{abc}	B2K2M1C2S2	93.30^{abc}
B2K2M1C3S2	96.70^{abc}	B2K2M1C3S2	96.70^{abc}
B2K2M2C1S2	96.70^{abc}	B2K2M2C1S2	98.00^{ab}
B2K2M2C2S2	99.10^{ab}	B2K2M2C2S2	99.70^{a}
B2K2M2C3S2	99.80^{a}	B2K2M2C3S2	99.70^{a}

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%
* [1]Mean 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 Feragness 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% for4°C, 20°C and -80°C respectively. Differences in pollen germination following storage at 4, -20and -80 °C were significant but there was no significant difference between pollen germination following storage at -20and -80 °C.

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

pollen germination (%)

Cultivars	3months after maintenance at temperatures(°C) in optimized medium[1]				Fresh at temperatures(°C)		
	0	-20	-80	Average	15	24	30
Rabie	70.76a	80. 76a*	88.56a	80.02a	98.35a	94.54a	80.63b
Tuono	64.56a	74.56a	82.66a	73.92b	94.52a	95.35a	79.00b
Ferragness	55.00b	70.00b	74.00b	66.33c	91.52a	90.65a	71.25b
Average	63. 44 a	75. 10 a	81. 74b	73.42	94.73a	92.84a	76.96b

[1] optimized medium :100 mg/l boric acid, 0.0mg/l sulphate magnesium, 0.0 mg/l potassium nitrate, 300 mg/l calcium nitrate ,15 % sucrose and 1% agar at 24°C

*Means with 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 boric 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 100mg/l compared with 0mg/l and calcium nitrate 300 compared to low concentration of calcium nitrate was more effective .According to the report of Brewbacker 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 (Brewbacker 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; Ranhawa et al., 1962; Snope and Ellison, 1963).

Result from Table 2 showed that differences in pollen germination of cultivars following storage at 4, -20and -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 was63. 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.

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