AcademicPres

Available online: www.notulaebiologicae.ro

Print ISSN 2067-3205; Electronic 2067-3264

Not Sci Biol, 2014, 6(2):158-162

Notulae Scientia Biologicae

In vitro Pollen Grain Germination and Tube Growth of Ten Iranian Jujube (*Ziziphus jujuba*) Cultivars

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Abstract

Jujube (*Ziziphus jujuba*) is a species particularly prone to erratic fruit set. Several causes such as pollen germination and tube growth involve in this behaviour. Thus, the present research aimed to evaluate and compare of pollen grains germination and tube growth of ten Iranian jujube cultivars on *in vitro* condition. A factorial trial based on completely randomized design with five replications was carried out. The pollen grains were collected from un-opened-flowers of 'Al-Ghour', 'Bardaskan', 'Doroh', 'Gazik', 'Ghom', 'Giouk', 'Kangan', 'Kalkestan', 'Magham Sari' and 'Noghab' cultivars at balloon stage. This study showed that there were significant differences among the cultivars in all measured factors. The highest percentage of pollen grains germination was observed for 'Gazik', followed by 'Bardaskan', while the lowest was in 'Al-Ghour'. The percentage of pollen grains germination increased with the increasing time of incubation period up to 36 h. Also, flowering times are found to be effective in germination of pollen grains, which 1 flash had highest percentage of germination of pollen grains than in comparison to other flowering times. The results also showed that the mean time germination varied from 24.52-25.92 h, mean hourly germination from 0.67-1.55, hourly germination speed from 0.64-1.48 and coefficient of velocity of germination of pollen grains from 0.058-0.061. The length of pollen tube was found between 44.4 and 624.8 µm. These data demonstrated that cultivar is the main factor determining pollen grains germination and tube growth in jujube, which this important information can be useful for selection of superior desirable jujube genotypes for bringing to commercial cultivation.

Keywords: incubation period, flowering time, properties *Ziziphus jujuba*

Introduction

Ziziphus jujuba M., named jujube, belongs to the Rhamanaceae family. This genus includes about 40 species of spiny shrubs and small trees, distributed in tropical and sub-tropical regions throughout the world (Soliman *et al*., 2013), such as Iran.

Besides, the high amounts of vitamin A, C and B complexes and minerals in *Ziziphus delicious* fruits (Pareek, 2004), alkaloids, flavonoids, sterols, tannins, and fatty acids have been isolated from its different species (Croueour *et al*., 2002; Abdel-Zaher *et al*., 2005; Bhargava *et al*., 2005; Kakani *et al*., 2005). On the other hand, existence the high levels of antioxidant activity, reducing power, and scavenging effect on free radicals (Li *et al.,* 2005) have been led to be used in folk medicine for the treatment of some diseases in the world (Belford, 1994; Crouėour *et al*., 2002; Abdel-Zaher *et al*., 2005; Li *et al*., 2005).

Jujube is talented to produce erratic fruits which lead to lower yield (Wang *et al*., 2009). There are various reasons for erratic fruit production in jujube including: a) Strong incompatibility, which results in problems such as reduction or irregularity in fruit production and decrease in yield owing to absence or inadequate compatible pollen grain; b) Cytoplasmic male sterility; unviable pollen grain percentage varied in Chinese jujube cultivars (Mengjun, 2003). It was reported some cultivars had completely unviable pollen grain.

Generally, *in vitro* pollen germination mean of jujube cultivars is variable. Various factors effect on pollen germination such as type and amount of media components and environmental temperature has been reported by Soliman *et al*. (2013). In jujube, it was also reported that pollen germination and tube growth extremely enhanced with increasing sucrose concentration up to 10% and then reduced in higher concentrations for some cultivars (Mengjun, 2003). Addition of 35 ppm boric acid to

medium increased pollen tube growth (Tangmicharoen and Owens, 1997). Dane *et al*. (2004) stated the highest pollen germination (39%) was observed in medium containing 1% agar and 15% sucrose at 25-30 °C for 24 hours.

Jujube is a traditional medicinal plant all over the world, thus successful pollination and fruit set which lead to higher yield, is of importance. In the other hand, Iran is one of the germplasm pools of this medicinal fruit. Therefore, studying the pollen germination and tube growth of Iranian seems necessary. The present research aimed to evaluate the variation of cultivars and flower time affecting pollen grains germination and tube growth. Finally, selection of suitable cultivars with high potential of pollen grains production among important Iranian Jujube cultivars has been carried out.

Materials and methods

Plant samples

Ten jujube cultivars were studied: 'Al-Ghour' (A), 'Bardaskan' (B), 'Doroh' (D), 'Gazik' (G), 'Ghom' (Gh), 'Giouk' (Gi), 'Kangan' (K), 'Kalkestan' (Ka), 'Magham Sari' (M) and 'Noghab' (N). Mature trees randomly selected to represent the population of the plantation in the jujube collection orchard, Agricultural Research Center of Birjand (South Khorasan province, Iran). Birjand is located on the south east part of Iran at 32° 52' 26" N latitude and 59° 12' 51" E longitude. The average temperature, the amount of rainfall and relative humidity in growing season of 2011 were 28.65°C, 20 mm and 28%, respectively. Soil characteristics were texture being sandy-loam, EC = 4.12 (ds/m) and soil pH = 7.21. The trees were spaced 7 and 5 m between and along the rows, respectively. Trees were grown under traditional irrigation and routine cultural practices suitable for commercial fruit production. All cultivars were grown under the same geographical conditions and with the same applied agronomic practices.

In jujube, the flowering period was between late May and late July. The flowering occurs in three distinct waves (namely; 1, 2 and 3 flash). Freshly flowers (not opened) were collected randomly from the different cultivars in 1 (22 May), 2 (22 June) and 3 flash (22 July) at the balloon stage. These times were chosen to find the effects of flowering time on pollen grain germination and tube growth. The flowers were transferred to the laboratory immediately. The Anthers were removed and placed among silica pellets in a glass jar and left to dry for 2-3 days at room temperature (20-25 °C), which allowed the anthers to dehisce. The pollen grains were kept into silica pellets in glass jars fitted with air-tight caps at 3-5 °C until analysis.

Pollen grain germination and tube growth

The pollen grains sowed with a clean brush in Petri dishes (5 cm diameter) containing medium (1% agar, 15% sucrose and 0.01% boric acid) according to the method of Dane *et al*. (2004). The Petri dishes were incubated at the constant temperature of 25°C under usual light conditions (Davarynejad *et al*., 2008).

The effect of flowering time (1, 2 and 3 flash) and incubation period (12, 24 and 36 h) on germination of pollen grains were evaluated. The pollen grains were recorded as germinated when the length of pollen tube was equal to or longer than the pollen diameter (Kakani *et al*., 2002). The percentage of pollen grains was calculated as follows: Germination percentage (%) = [A/B] × 100, where A: number of germinated pollen grains per Petri dish and B: total number of pollen grains per Petri dish.

Mean time germination (MTG), mean hourly germination (MHG), hourly germination speed (HGS) and coefficient of velocity of germination (CVG) of pollen grains for 1 flash (flowering at 22 May) measured by 1-4 formula:

1. $MTG=\frac{(12\times G_{12})+(24\times G_{24})+(36\times G_{36})}{G_{36}}$ (Ellis and Robert, 1981)
2. $MHG=\frac{G_{36}}{36}$ (Scott, 1984)
3. $HGS=\frac{1}{MHG}$ (Huntr, 1984)
4. $CVG=\frac{G_{12}+G_{24}+G_{36}}{(12\times G_{12})+(24\times G_{24})+(36\times G_{36})}$ (Maguire, 1962)

G12, G24 and G36 indicate the number of germinated pollen grains in 12, 24 and 36 hours after incubation period, respectively.

For growth of pollen tube, samples were prepared as described above, and the 6 slides of samples were observed using a Zeiss microscope, when their elongation was greater than the pollen diameter (Davarynejad *et al*., 2008). The length of pollen tube was recorded in 24 h after incubation period for 1 flash (flowering at 22 May).

Statistical analysis

This experiment was conducted according to factorial based on completely randomized design with 5 replications. Data were analyzed by statistical analysis system (SAS) software version 9.1 using analysis of variance (ANOVA) and differences among means were determined for significance at $P < 0.05$ using Tukey's test.

Results and discussion

Germination of pollen grain

The effects of cultivar and incubation period on pollen grains germination of jujube cultivars are described in Fig. 1. Significant differences were revealed among the different cultivars, incubation periods and their interactions for germination of pollen grains. The highest percentage of pollen grains germination was observed for 'Gazik' (with mean of 53%), followed by 'Bardaskan' (with mean of 47.4%), while the lowest was in 'Al-Ghour' (with mean of 21%). Among the studied incubation period, 36 h (with mean of 43.3%) had the highest percentage of pollen grains and 12 h (with mean of 34.56%) had the lowest percentage of pollen grains

The influences of cultivar and flowering time on pollen grains germination of jujube cultivars are given in Fig. 2. According to results, significant differences were revealed among the different cultivars, flowering times and their interactions for germination of pollen grains. The germination of pollen grains was affected by cultivar, since the highest percentage of pollen grains germination was detected in 'Gazik' (with mean of 31%), while the lowest

was observed in 'Al-Ghour' (with mean of 13.66%). The highest percentage of pollen grains germination was observed for 1 flash (with mean of 41.06%), followed by 2 flash (with mean of 23.06%), while the lowest was in 3 flash (with mean of 7.4%).

As shown in Tab. 1, significant differences were revealed among the jujube cultivars for mean germination time, hourly germination, mean hourly germination speed, coefficient of velocity of germination of pollen grains from ten jujube cultivars. The results also showed that the mean time germination (MTG) varied from 24.52 ('Gazik') - 25.92 ('Al-Ghour'), mean hourly germination (MHG) from 0.67 ('Al-Ghour') -1.55 ('Gazik'), hourly germination speed (HGS) from 0.64 ('Gazik')-1.48 ('Al-Ghour') and coefficient of velocity (CVG) of germination of pollen grains from 0.058 ('Al-Ghour')-0.061 ('Gazik') (Tab. 2).

Previous studies have also reported variable ranges of pollen grains germination among different cultivars of apricot, sweet cherry, sour cherry (Bolat and Pirlak, 1999), sour cherry (Tosun and Koyuncu, 2007; Davarynejad *et al.,* 2008), pistachio (Acar and Kakani, 2010), and almond (Sorkheh *et al.,* 2011). Our results are in general agreement with these studies. With regards to the results, the jujube cultivars were easily separated into three groups according to their percentage of pollen grains germination: high ('Bardaskan', 'Gazik', 'Kangan' and 'Magham Sari'), medium ('Doroh', 'Kalkestan', 'Ghom' and 'Noghab') and low ('Al-Ghour' and 'Giouk'). These results indicated that the pollen grains germination varied among different cultivars of jujube. Thus, cultivar type plays an important role in terms of pollen grains germination.

Growth of pollen tube

When pollen grains were incubated in a germination medium for 24 h in darkness, a great variation in terms of length of pollen tube was observed among the jujube cultivars and the differences were statistically significant (Tab. 1). The highest amount of pollen tube length was observed in 'Gazik', followed by 'Bardaskan' and 'Kangan', while the lowest was in 'Al-Ghour' (Fig. 3).

Bolat and Pirlak (1997) reported that the pollen tube lengths ranged from 198 to 357 μm, 209 to 335 μm and 174 to 188 μm in apricot, sweet cherry and sour cherry, respectively. Also, Acar and Kakani (2010) indicated that the length of pollen tube varied significantly according to cultivar. Their results were in agreement with our results. The differences in length of pollen tube observed in the present study were a reflection of cultivar variability. Therefore, there was a high genetic heterogeneity within the studied cultivars.

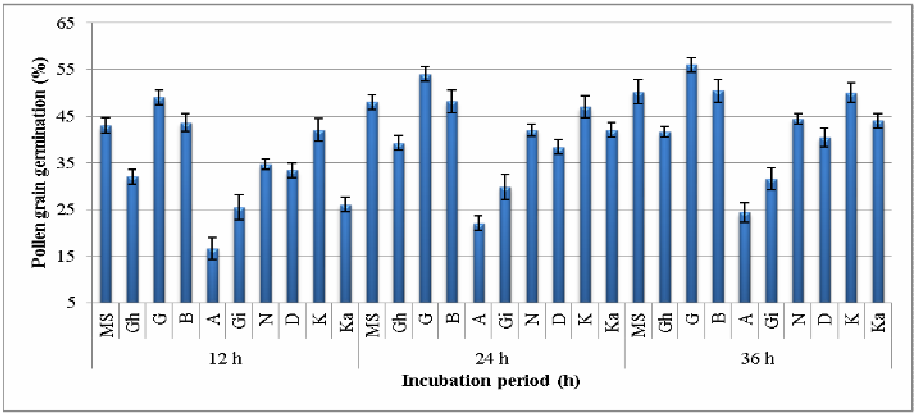

Fig. 1. Effect of cultivar and incubation period (h) on pollen grain germination (%) for 1 flash. Bars show standard error (SE). (MS-Magham Sari; Gh-Ghom; G-Gazik; B-Bardaskan; A-Al-Ghour; Gi-Giouk; N-Noghab; D-Doroh; K-Kangan; Ka-Kalkestan)

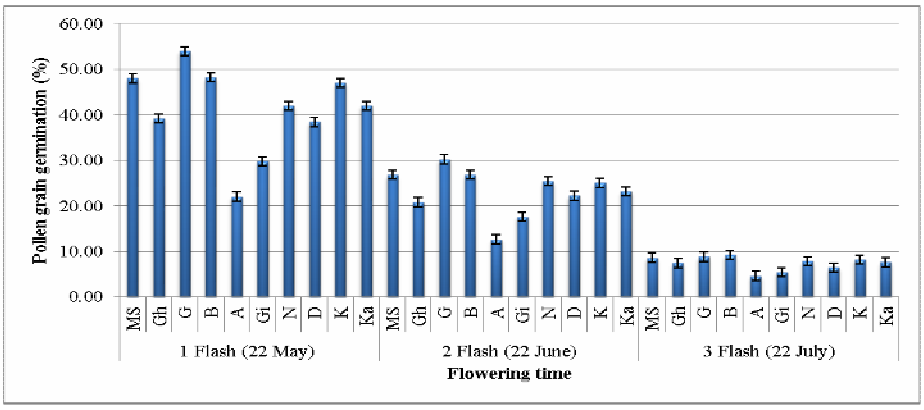

Fig. 2. Effect of cultivar and flowering time on pollen grain germination (%) after a 24 h incubation period. Bars show standard error (SE). (MS-Magham Sari; Gh-Ghom; G-Gazik; B-Bardaskan; A-Al-Ghour; Gi-Giouk; N-Noghab; D-Doroh; K-Kangan; Ka-Kalkestan)

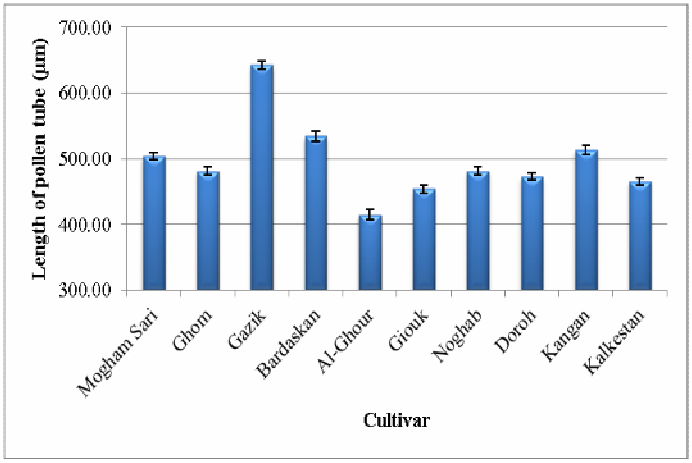

Fig. 3. Effect of cultivar on length of pollen tube (µm) after a 24 h incubation period for 1 flash. Bars show standard error (SE)

Table 1. ANOVA for dependent variables for cultivar in 1 flash

Source	Degrees of freedom (DF)	Parameters				
		MTG[a]	MHG	HGS	CVG	LPT
Cultivar	9	1.00**[b]	0.34**	0.32**	0.01**	18819.01*
Error	20	0.02	0.005	0.004	0.0002	15.6
Total	29					

[a] MGT, mean germination time, MHG, mean hourly germination, HGS, hourly germination speed; CVG, coefficient of velocity of germination; LPT; length of pollen tube. [b] Sum of squares, **: significant at 0.01 level, *: significant at 0.05 level.

Table 2. Mean germination time (MGT), mean hourly germination (MHG), hourly germination speed (HGS) and coefficient of velocity of germination (CVG) of pollen grains of ten Iranian jujube cultivars in 1 flash

Cultivars	Parameters			
	MGT (h)	MHG	HGS	CVG
Mogham Sari	24.61 e	1.39 b	0.71 de	0.0607 ab
Ghom	25.02 c	1.15 c	0.86 c	0.0599 bc
Gazik	24.52 e	1.55 a	0.64 e	0.0610 a
Bardaskan	24.57 e	1.39 b	0.72 de	0.0608 ab
Al-Ghour	25.92 a	0.67 e	1.48 a	0.0584 d
Giouk	24.85 cde	0.87 d	1.15 b	0.0601 ab
Noghab	24.97 cd	1.23 c	0.81 cd	0.0600 b
Doroh	24.75 cde	1.12 c	0.89 c	0.0604 ab
Kangan	24.69 de	1.38 b	0.72 de	0.0603 ab
Kalkestan	25.49 b	1.22 c	0.82 cd	0.0590 cd

Means in each column followed by different letters are not significantly different (P≤0.05).

Conclusion

Pollen grain performance (includes pollen grains germination and tube growth rate) is most important component in jujube tree fertilization success. In this research, pollen grains germination and tube growth of ten Iranian jujube cultivars on *in vitro* condition were investigated. These results demonstrated that the cultivar is the main factor determining the pollen grains germination and tube growth in jujubes.

Among the ten cultivars studied, 'Gazik', 'Bardaskan', 'Kangan' and 'Magham Sari' cultivars showed the highest amount of pollen grain germination and tube growth, which could be used as a good pollen donor for others cultivars. In addition, the results provide important information of pollen grains germination and tube growth in ten Iranian jujube cultivars, which can be useful for selection of superior desirable jujube genotypes for bringing to commercial cultivation.

Acknowledgements

The authors would like to acknowledge Ferdowsi University of Mashhad for the funding of this study.

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