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Investigation on Pollen Viability, Germination and Tube Growth in Some Apple Cultivars in Climate Conditions of Shirvan

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ABSTRACT

This study purpose is determining germination and pollen tube growth in two independent experiments, in experiment 1:staining test has been used to determine pollen viability percentage in 2 factors of cultivars and staining test material, and in experiment 2: in vitro culture has been used in two factors of cultivars in7 levels (Golab e Isfahan, Golden Delicious, Red Delicious, Mahali sheikhi, Malakeh, Sheikh Amiri and Sib Gol) and sucrose concentration in 4 levels (0%, 5%, 10%, 15%) in completely randomize design with 3 replication, has been carried out. Results showed that various cultivars effect, various sucrose levels on pollen germination percentage, pollen tube growth and their interaction effects on germination percentage were significant in 1% level. Data mean comparison showed that Mahali- Sheikhi cultivar with 47.76% and Golabe Isfahan cultivar with 18.86% have the most and the least percentages of germination, respectively, and the highest pollen tube growth was for Sib Gol cultivar (153.62 µm) and the lowest growth was for Golab e Esfahan (99.65 µm). The most value of germination percentage and pollen tube growth was obtained in sucrose 15%. In viability test(staining test), the higher percentage of pollen viability was for safranin that in Mahali Sheikhi it was 75.53%, and the least viability was obtained for IKI in Sib Gol cultivar 50.13%. Totally, the medium culture containing sucrose 15% and staining test with safranin has been suggested respectively to determine pollen germination, tube growth and pollen viability percentage in pollen apple cultivars.

KEY WORDS: apple, viability, pollen germination, pollen tube growth

INTRODUCTION

The great majority of apple cultivars are self-incompatible (Broothaerts,2003), and for fruit set needs the pollination of flowers and fallowed by pistil fertilization (Calzoni et al.,1979) So, in breeding programs for this species, manual pollination could be carried out in the field or laboratory(Sharafi,2011). The viability, tube growth and morphological homogeneity related to pollen quality are the most important properties in fruit trees. These properties are useful for plant breeders, geneticists, and growers (Bolat and Pirlak, 1999). For successful pollination the high quantities and qualities pollen must be transferred to the stigma when it is receptive (Dekers and porreye, 1984). However, some times, the pollen is deposited before the receptive period and the pollen should remain viable for a period long enough (Stosser et al. 1996). There are several methods for determining the viability of pollen (Dafni and firmage; 2000). Fastest way to interpret pollen viability using the staining reaction to pollen with enzymes and therefore represents the full contents of the pollen cells (Shivanna and Heslop-Harrison1981).since they are faster and easier than pollen germination tests. But, in some cases viability tests gave inconsistent results with the germination status of pollen (Parfitt and Ganeshan, 1989). Another method used to determine viability, in vitro culture conditions for the germination of pollen grains of many species because the Boric acid is readily osmotic environment and germ does this method widely is used (Taylor and Hepler, 1997). Germination tests can be considered as more reliable way to determine the exact amount of viable pollen (Bolat and Pirlak, 1999).

Petrisor et al., (2012) have shown pollen germination of 10 cultivars in in vitro condition culture contains 15% sucrose and 10 ppm acid Boric and 1.5% agar between 52.55 and 89.92.

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 (Malho 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). However, the basic components of a medium containing boric acid and sucrose during pollen tube elongation, sugars are utilized as an energy source for synthesis of cell wall materials such as pectins, cellulose and callose (Mascarenhas, 1993;

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Derksen et al., 1995). The application of boron 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. In addition to the elements, PH and temperature of growth medium are two important factors that can affect the germination and growth (Boavida and McCormick2007; Chebli and Geitmann, 2007).

In order to characterize pollen viability and germinability by biochemical parameters, an introductory investigation was made of the germinability in vitro of pollen of 2 apple cultivars, 'Golden Delicious' and 'Starkrimson' obtained for both cultivars after 120 min incubation in Petri dishes at 30°C in a medium containing 0.2 M sucrose, 20 µg/ml H3BO3, 300 µg/ml Ca (NO3)2 · 4H2O. Optimum pH was 6.0 for 'Starkrimson' and 7.0 for 'Golden Delicious. (Calzoni and et al, 1997)

It was indicated in pollen germination test that germination percentage plays an important role in culture containing sucrose, boric acid, nitrate calcium, and calcium (Brewbacher and Kwack,1963).

MATERIALS AND METHODS

Branches with unopened flowers were collected from trees of 7 apple cultivars (Golab e Isfahan, red delicious, Golden delicious , Mahali Sheikhi , Sheikh Amiri, Malakeh, Sib Gol) and leaving them in a vase with water. Flowers collected at the balloon stage just before the petals expand, and before the anthers dehiscence. Anthers were carefully removed using forceps, the collected anthers, desiccated at room temperature for 24 - 48 h and then placed in glass bottles and stored at 4°C until use.

Experiment 1: Evaluation of pollen viability by stain tests

In this research IKI (iodine+potassium iodide) and safranine stain tests were used to determine pollen viability. The grains of pollen were counted to determine viability after a couple of minutes in the IKI medium (1gKI and 0.5g I dissolved in 100ml distilled water),(Eti., 1991). The pollen viability was obtained one hour following sowing in the safranin medium [(1g safranin dissolved in 95%alcohol (40 ml) and the final volume was made up to 100 ml. One part safranine was mixed with two parts gliserol and one part distilled water (1:2:1) (Bolat and Pirlak.,1999). A drop by Pasteur pipettes put on microscope slides and pollen were shaked with a slim brush (each brush used only one plant type) covered with a coverslip. To determine viability about three hundred pollen grains of each replicate from four different areas were counted under a light microscope. Viable pollens were dyed in red; dead pollens were not dyed in safranin and viable pollens were dyed in dark brown and dead pollens were not dyed or pale yellow in IKI .

Experiment 2: Evaluation of pollen germination and tube growth in solid medium culture

For the cultivation of pollen apple and to determine the best medium for varieties used the sterile Petri dishes with solid medium containing sucrose, 0%, 5%, 10%, 15% + acid Boric 0.2 (g) + 0.3 g of calcium nitrate(PH=5.7), under the hood and sterile environment with the help of a brush, very low pollen density on the surface of the medium culture were sprayed, immediately with a Teflon Petri dish seamed and were incubated in 24°c about 36 hours. Pollen germination percentage and pollen tube length were measure under light microscope with 10x ocular. Five microscopic areas were counted randomly for evaluation of pollen germination percentage and pollen tube length. Standard germination when the pollen tube length was greater than the diameter of the pollen. Tube length was recorded based on micrometer (µm) that fitted to the eyepiece on microscope. The length was measured in 20 randomly selected pollen tube pollen tube length was calculated as the mean.

Statistical analysis was performed using Microsoft Excel (2007) and SAS software and means were compared using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Pollen viability percentage

Variance analysis results of various cultivars showed the type of stain test material on pollen viability percentage in 1% significance level (table 1). The comparison between the average of stain test material type and pollen viability percentage showed that the highest percentage of viability (58.74%) was in stain test with safranin and the least percentage was for IKI (43.01%) (Figure1).

The comparison between pollen viability showed that the highest viability percentage is for Mahali Sheikhi cultivar with 75.53% and the least percentage was for Sib Gol cultivar (50.13%) which doesn't have significant difference with Golab e Isfahan, Malakeh, and Golden Delicious (Figure 2). Therefore, safranin can be used to determine pollen viability in apple cultivars. (Karakaya et al, 2000), by study on strawberry pollen viability with TTC, safranin and IKI indicate significant difference among type of staiting, and safranin has higher percentage

than IKI for pollen viability. (Dalkilic and Mestva, 2011) in study 7 cultivars viability percentage to (Quience) in IKI was between 90.8% and 98.1%. Results showed that pollen viability percentage was different according to stain test and cultivars. Such results reported by Bolat and Pirlak (1999), Parfitt and Ganeshan(1989), Oberle and Watson (1953) and were compatible.

Table 1: Analysis of variances stain test with safranin and IKI effect on pollen viability percentage

Source of variance	DF	Mean- square	The sum of squares	F
Cultivars	6	2544.77	424.12	5.08**
Stain test	1	10466.56	10466.36	132.32**
Cultivars. Stain test	6	944.44	157.40	2.27 ns
Error	34	2884.07	84.82	
Total	41	15855.40		
CV(%)	9.21			

**, ns show significant and no significant difference in 1%, respectively.

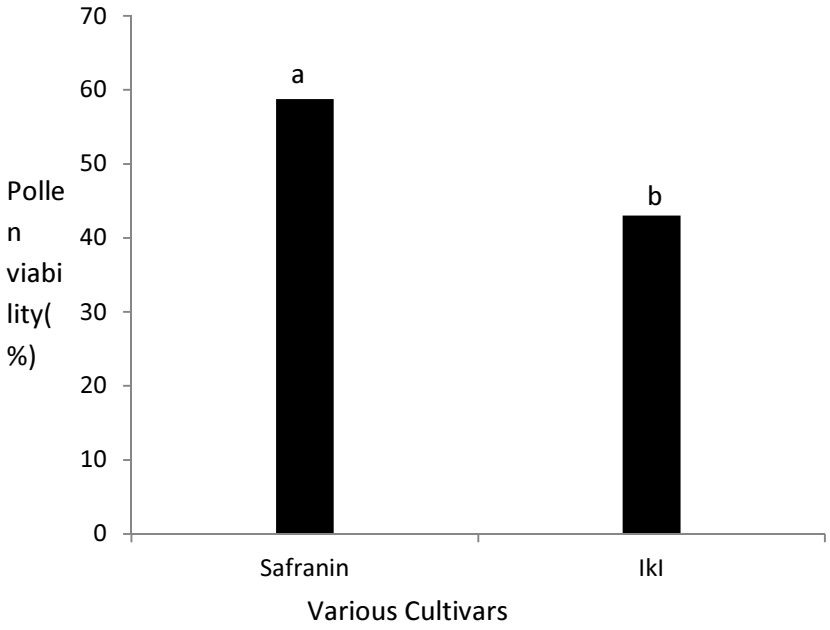

Figure1: stain test effect on pollen viability percentage

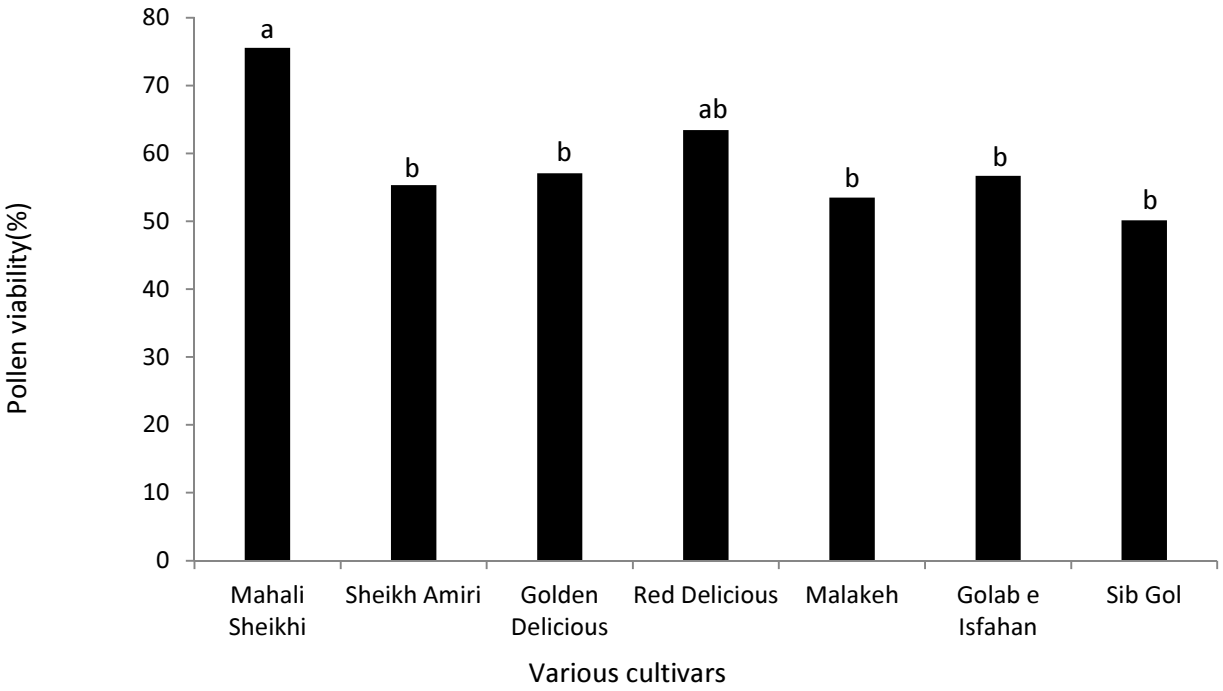

Figure 2: stain test effect in various cultivars on pollen viability percentage

Germination and Pollen Tube Growth Percentage

Variance analysis results showed that various cultivars effect, sucrose various levels on germination percentage, pollen growth and their polar effects on germination percentage were significant in 1% (table

Table2: Analysis of variances sucrose different levels effect in cultivars on pollen germination and tube growth percentage.

Source of Variations	DF	Pollen germination (%) Mean-square	pollen tube growth(µm)
Cultivar	6	1454.23**	4705.87**
Sucrose different levels	3	22731.53**	15523.1**
Sucrose percentage .various cultivars	18	395.08**	1171.09^{ns}
Error	56	2110.22	62083.62
Total	83		
CV (%)		6.13	33.29

**, ns show significant and no significant difference in 1%, respectively.

The mean comparison between different cultivars effect and pollen germination percentage has shown that Mahali Sheikhi cultivar with 47.76% and Glob eIsfahan with 18.86% have the highest and the lowest pollen germination, respectively, and the highest and lowest pollen tube growths were for Sib Gol (153.62µm) and Golab e Isfahan cultivar (99.65 µm), respectively (table 3).

Table 3- the mean comparison between various cultivars effects on pollen germination and tube growth percentages.

Cultivar	pollen germination(%)	pollen tube growth (µm)
Mahali Sheikhi	47.76a	109.26bc
Sheikh Amiri	23.78c	111.47abc
Golden Delicious	45.29a	142.76ab
Red Deliciouse	36.18b	142.81ab
Malakeh	25.39c	121.87abc
Golab e Isfahan	18.86c	99.65c
SibGol	33.96b	153.62a

Abc means with similar letters had no significant difference by Duncan test (P<0.01).

The comparison between sucrose various concentrations on pollen germination and pollen tube growth showed that the most pollen germination (71%) was in sucrose15% and the least (0, 10.69)% was obtained for witness and 5% sucrose concentration respectively . The highest growth of pollen tube in sucrose 15% was 180.72 µm and the lowest growth in sucrose 0% was 1% (Table4).

Table4: the mean comparison between different levels of sucrose effects on pollen germination and tube growth percentage.

Different levels of sucrose	Pollen germination (%)	Pollen tube growth(µm)
0%	1.00d	0.00c
5%	10.69c	145.58b
10%	49.68b	178.92a
15%	71.00a	180.72a

abc Means with similar letters had no significant difference by Duncan test (P<0.01)

The comparison between sucrose various levels effects on pollen germination percentage have shown that the highest percentage of germination in Mahali Sheikhi cultivar in 15% concentration was 90.99% and the lowest germination percentage in Malakeh and Golab e Isfahan cultivars were 0% and (5.66 % and 4.00%) in 0% and 5% sucrose, respectively. (figure 4)

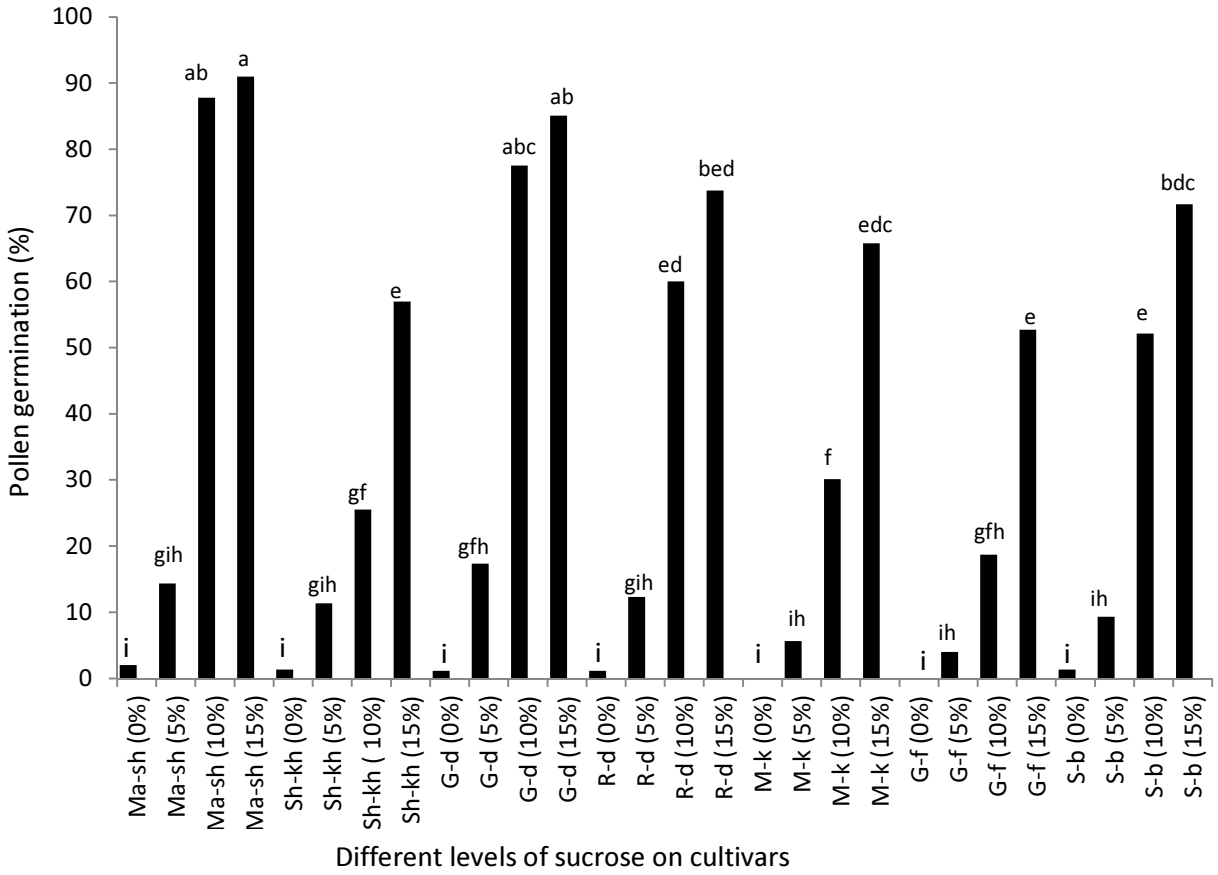

Figure3: Different levels of sucrose on pollen germination in apple cultivars

In Bloat I, Pirlak L (1999) study, significant difference was seen in the some stone fruits in different sucrose concentrations on pollen germination. The highest pollen germination percentage for apricot, cherry, and sour cherry was obtained in solid medium sucrose 15% concentration. Petrisor, et al (2012),study result on pollen germination

and pollen tube growth of apple 7 cultivars in in vitro condition by combination of 15% sucrose and 10 ppm acid Boric and 1.5% agar showed significant difference among cultivars which is in accordinate with our results. Imani et al(2011), showed in a research that mediume culture containing sucrose 15% is more effective than containing sucrose 10%.The results of investigation pollen viability and germination of 8 in apricot cultivars showed, these cultivars have the highest germination in 20 °C in sucrose 15%.(Asma 2008) .

Chauhan et al., 2008: study on some apple cultivars showed that all cultivars in sucrose 10% have the most percentage than 15% and 20%; in many cultivars sucrose concentration higher than 10% lead to reduce germination that is not in agreement with our results which reason is using more combination of nutrient in pollen culture medium.

The Pollen some apple cultivars germinated in sucrose 0% and by increasing sucrose concentration to 15%, germination increases too, but pollens which tubes growth was more than their pollen diameter were counted (Ahmadi 1999). Study results on pollen germination of apple and pear and significant difference between germination and pollen tube in solid culture medium have shown that among cultivars, pollen germination amplitude was between 50.2% and 96% and pollen tube growth was between 181.3µm to 721.2µm (Sharafi., 2011).Pollen germination and tube growth rate are the most important characteristics related to pollen quality, and successful fertilization needs high germination rates and fast tube growth because, low rates may lead to low fruit set caused by ovul (Cheung, 1996; Sharafi et al., 2011). In this research, the means of pollen apple cultivars germination in sucrose 15% was between 52.72% and 90.99% which shows high viability of pollens, and no cultivar has been identified to have male sterility which zero pollen germination (Saghali et al., 2013) and the highest means of pollen growth was 180.72 µm, that the reason of low pollen tube growth than the other researches like germination and pollen tube growth is dependent on factors such as medium culture combination, genotype, temperature, humidity, grains nutrition conditions and varieties, pollen gathering time, drying technics and maintaining pollens (Ahmadi et al., 2001).In this research, cultivars with high pollen germination percentage had not shown high pollen tube length, This phenomenon indicates genetically differences among the studied pollen apple cultivars which have been reported by some researchers in many fruit tree cultivars (Stoser et al., 1996, Pirlak and Polat, 1999; Viesser and Oost, 1981;sharafi et al;2013).

In present study the pollen tube growth in some medium cultures higher than the other, probably, because of the existence of nutrition combination or osmotic pressure that have mostly happened in medium culture containing sucrose, but statistically there was no difference between cultivars and sucrose concentrations. Totally, pollen apple cultivars germination rate in vitro was different, in additional cultivar, the factors such as type, component and temperature of medium culture can be referred.

Conclusion

In this study, the method of pollen culture has higher variability percentage than stain test, contrary the stain test is faster in determine pollen viability and using safranine in stain test shows better result than IKI in exploring viability of apple. Sucrose 0% has the least percentage of germination, and shows the necessity of sucrose existence in pollen apple culture for germination and tube growth. By increasing sucrose concentration germination percentage and pollen tube growth are increased so that the medium culture containing sucrose 15% has higher germination and pollen tube growth than sucrose with low concentrations. Therefore, in laboratory it seems that the medium cultures containing sucrose, influence more on germination and pollen tube growth. Various cultivars have different germination and pollen tube growth," Mahali Sheikhi" cultivar with the highest viability, germination and pollen tube growth could be selected as suitable pollinator cultivar if compatibility with receiver . As many studies have been done on pollen viability of many cultivars of fruit trees, but this research has been done first on these 7 cultivars in climate conditional of Shirvan. It is hoped this method is used in inbreeding program and investigation pollen apple viability cultivars that storage for a longtime, until pollination.

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