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Full Length Research Paper

Storage of apple pollen and *in vitro* germination

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Temperate fruits including pome fruits, especially apple are important fruit 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. For that reason, examination to optimize the pollen medium of apple and determination of the best medium was carried out with 16 types of culture medium containing different concentrations of boric acid (0 and 100 mg/1), calcium nitrate (0 and 300 mg/l), magnesium sulphate (0 and 200 mg/l), potassium nitrate (0 and 100 mg/l), sucrose (15%) and agar (1%) in the *In vitro* using random complete design with three replications. The results showed that maximum germination was in combination medium B2M1K1C2S (100 mg/l boric acid, 0mg/l sulphate magnesium, 0.0 mg/l potassium nitrate, 300 mg/l calcium nitrate, 15% sucrose and 1% agar) with 100%, and lowest germination medium in combination B1M2K2C1S (0.0 mg/l boric acid, 100 mg/l potassium nitrate, 200 mg/l magnesium sulphate, 0 mg/l calcium nitrate, 15% sucrose and 1% agar with 13.30% occurred. The viability of pollen of 4 apple cultivars, three and seven months after maintenance at 3 temperatures (4°C, - 20°C and -80°C) showed that 3 months after storage, maximum germination was in Primgold Pollen stored at -80°C with 96.21% and the lowest germination (58.33%) in Northern Spy pollen stored at 4°C, but 7 months after storage, maximum germination was in Primgold Pollen stored at -80°C with 90.66% and the lowest germination (36.67%) in Northern Spy pollen stored at 4°C.

Key words: *In-vitro*, pollen germination, apple.

INTRODUCTION

Temperate fruits including pome fruits, especially apples are important fruit crops which mostly for fruit set needs the pollination of flowers and followed by pistil fertilization (Calzoni et al., 1979) . Therefore, pollen viability and its germination capability are essential. The biological review indicated that the pollen grains in the special environment have the good growth and germination (Boavida and McCormick, 2007). On the other hand, the basic components of pollen medium contain calcium, boric acid, magnesium, potassium and sucrose. In general, compounds in the pollen medium at different concentrations are found (Linskens, 1964) . In addition to these elements, pH and temperature are two important factors that affect germination and growth (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 the proposed structure prerequisite in the development of cell walls of pollen participate (Matoh et al., 1996; Fleischer et al., 1998).

It has also 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 i*n 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). The application of boron

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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. The role of calcium in pollen tube growth also has been reported (Malho et al., 1994; Malho and Trewavas, 1996; Malho et al., 2000).

Pollen viability and germination capability of 2 commercial apple cultivars Golden delicious, and Starkrimson *in vitro* showed that the best germination results was for the two digits in the medium temperature of 30°C and including 0.2 mol sucrose, 20 g/ml of H_3BO_3 and 300 g/ml of $CaNO_3$ (Calzoni et al., 1979; Watters and Sturgeon, 1990). On the other hand, it was 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). To the available 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, has been given more attention (Khosh-Khui et al., 1976) . Preserving the pollen germination ability depends on the storage conditions like humidity, temperature, and air pressure (Linskens, 1964; Snope and Ellison, 1963).

Pollen viability is determinate by different 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 10 ppm Boric acid at 20°C for germination and pollen tube growth as an 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 the present research were optimization of the pollen culture medium and the viability pollen of apple, after short times maintenance under different temperatures.

MATERIALS AND METHODS

Branches with unopened flowers were collected from trees of 4 apple cultivars (M9, Northern Spy, Primgold and Golab) growing in commercial orchards. After 24 h, pollen was collected in large quantity from freshly opened blossoms in those cuttings. To optimize pollen germination media for each apple cultivars, 16 media were designed with different combinations of boric acid (0 and 100 mg/1), calcium nitrate (0 and 300 mg/l), magnesium sulphate (0 and 200 mg/l), potassium nitrate (0 and 100 mg/l), sucrose (15%) and agar (1%). Fresh pollen culture were used to determinate the optimize media, after 24 h fresh pollen culture, pollens were counted under the light microscope in 3 scopes for each media, and germination percentage was determined, pollen tubes equal to or longer than twice of the diameter of pollen grains were considered as germinated.

The storage of pollen viability of 4 apple cultivars was assessed after three months storage under 3 temperature conditions (4°C, -20°C and -80°C), using the above optimized media to determine their germination percentage. Pollen germination count was carried out under Nikon type-2 microscope. Statistical analysis was performed using Microsoft Excel (2007) and SAS software (SAS Institute Inc, 1996) and means were compared using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSIONS

The results from 16 types of the medium composition on pollen germination of 2 apple cultivars in Table 1 showed that maximum germination of apple cultivars Northern Spy and M9 was in combination medium $B_2M_1K_1\ C_2S$ (100 mg/l boric acid, 0.0 mg/l magnesium sulphate, 0.0 mg/l potassium nitrate, 300 mg/l calcium nitrate, 15% sucrose and 1% agar) with 100% and 98.3% (Figure 1), respectively. While the lowest germination in medium combination $B_1M_2K\ _2C_1S$ (0.0 mg/l boric acid, 100 mg/l potassium nitrate, 200 mg/l magnesium sulphate, 0 mg/l calcium nitrate , 15% sucrose and 1% agar) occurred 13.30% and 18.30%, respectively. Significant difference between cultivars and the ability for germination and pollen tube growth was not observed (Table 1). So we can say with certainty medium optimized for two varieties of M9 and Northern Spy about the same and can be used to test viability of other varieties of apple, although the ability to compare germination between cultivars of various species have been reported different (Weinbaum et al., 1984).

The pollen was determined by both varieties of apple Northern Spy and M9 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, absorb, and transport 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 showed that germination in the culture media containing boric acid 100 mg/l compared with 0 mg/l and calcium nitrate 300 compared to the 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 if applied to an important role in pollen germination plays, but if not used with optimal concentration of inhibitory effects of different and sometimes to cause toxicity in the medium that occur in this case study, pollen germination of some cultivars and species have been reported (Brewbacker and Kwack, 1963; Mehan and Malik, 1975; Khan and

Table 1. Comparisons of different culture media on pollen germination of 2 apple cultivars, Northern Spy and M9.

Northern Spy	Medium culture	B2M1K1C2S	B2M2K1C1 S	B2M2K2C1 S	B2M2K1C2 S	B2K2M2C2 S	B2M1K1C1 S	B2M1K2C2 S	B2M1K2C1 S	B1M1K1C2 S	B1M1K2C2 S	B1M2K1C1 S	B1M2K2C2 S	B1M1K1C1 S	B1M2K1C2 S	B1M1K2C1 S	[illegible] S
	%germination	a*00.100	ab00.90	ab00.90	ab00.90	b30.88	b00.85	c70.61	cd03.58	c30.53	e30.43	ef70.41	ef70.41	ef00.35	f30.33	g00.25	h30.13
		Stand. deviation = 0.2717															
M9	Medium culture	B2M1K1C2S	B2M2K1C1 S	B2M2K1C2 S	B2M2K2C1 S	B2M1K2C2 S	B2K2M2C2 S	B2M1K1C1 S	B2M1K2C1 S	B1M1K2C2S	B1M1K1C2 S	B1M1K1C1 S	B1M2K1C2 S	B1M1K2C1 S	B1M2K1C1S	B1M2K2C2 S	[illegible] S
	%germination	a30.98	a00.95	ab20.93	ab70.91	bc56.68	c30.83	d?73.30	e70.46	e70.46	ef00.40	fg30.33	fg30.33	fg30.33	fg30.33	g70.31	h30.18
		Stand. deviation = 0.2946															

* Means with similar letters have no significant difference by Duncan test (P<0.05), MKBCS: M = Magnesium sulphate, K = Potassium nitrate, B = Boric acid, C = Calcium nitrate, S = Sucrose; K1 = 0 mg/l, C1 = O mg/l, B1 = O mg/l, M1= 0 mg/l, M2 = 200 mg/l, K2 = 100 mg/l, C2 = 300 mg/l, B2 = 100 mg/l, S=15%.

Perveen, 2006a).

As is seen (Table 2) that the viability of pollen of 4 apple cultivars, three and seven months after maintenance at 3 temperatures (4°C, -20°C and -80°C) (Table 2) was different. Following 3 months after storage, maximum germination was in Primgold Pollen stored at -80°C with 96.21% and the lowest germination (58.33%) in Northern Spy pollen stored at +4°C, while 7 months after storage, maximum germination was in Primgold Pollen stored at -80°C with 90.66% and the lowest germination (36.67%) in Northern Spy pollen stored at +4°C. Germination in early was high but germination further decreased.

The proportion of viable pollen exceeded 90% for all cultivars evaluated before storage, but mean of pollen germination of Primgold, Golab, M9 and Northern Spy Cultivars 3 months after maintenance at 3 temperature conditions (the sum from three temperatures) was 84.22%, 84.26%, 67.09% and 54.33% respectively (Table 3) . However these amounts of pollen germination at same of 3 above temperature conditions after 7 months of storage for Primgold, Golab, M9 and Northern Spy Cultivars were 78.22%, 68.43%, 62.17% and 47.22% respectively. It was found that germination levels of cultivars varied significantly in their viable pollen storage. As in Table 4 was observed, mean of pollen germination following 3 months of storage decreased to 89.55%, 83.59% and 61.64% at 4°C, -20°C and -80°C, respectively. In opposition to mean of pollen germination in above temperatures after 7 months of storage 71.83, 67.03 and 52.43% for 4°C, -20°C and -80°C respectively. Differences in pollen germination 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 mean of pollen germination of apple cultivars following 3 months of storage decreased to 89.55%, 83.59% and 61.64% for 4°C, -20°C and -80°C, But these amounts of pollen germination in above temperatures after 7 months of storage 71.83, 67.03 and 52.43% for 4°C, -20°C and -80°C, respectively (Table 4).

These findings with the results of germination capacity of stored pollen of *Abelmoschus esculentus* (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., 2002), 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 -80°C

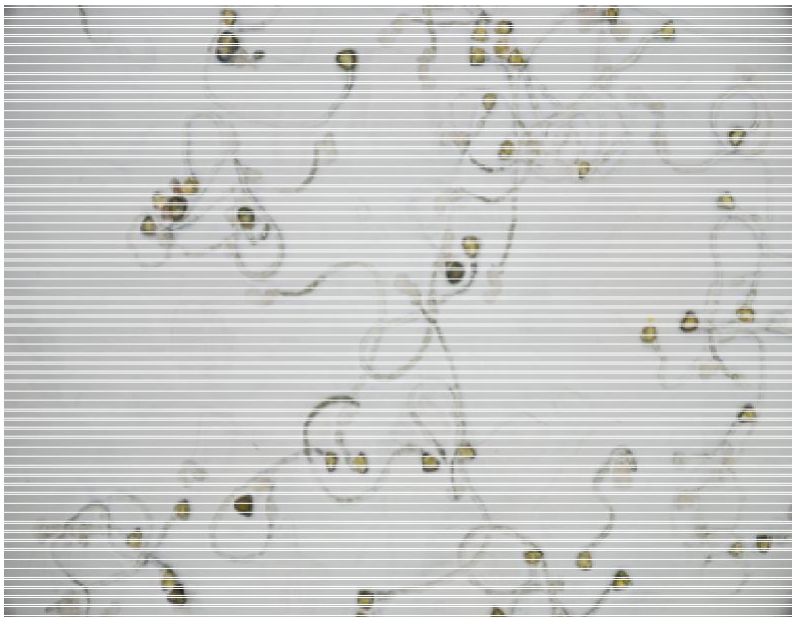

Figure 1. Pollen germination of M9 in medium B2M1K1C2S.

Table 2. Pollen germination ability of 4 apple cultivars after 3 and 7 month storage under 3 temperature conditions (4°C, -20°C and -80°C).

Cultivar	Maintenance at temperature (°C)	3 months after storage	7 months after storage
		Pollen germination (%)	
Primgold	-80	96.21a*	90.66a
Primgold	-20	90.12ab	84.66b
Primgold	4	66.35d	59.36cb
Golab	-80	95.32a	75.00b
Golab	-20	92.23ab	66.96bc
Golab	4	65.24d	63.33c
M9	-80	78.33c	68.33bc
M9	-20	66.27d	64.86bc
M9	4	56.67e	50.36d
Northern Spy	-80	88.34ab	53.33d
Northern Spy	-20	85.74bc	51.66d
Northern Spy	4	58.33d	36.67e

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

Table 3. Mean of pollen germination ability of 4 apple cultivars after 3 and 7 month storage under 3 temperature conditions.

Cultivar	3 months after storage	7 months after storage
	Pollen germination (%)	
Primgold	84. 22a*	78. 22a
Golab	84 26a	68.43a
M9	67.09b	62. 17c
Northern Spy	54. 33c	47.22b

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

Table 4. Mean comparison of pollen germination of 4 apple cultivars, 3 and 7 months after maintenance at 3 temperatures (+4°C, -20°C and -80°C).

Maintenance at temperature (°C)	Pollen germination (%) of storage	
	3 months after storage	7 months after storage
	Pollen germination (%)	
-80°C	89.55a*	71.83a
-20°C	83.59b	67. 03b
+4°C	61.64c	52.43c

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

showed better germination percentage than -20°C and 4°C. This condition seems to have more potential to maintain viability as compared to other conditions. Its been hoped that results of this research is used in pollination management and hybridization programs of apple.

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