International Journal of Nuts and Related Sciences 2(2): 68-72, 2011
ISSN 2008-9937

Evaluation of Germination Capacity of Stored Pollen of Almond and Peach

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Abstract: Pollen viability and its germination capability for fruit set in are almond and peach essential. To optimize the pollen culture medium of almond and peach and selection of best medium, the present study was carried out with 48 different culture media containing different compositions including diverse levels of boric acid (0 and 100mg/1), calcium nitrate (0,150and300mg/l¡), magnesium sulphate (0 and200mg/l), potassium nitrate (0 and100 mg/l), sucrose (10 and15 %) and agar (1 %). Following optimization of the best medium, in order to determine the viability of stored pollens of almond and peach cultivars, three months after maintenance at 4°C,-20°C and -80°C was assessed through evaluation of their germination percentage using optimized medium. Maximum pollen germination (99.80 %) was recorded in B2M2K1C2S medium (boric acid 100 mg/l, magnesium sulphate 100 mg/l, potassium nitrate 0.0 mg/l, calcium nitrate 0.0 mg/l, sucrose 15 % and agar 1%). Lowest germination percentage (30.57 %) was found in B1K1M2C3S1 medium (boric acid 0.0 mg/l, potassium nitrate 0.0 mg/l, magnesium sulphate 100 mg/l, calcium nitrate 150 mg/l, sucrose 10 % and agar 1 %). Data recorded for pollen germination of stored pollen showed that Rabie Pollen stored at -80°C gave highest germination (90.66 %) while the lowest germination (64.00 %) was observed in Anjiri pollen stored at +4°C. The findings of the present study may help the fruit breeders and whoever is involved in pollen analysis studies.

Key words: *In vitro* % Pollen viability % Almond % peach

INTRODUCTION

Almond and peach for fruit set needs the pollination of flowers followed by fertilization. Therefore, pollen viability and its germination capability are essential (Kester *et al*., 1991; Martinez-Gomez *et al*., 2002). The biological review indicated that the pollen grains in the special environments have the good growth and germination (Boavida & McCormick, 2007). On the other hand, the basic components of pollen culture 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 of the growth medium are two important factors that germination and growth are significantly affected (Boavida & McCormick, 2007; Chebli & Geitmann, 2007).Among the elements of the primary role, boron (B) has a considerable task in the development of pollen so that it is a proposed structure prerequisite in the development of cell walls of pollen participate (Matoh *et al*., 1996; Fleischer *et al*., 1998; Chene *et al*., 1998). The importance of B during *in vitro* and *in vivo* pollen germination studies was already pointed out (Nyomora *et al*., 2000; Jayaprakash & Saria, 2001; Wang *et al*., 2003). Boron application for germination of pollen grains is considered as an effective strategy in fruit trees (Hanson, 1991; Picchioni & 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 & 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 & Trewevas, 1996; Malho *et al*., 2000).

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To storage the pollen in suitable condition for preserving its viability in order to eliminate the problem in time and place of artificial pollination, is very essential (Khosh-khui *et al*., 1976). Preserve the ability of pollen germination depends on the storage conditions like humidity, temperature and air pressure (Linskens, 1964; Snope & Ellison, 1963). Pollen viability is determined by different methods including culture on a drop through the sucrose solution (2.5 to 20%) (Amma & Kulkarni, 1979), staining (Ganeshan & Alexander, 1991; Alexander,1996), *in vitro* and so on (Stanley & Linskens,1974). 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 & Linskens, 1974). It was found that pollen germination in culture media containing sucrose, Boric acid and calcium nitrate and calcium plays an important role (Brewbacker & 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 & Malik, 1975; Brewbacker &Kwack, 1963; Khan & Perveen, 2006a).

Germination capacity of stored pollen of plants has also studied by maintaining of pollens in different temperatures) Stanley & Linskens, 1974; Amma and Kulkarni, 1979; Pinney & Polito, 1990; Martinez-Gomez *et al*., 2002; Aslantus & Pirlak, 2002;Khan & Perveen, 2006a; Khan & Perveen, 2006b) where pollen stored at low temperature presented better germination capacity than high temperature

The objectives of the present study were optimization of the pollen culture medium and the viability pollen grains of some almond and peach cultivars following maintenance under variable temperatures.

MATERIALS AND METHODS

Branches with unopened flowers were pruned off from trees of almond (Rabie) and peach (Anjiri) cultivars growing in orchards of KamalShahr, Karaj. Pollen was collected in large quantities from these cuttings, 24 h following pruning from freshly opened blossoms. To optimize the pollen medium of almond and standardization of best medium, 48 different culture media were prepared containing different compositions including diverse levels of boric acid (0 and 100mg/1), calcium nitrate (0,150 and 300mg/l_i), magnesium sulphate (0 and200mg/l), potassium nitrate (0 and100 mg/l), sucrose (10 and15 %) and agar (1 %).The experiment was laid out as complete randomized design including three replications.

Following optimization of the best medium, in order to determine the best viability of stored pollens of almond and peach cultivars, three months after maintenance at 4°C,-20°C and -80°C was assessed through evaluation of their germination percentage using optimized medium. Light microscopy was carried out under Nikon type-2 microscope. The statistical analysis was performed using Microsoft Excel (2007) and SAS software (SAS Institute Inc, 1990) and means were compared using Duncan's Multiple Range Test (DMRT).

RESULTS

In vitro pollen germination of three almond cultivars in various media is shown in Table 1. *In vitro* pollen germination of almond and peach cultivars in various media is shown in Table 1.It is clear that maximum pollen germination (98%) for almond cultivar Rabie was recorded in B2M2K1C2S medium (boric acid 100 mg/l, magnesium sulphate 100 mg/l, potassium nitrate 0.0 mg/l, calcium nitrate 0.0 mg/l, sucrose 15 % and agar 1%). Lowest germination percentage (27 %) was found in B1K1M2C3S1 medium (boric acid 0.0 mg/l, potassium nitrate 0.0 mg/l, magnesium sulphate 100 mg/l, calcium nitrate 150 mg/l, sucrose 10 % and agar 1 %) for this cultivar. Pollen germination for peach cultivar cultivars (Anjiri) was almost similar at above media. Considerable differences among cultivars in the ability for germination and pollen tube growth were not observed.

The pollen viability of almond and peach cultivars was found to be significantly affected during storage in variable temperatures (+4, -20 and -80°C). Maximum germination was recorded in Rabie Pollen stored at -80°C (88.56%) and the lowest germination (64. 00 %) in Anjiri stored at +4°C (Table 2).

The Pollen viability exceeded 90% for both cultivars evaluated before storage while average pollen germination percentage in Rabie and Anjiri cultivars, following storage in +4, -20 and -80°C was 80.02 % and 69.00% respectively (Table 2).

Almond and peach showed significantly different response with regard to their pollen viability following storage. According to Table 2 mean values for pollen germination, 3 months after storage decreased to 63.44%, 75.10% and 81.74% for+4, -20 and -80°C respectively. It was observed that 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 -20and -80°C.

Table 1: Means comparisons of different culture media on pollen germination of almond and peach cultivars

Medium culture	Rabie	Medium culture	Anjiri
B2K1M2C1S2**	99.70 a*	B2K1M2C1S2	95.12 a
B2K1M2C3S2	98.30 ab	B2K1M2C3S2	93.50 a
B2K1M1C2S2	98.00 ab	B2K2M1C2S2	89.00 ab
B2K2M1C1S2	98.00 ab	B1K1M2C2S2	86.70 ab
B2K1M2C2S1	96.70 ab	B2K2M2C2S2	82.30 abc
B2K2M2C2S2	96.70 ab	B2K2M1C1S2	82.30 abc
B2K1M1C3S1	96.00 ab	B1K2M1C3S2	80.70 abcd
B2K2M2C3S1	94.70 ab	B2K2M2C1S2	80.70 abcde
B2K1M2C3S1	91.70 ab	B2K2M2C2S1	79.30 abcdef
B2K2M1C2S2	91.70 abc	B2K1M1C2S2	79.70abcdef
B1K1M2C2S2	90.00 abc	B1K1M1C1S2	77.70 abcdefg
B2K2M1C1S1	90.00 abc	B2K2M1C2S1	75.00 abcdefg
B2K1M1C2S2	90.00 abc	B1K2M1C2S2	75.00 abcdefg
B2K1M2C2S2	90.00 abc	B2K1M2C3S1	75.00 abcdefg
B1K2M1C2S2	88.30 bcd	B2K1M2C2S2	73.30 abcdefgh
B2K2M2C3S2	88.30 bcd	B1K1M1C2S2	73.30 abcdefgh
B2K2M2C1S2	88.30 cde	B1K1M2C2S1	73.30 abcdefgh
B1K2M1C3S2	88.30 cde	B2K1M1C2S1	71.60 bcdefghi
B1K1M1C1S2	81.70 cde	B2K2M1C1S1	68.40 cdefghij
B1K2M1C3S1	81.70 cde	B1K1M2C1S1	66.50 cdefghijk
B1K1M2C1S1	78.30 def	B2K1M2C2S1	66.50 cdefghijk
B2K2M1C2S1	78.30 def	B2K2M2C3S1	66.50 cdefghijk
B2K1M1C2S1	78.30 def	B1K2M2C2S2	66.50 cdefghijk
B1K2M2C1S2	78.30 def	B1K2M2C2S1	66.50 cdefghijk
B1K2M1C1S2	78.30 def	B1K1M2C1S2	66.50 cdefghijk
B1K2M2C3S2	78.30 def	B1K2M1C3S1	65.02 defghijk
B1K1M2C1S2	76.70 ef	B2K2M2C2S1	63. 33efghijkl
B1K2M2C2S2	76.70 ef	B1K2M2C1S1	63.37 efghijkl
B1K1M1C2S2	76.70 ef	B1K2M2C1S2	61.76 efghijkl
B2K2M2C2S1	75.00 ef	B2K2M1C1S2	61.77 efghijkl
B2K2M2C2S1	75.00 ef	B1K1M1C3S1	60.00 fghijklm
B1K1M2C2S1	75.00 ef	B2K1M1C1S2	60.00 fghijklm
B1K2M2C3S1	73.30 efg	B2K1M1C2S2	58.30 ghijklm
B2K1M1C1S1	68.30 fg	B1K2M2C3S2	56.50 hijklm
B1K2M2C1S1	68.30 fg	B1K2M1C1S2	55.00 ijklm
B1K1M1C3S2	65.00 g	B1K1M1C2S1	53.30 jklmn
B1K1M2C3S2	55.00 h	B2K1M1C1S1	53.30 jklmn
B2K2M1C1S2	53.30 hi	B1K2M2C3S1	53.30 jklmn
B1K1M1C3S1	53.30 hi	B1K1M1C1S1	50.00 klmno
B2K1M1C1S2	48.30 hij	B1K1M1C3S2	46.40 lmnop
B1K1M1C2S1	45.00 ij	B1K2M2C3S1	46.40 lmnop
B1K2M2C2S1	45.00 ij	B1K2M1C1S1	46.20 lmnop
B2K2M2C1S1	45.00 ij	B2K1M1C3S1	44.00 mnopq
B1K1M1C2S1	43.30 j	B1K1M2C3S2	43.90 mnopq
B1K2M1C1S1	43.30 j	B2K1M1C1S1	38.70 nopq
B1K1M1C1S1	43.30 j	B1K1M1C2S1	36.80 opq
B2K1M1C1S1	41.70 j	B2K2M2C1S1	31.90 pq
B1K1M2C3S1	33.00 ks	B1K1M2C3S1	30.60 q

Table 2: Mean comparison of pollen germination of 3 Almond cultivars, 3 months after maintenance at temperatures

	Pollen germination (%)			
Cultivar	3months after maintenance at temperatures (°C) in optimized medium[1]			
	+4	-20	-80	Average
Rabie	70.76a	80. 76a*	88.56a	80.02a
Anjiri	64.00b	73.00b	70.00b	69.00c
Average	67. 38 a	76. 88 a	79. 28b	74.51

[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 followed by the similar letter(s) are not significantly different by Duncan test (P<0.05).

DISCUSSION

Data presented in Table 1 demonstrated that the medium optimized for three cultivars of Rabie and Anjiri may be applied to pollen germination test in other cultivars of almond, however Weinbaum *et al*.(1984) reported that the ability to compare germination between cultivars of different species would be different.

The pollen of Rabie and Anjiri cultivars showed lowest germination in most media lacking boric acid as compared to media supplemented with boric acid (Table 1). These observations agree with previous researches (Chene *et al*., 1998; Wang *et al*., 2003) concluded that for pollen viability test some media are effect compare to others.

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 the inhibitory effects could be arose (Brewbacker & Kwack, 1963; Mehan & Malik, 1975; Khan & Perveen, 2006a) and sometimes to cause toxicity in the medium that occur in this study.

Rabie and Anjiri cultivars were significantly different with respect to their pollen viability following a three months period storage (Table 2). Pollen germination was initially high but it was declined later on. Corroboratory results also were already reported by Snope and Ellison (1963), Linskens (1964), Pinney & Polito (1990), Martinez-Gomez *et al*. (2002) & Khan & Perveen (2006a) who assessed the pollen viability in different conditions.

It is clear from our data (Table 2) that three different temperatures (+4, -20 and -80 °C) in a different way influenced the pollen viability, so that, lowering of temperature tends to increase the level of viability. As a result, average pollen germination of almond and peach cultivars, 3 months after maintenance at +4, -20 and -80°C was determined to be 67.38% and 79. 28 %, respectively (Table 2).

These findings confirm the results of germination capacity of stored pollen of *Abelmoschus esculentus* L.) Khan & Perveen, 2006a), germination capacity of stored pollen of *Solanum melongena* L.) Khan & Perveen, 2006b), low temperature storage of almond pollen (Martinez-Gomez *et al*., 2002), olive pollen storage and *in vitro* germination (Pinney & Polito, 1990), increasing germination capacity of strawberry pollen in low temperature(Aslantus & Pirlak, 2002) also concur with those of (Stanley & Linskens, 1974; Amma & 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 as compared to -20°C and 4°C. This condition seems to have more potential to maintain viability as compared to other conditions.

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

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