

Full Length Research Paper

# Impact of Storage Conditions and Cultivar Genotype on *Vitis vinifera* Pollen Performance

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The grapevines (*Vitis vinifera*, L.) are cultured widely in Maragheh and are made one of the most important commercial fruit crops of its regions. Most of the cultivars are growing with their fruits or are used in rasine, juice and fresh fruits forms. However, pollination is the main factor affecting fruit set and knowledge about pollen traits of cultivars is necessary to plan the vineyards establishment and breeding programs. This study was accomplished to investigate pollen traits of 15 main grape cultivars ('Fakhri', 'Hosaini', 'Khalili', 'Keshmeshi' ('Sefide bidaneh'), 'Lale bidaneh', 'Garmian', 'Gizil uzum', 'Sahebi', 'Jigh jigha', 'Shahani', 'Soltanin', 'Rish baba', 'Syahe malahi', 'Razeghi' ('Peikami') and 'Tabarzeh') which are grown in Maragheh. Pollens of cultivars gathered and cultured in the *in vitro* medium contained sucrose, boric acid and agar. Pollen germination percentage and tube growth were studied by light microscope. Experiment was carried out based on completely randomized design was (CRD) and data were analyzed with SAS software. Pollen germination percentage and tube growth rate showed significant differences among all of the studied cultivars. Finally, cultivars 'Fakhri', 'Khalili', 'Keshmeshi' 'Lale bidane' and 'Rish baba' showed the highest range of pollen germination, tube growth rate, longevity and selected for vineyards establishment and breeding programs.

**Key words:** Grapevine, vineyards, pollen germination, pollen tube growth, pollen longevity, breeding programs

## INTRODUCTION

*Vitis vinifera* L. is a widespread species that belongs to Vitaceae family and has main edible cultivars with many various usages (Anonymous, 1997). Pollination and fertilization are the basic factors affecting fruit setting volume and the most important goal of fruit growers is obtaining high quantity and quality yield in fruit industry which depend on sufficient fruit setting. Therefore, knowledge about pollen traits of the species and cultivars is one of the main issues for growers and breeders (Kozma et al., 2003; Szabo, 2003). For successful pollination, the high quantities and qualities of pollen must be transferred to the stigma when it is receptive

(Taylor, 1997; Wang et al., 1993). However, sometimes, the pollen is deposited before the receptive period of stigma and the pollen should remain viable for a period long enough to germinate although some of *V. vinifera* L., species cultivars set parthenocarpic or stenospermocarpic fruits genetically or occasionally based on specific physiological-environmental conditions (Kelen and Demitas, 2003; Stosser et al., 1996).

Furthermore, in breeding programs, breeders sometimes should maintain pollens for applying in the controlled artificial pollination methods whereas pollens should protect their viability and germination capacity. Many researchers have been performed to determine quantitatively and qualitatively the components necessary for the best composition of culture medium in pollen grain germination and the best storage conditions for different species pollens (Dane et al., 2004; Eti, 1991; Eti et al.,

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1998; Liu and Zhu, 1985; Sharafi, 2011).

Moreover, temperature is a very basic factor in the control of the environmental conditions and influences pollen grain germination and longevity in stored pollens (Kelen et al., 1996; Odabas, 1976; Vasilakakis and Porlingis, 1985). Based on these, it could be stated that, pollen traits especially germination percentage and tube growth in stored pollens should be carried out for confidence, their viability and longevity in different researches and horticultural exercises. Previously in different species, many cultivars and genotypes with unfavorable pollens such as sterile pollens, pollens with low germination percentage or low tube growth rate (versus to cultivars with high pollen germination and tube growth) have been reported by breeders and researchers (Kelen and Demitas, 2003; Maghradiz et al., 2009a; Sharafi and Bahmani, 2010; Shivanna, 2003; Stosser, 1996; Szabo, 2003).

Several researchers previously have studied the pollen viability of some tree fruit species in different storage conditions such as liquid nitrogen (-196°C), refrigerator (+4°C), freezer (different minus temperatures) and freeze dried, organic solvents, (Anjum and Shaukat, 2008; Alburque et al., 2007; Jain and Shivanna, 1988; Hedhly et al., 2005; Mert, 2009; Parfitt and Almehdia, 1984; Sharafi and Bahmani., 2010; Shivanna, 2003).

Except for some parthenocarpic fruit cultivars, pollination and fertilization are certainly necessary for fruit set and except for some special conditions, a linear relation between pollen viability and germination capability in many fruit species have been reported (Chkhartishvili et al., 2006; Sharafi et al., 2010, 2011; Sutyemez and Kelen, 1996; Voullamoz et al., 2006; Wang et al., 1993). Germination capability of pollen is related to cultivars, nutrition conditions, and environmental factors (Polat and Pirlak, 1999; Dafni and Firmago, 2000; Kelen and Demitas, 2003). There is a big variation in optimum germination conditions of pollen among plant species and cultivars (Kelen and Demitas, 2003). Pollen viability levels, environmental conditions, and compatibility among cultivars are important for the normal fruit set in all of the fruit trees and grapes (Dantas et al., 2005; Kelen and Demitas, 2003). Different nutrition conditions and germination methods for many plant species and varieties were used by researchers (Abreu et al., 2006; Kelen and Demitas, 2003).

Carreño et al. (2010) studied the influence of some compounds and temperature on pollen germination capability in some table grape cultivars and reported that optimum temperature for pollen germination depend on the genotype, but in general ranged 25 to 30°C. Also, they resulted that pollen stored at -80°C maintains a good germination capability.

In this research, pollen germination, tube growth and

longevity were studied in some cultivars of grapevine after two weeks maintenance in 0°C, using *in vitro* medium containing 15% sucrose, 50 ppm acid boric and 1% agar.

## MATERIALS AND METHODS

### Plant materials and research area

Totally fifteen cultivars of *V. vinifera* L. which are grown in different regions of Maragheh town of East Azarbaijan, Iran were selected including 'Fakhri', 'Hosaini', 'Khalili', 'Keshmeshi' ('Sefide bidaneh'), 'Lale bidaneh', 'Garmian', 'Gizil uzum', 'Sahebi', 'Jigh jigha', 'Sahani', 'Soltanin', 'Rish baba', 'Syah malahi', 'Razeghi' and 'Tabarzeh'. Research was carried out in department of Horticultural Sciences, Islamic Azad University of Maragheh, Iran.

### Pollen collection and germination test

In May 2009, in determining the pollen germination and pollen tube growth rate of cultivars, well-grown flower clusters from each cultivar were picked in the full flowering time. They were put in paper bags and were transferred to the laboratory. Petals and sepals were separated and anthers placed in Petri dishes for releasing pollens. Pollens gathered and their germination and tube growth rate were tested immediately and then, stored two weeks in 0°C. Pollens were planted *in vitro* medium containing 1% agar, 15% sucrose and 50 ppm boric acid and incubated in 25°C about 24 h and then tube growth was stopped with adding chlorophorm.

Seven microscopic areas were counted randomly for evaluation of pollen germination and tube growth in each Petri dish. Pollen tube long at least as its diameter was considered to be germinated and measurements of pollen tube length were recorded directly by an ocular micrometer fitted to the eyepiece on microscope based on micrometer scale ( $\mu\text{m}$ ).

### Experimental design and data analysis

The experiment was carried out as in completely randomized design (CRD) with fifteen treatment (each cultivar) and five replications (5 Petri dishes for each cultivar). Data were analyzed using SAS software and comparison of means was carried out with Duncan's multiple range tests.

## RESULTS AND DISCUSSION

Analysis of variances in Table 1 indicated significant differences among fifteen studied cultivars of *V. vinifera* L. in pollen germination percentage and pollen tube growth rate after two weeks storage in 0°C. Among cultivars, means of pollen germination percentage and pollen tube length were ranged between 23.6 to 83.1% and 86.2 to 538.2  $\mu\text{m}$ , respectively. It should be stated that, the means of pollen germination percentage of all cultivars were higher than 80% immediately after gathering in laboratory (data not shown). Difference in the

**Table 1.** Variance analysis of pollen germination percentage and pollen tube length (based on micrometer) in fifteen cultivars of grapevine in Maragheh.

Source of variation	DF	Pollen germination percentage (%)	Pollen tube length (µm)
Cultivars	14	1056.3**	3702.1**
Experimental error	60	98.8	463.2
Coefficient Value (%)		17.8	16.4

\*\* : Significant in P < 0.01% level.

**Table 2.** Comparison of means for pollen germination percentage and pollen tube length (based on micrometer) in fifteen cultivars of grapevine in Maragheh.

Cultivar	Pollen germination percentage (%)	Pollen tube length (µm)
'Fakhri'	83.1 <sup>a</sup>	443.7 <sup>ab</sup>
'Hosaini'	59.3 <sup>cd</sup>	538.2 <sup>a</sup>
'Khalili'	67.2 <sup>bc</sup>	318.1 <sup>c</sup>
'Keshmeshi'	74 <sup>ab</sup>	175.2 <sup>e</sup>
'Lale bidaneh'	71.2 <sup>b</sup>	90.3 <sup>f</sup>
'Garmian'	23.6 <sup>f</sup>	185.9 <sup>df</sup>
'Gizil uzum'	27.8 <sup>ef</sup>	122.3 <sup>df</sup>
'Sahebi'	35.6 <sup>def</sup>	215.1 <sup>d</sup>
'Jigh jigha'	47.2 <sup>ecd</sup>	319.4 <sup>bc</sup>
'Shahani'	57.1 <sup>c</sup>	229 <sup>d</sup>
'Soltanin'	46.5 <sup>cd</sup>	421.3 <sup>b</sup>
'Rish baba'	62.1 <sup>d</sup>	115.8 <sup>df</sup>
'Syahe malahi'	58.4 <sup>cd</sup>	208.3 <sup>d</sup>
'Razeghi'	34 <sup>def</sup>	215.1 <sup>d</sup>
'Tabarzeh'	33.7 <sup>def</sup>	86.2 <sup>f</sup>

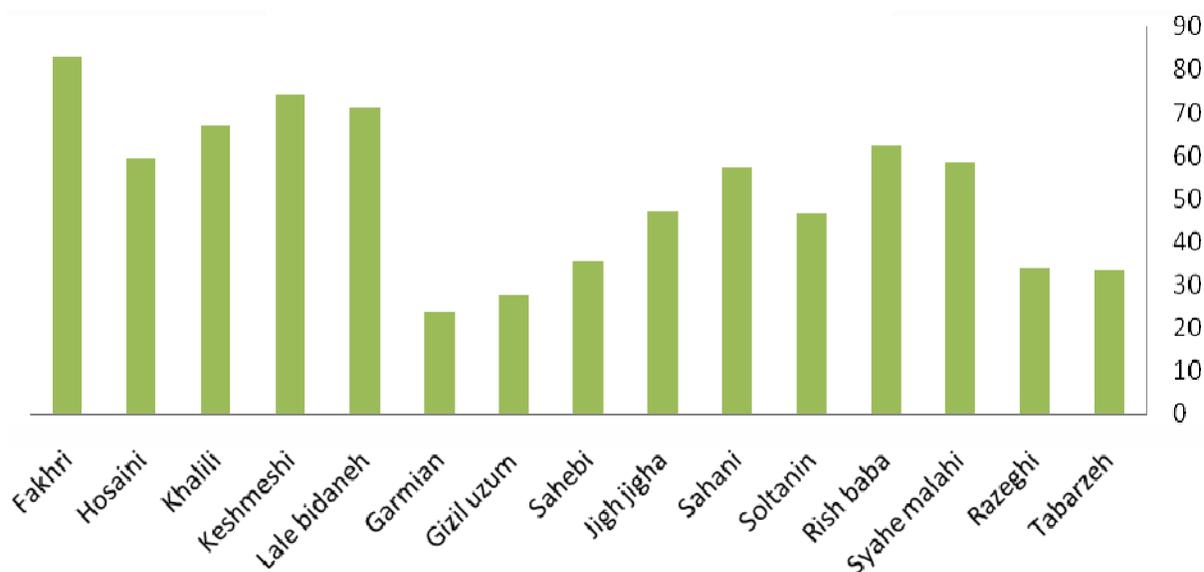
Same letters show no difference among cultivars in each column.

means of pollen germination percentage and pollen tube length showed higher variety in tube length in compared with germination percentage (Table 2, Figures 1 and 2). Based on data which are shown in Table 2, maximum pollen germination was observed in 'Fakhri' cultivar (83.1%) while minimum was observed in 'Garmian' cultivar (23.6%), respectively. Maximum pollen tube length was observed in 'Hosaini' cultivar (538.2 µm) while minimum was observed in 'Tabarzeh' cultivar (86.2 µm), respectively. However, high pollen germination percentage of these cultivars after two weeks maintenance in 0°C showed their extensive longevity, and they could be selected for vineyard establishment and breeding programs as a pollinizer for pollination of other cultivars.

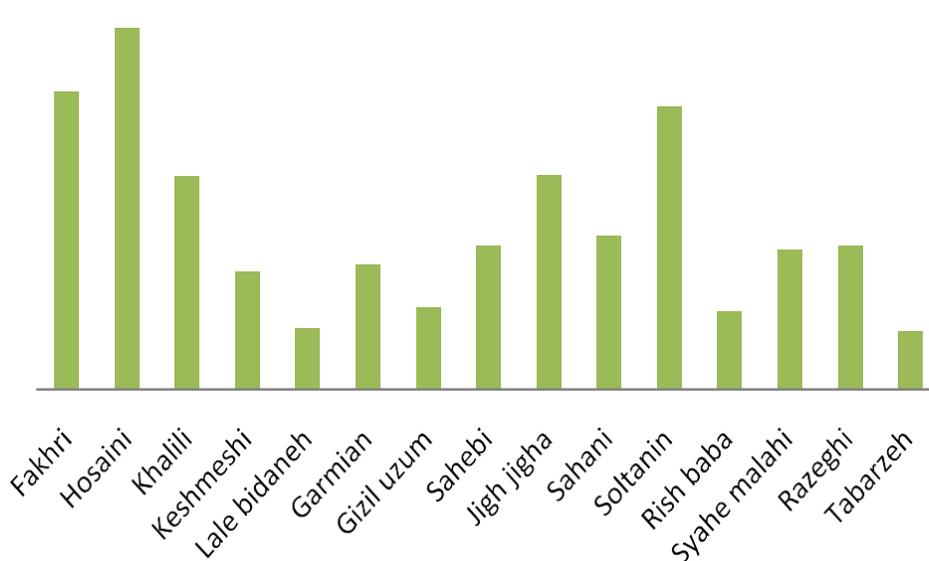
Pollen germination and tube growth rate are the most important characteristics related to pollen quality and successful fertilization leads to high germination rates and fast tube growth because low rates may lead to low

fruit set caused by ovule degradation before the pollen tube reaches to the ovary (Sharafi et al., 2010, 2011). According to report of Sharafi and Bahmani (2011), in some species of genus *prunus*, in this research, cultivars with high pollen germination was not followed by high pollen tube growth. This phenomenon indicates genetically differences among the cultivars which was reported by many researchers in numerous of the fruit tree species and cultivars (Albuquerque et al., 2007; Polat and Pirlak, 1999; Sharafi et al., 2010; Stosser et al., 1996).

Carreño et al. (2010) studied the influence of some compounds on pollen germination capability *in vitro* and its preservation in some table grape cultivars such as 'Sugraone', 'Mistery', 'Autumn Royal', and 'Crimson Seedless'. Also, they studied the influence of temperature on pollen germination. Their results showed that the best medium for pollen germination contains 20% sucrose + 100 mg/L Boric Acid + 300 mg/L calcium nitrate.



**Figure 1.** Comparison of means of pollen germination percentage in fifteen cultivars of grapevine in Maragheh.



**Figure 2.** Comparison of means of pollen tube length (based on micrometer) in fifteen cultivars of grapevine in Maragheh.

Optimum temperature for pollen germination depended on the genotype, but in general ranged 25 to 30°C. They also studied the decay of germination capability in dry pollen stored at temperatures of -20, -40 and -80°C. The results showed that pollen stored at -80°C maintains a good germination during the same season.

Abreu et al. (2006) investigated the causes of low

productivity of *V. vinifera* cv. 'Loureiro' pollen by studying pollen viability using fluorochromatic reaction and pollen germination ability by *in vitro* assays and reported that the acopolated pollen grains of mentioned cultivar are viable, but no germination was recorded.

Sharafi (2011) and Sharafi and Bahmani (2010), investigated the pollen germination percentage, longevity

and pollen tube growth rate after different short storage times in low temperatures in some almond, apricot, hawthorn, loquat, peach, plume, prune, sour cherry and sweet cherry genotypes and reported similar results which observed in this works.

Sometimes, cultivars produce high quantity of pollens but not with high quality such as low pollen germination percentage or low tube growth also, some of the pollens may be sterile or not viable (Sharafi et al., 2010, 2011).

Anjum and Shaukat (2008), with studding pollen germination of *Malus pumila*, beyond 48 weeks in the refrigerator (+4°C), freezer (-20°C, -30°C) and freeze drier (-60°C) in different concentration of sucrose and boric acid solution, which resulted to pollens that were stored at low temperature had higher germination percentage when compared with pollens stored at +4°C. Also, in fresh pollen, freeze dried pollen (-60°C) showed the highest germination percentage.

## Conclusion

Finally, it was concluded that pollen germination and pollen tube growth rate were standard in all fifteen cultivars of *V. vinifera* L., after two weeks storage in 0°C although, some decrease was observed in some of them. Five cultivars including 'Fakhri', 'Khalili', 'Keshmeshi' 'Lale bidane' and 'Rish baba' showed the highest range of pollen germination, tube growth rate and longevity among fifteen cultivars and cultivars with high pollen germination have not shown high pollen tube growth necessarily. However, mentioned cultivars with high pollen germination percentages and high pollen tube growth rate were selected for vineyards establishment and breeding programs.

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