J. King Saud Univ. Vol. 10, *Agric. Sci.* (1), pp. 73-81, (A.H. 1418/1998)

Pollen Viability, Germination and Rates of Pollen Tube Growth in Some Pomegranate Cultivars (*Punica granatum*, L.)

Mahmoud A. Ali, Mohamed A. Bacha and Farahat A. Farahat

Plant Production Department, College of Agriculture,
King Saud University, Riyadh, Saudi Arabia

(Received 26/8/1416; accepted for publication 20/8/1417)

Abstract. Pomegranate pollen grains obtained from five local cultivars (Balady red, Al-madina, Khob Al-jamil, Taeifi and Hamid abiod), two introduced cultivars from Spain (De Jativa and Molar) and four Egyptian cultivars (Banaty, Manfaluti, Mellasy and Succary) were evaluated for viability, germinability, shape and rates of pollen tube growth after 24, 48 and 72 hr. when cultured in sucrose media. The percentage of viable and germination of pollen and absolute pollen viability of De Jativa and Molar (Spanish cultivars) differed significantly from the other cultivars. This indicated genetic differences among the cultivars. The length/diameter ratio ranged from 1.01 to 1.05 in viable and from 1.11 to 1.84 in nonviable pollen grains. Pollen tubes length of Banaty, Hamid abiod and De Jativa cultivars grew more rapidly than those of other cultivars after 72 hr. when cultured in sucrose solution. Positive correlation was found between absolute pollen viability and pollen tube length among all tested cultivars, except in Hamid abiod, De Jativa and Succary.

Introduction

The pomegranate (*Punica granatum*, L.) is the second most important deciduous fruit tree (after grapes) in Saudi Arabia. Data on the viability of pollen grains may give breeders and growers a better chance for selection and exploitation of the genetic variabilities among cultivars, and for selecting improved pollinators.

Cruzan [1] found that the mean volume of pollen grains and total pollen production varied both within and between plants. Several researchers have evaluated the viability, germination and pollen tube growth of pollen grains in fruit trees [2, 3, 4]. Stott [5] reported that, in 32 cultivars of apple, there was good agreement between the

results of pollen viability assessed by sucrose hanging drop tests and by microscopic examination of germination on the stigma surface.

Although various stains have been used in studies of pollen viability, staining may not indicate true viability. Erlanson [6] reported that many apparently morphological perfect grains were unable to affect fertilization. On the other hand, Visser *et al* [7] called stainable pollen grains "normal pollen" but considered them as having only the potential to germinate.

The purpose of this study was to examine pollen grains for viability, germinability, pollen measurements and rate of pollen tube growth in 11 pomegranate cultivars with the aim of identifying the most common pollinators of pomegranate.

Materials and Methods

Pollen grains were collected from 11 pomegranate cultivars grown at the Experimental and Research Station in Dierab, College of Agriculture, King Saud University. The cultivars used were Balady red, Al-madina, Khob Al-jamil, Taeifi and hamid abiod (local cultivars), De Jativa and Molar (Spanish cultivars) and Banaty, Manfaluti, Mellasy and Succary (Egyptian cultivars). Pollen grains were collected from unopened flower buds, showing petal color and having reflexed sepals and placed in paper bags. In the laboratory, the mature unopened anthers were removed from the flowers and allowed to dry in a small plastic vials (5 g) at room temperature. When the anthers dehisced 2 to 3 days later, pollen grains were collected and stored in capped vials at 4°c over crystalline $CaCl_2$. Stainability of fresh pollen samples was determined by acetocarmine technique as described by Roberts [8]. Two hundered pollen grains were counted per slide, with 10 slides for each of the 11 cultivars. The pollen grains stained deeply and looking normal were counted as viable, while weakly stained were recorded as nonviable[9]. The percentage of viable and nonviable pollen grains were then determined. Germination of fresh pollen grains was assessed by sucrose hanging-drop culture with some modifications [7]. A drop of germination medium (15 % aqueous sucrose solution containing 200 mg H_3BO_4, 100 mg $Ca(NO_3)_2$, 100 mg $MgSO_4$, 100 mg KNO_3 and 50 mg EDTA) was placed on a coverslip and the pollen dusted onto the drop. The coverslip then was inverted and placed over a concave depression on a slide, using glycerol to seal the coverslip and prevent desiccation, then incubated for 24 hr. at 30° C. The 10 slides were scanned for each pollen cultivar. Germination counts and germination percentages were recorded. Pollen germination was considered to occur when a pollen tube was formed that was equal to or greater than the diameter of pollen grains [10]. Absolute pollen viability, or the effective germination capacity was calculated using the formula used by Visser *et al* [7, 11].

Absolute pollen viability = % stained pollen X % germinated pollen/100

Viable and nonviable pollen grains were examined under light microscope and length and diameter of grains were measured using an ocular micrometer. Shape of viable and nonviable pollen grains were calculated as length to diameter ratio.

Pollen tube growth in the 11 cultivars after 24, 48 and 72 hr. when cultured in hanging drop media were determined. One hundred pollen tubes were measured for length in each time for 11 cultivars under a microscopic equipped with an eyepiece micrometer.

Data was analyzed for variance using a completely randomized design. Least significant difference (LSD) was used to compare between means [12].

Results and Discussion

Viability and germination of pollen grains

Pollen viability measured by acetocarmine method in the 11 cultivars of pomegranate varied from 60 to 97%. Spanish cultivars (De Jativa and Molar) had 62.30 and 60.00 percent of poor pollen viability, respectively, and are thought possibly to have semisterile megaspores. Moreover, the Egyptian cultivars (Banaty, Manfaluti, Mellasy and Succary) had more than 90% viable pollen grains. The same trend was found in the local cultivars. Pollen grains viability ranged from 89.90 % in Al-madina cultivar to 96.65% in Khob El-jamil cultivar (Table 1). Analysis of variance indicated a highly significant difference for viability test by the use of acetocarmine technique among cultivars. The viable pollen percentage was significantly lower in the Spanish cultivars than that in the local and Egyptian cultivars. No significant differences were found between the local cultivars in one hand, and the Egyptian cultivars (Table 1).

Many investigators determined the pollen viability in some fruit trees species using the acetocarmine technique [13,14]. They found that, all the tested grape cultivars had more than 90% viable pollen grains. Very high variability in pollen viability was found by Randhawa and Ramakrishnan [15] in plum, Soost [16] in citrus, Mamedov [17], Sharma and Gaur [3] in pomegranate and Roberts [8] and Pearson and Harney [10] in rose.

The pollen germination percentage in the 11 cultivars of pomegranate using the sucrose hanging drop test, (Table 1) revealed that Banaty cultivar was found to have a high pollen germination among the 11 cultivars, followed by Khob El-jamil cultivar, while Hamid abiod, Al-madina, Molar and De Jativa cultivars recorded the lowest values. Moderate values (between 75 and 80 %) were recorded for Succary, Manfaluti, Balady red and Taeifi cultivars. The Spanish cultivars had the lowest values of pollen germination (Table 1), while the local and Egyptian cultivars except Banaty gave about equal values of pollen germination. Significant differences for the in vitro germination

pollen were found among the most different cultivars. The viability and germinability of pollen grains depended on the cultivar. This variation among the 11 cultivars, suggest the presence of genetical differences [18]. Many investigators found that the failure of germination of the pollen grains was related to the structure of the pollen exine [19,20,21].

Table 1. The viability[z], gremination[y] and absolute pollen viability[x] of pollen grains in 11 pomegranate cultivars[w]

Cultivars	Viable pollen %	Nonviable pollen %	Pollen germination %	Absolute pollen viability %
Balady red	91.57 bc ¶	8.43 bc	79.20 cd	72.52 cd
Al- Madina	89.90 c	10.10 b	72.28 fg	64.98 ef
Khob El jamil	96.65 a	3.35 de	85.16 b	82.31 b
Taeifi	93.26 abc	6.74 bcde	81.02 c	75.56 c
Hamid abiod	88.89 c	11.11 b	68.04 h	60.48 f
De Jativa	62.30 d	37.70 a	73.82 f	45.99 g
Molar	60.00 d	40.00 a	69.59 gh	41.75 g
Banaty	97.00 a	3.00 e	92.52 a	89.74 a
Manfaluti	95.51 ab	4.49 cde	76.95 de	73.49 c
Mellasy	90.22 c	9.78 b	74.65 ef	67.35 de
Succary	93.94 abc	6.06 bcde	76.63 de	71.99 cde

* Means of 10 replications, each containing 200 pollen grains.

¶ = Values have the same letters are not significantly different at 5% level.

[z]=Viable pollen percentage determined from counting acetocarmine stained pollen grains.

[y] = Pollen germination percentage determined by counts of germinated pollen grains in sucrose solution.

[x] =Absolute pollen viability = 100% stained X Germinated pollen/100

[w]=Means seperated by L.S.D. test.

In the 11 cultivars of pomegranate, a good agreement was found in the data between pollen viability assessed by acetocarmine technique and with pollen germination assessed by sucrose solution. These data confirm data reported by Stott [5] on apple cultivars and Soliman *et al* [4] on date palm. In the present research, the percentage of pomegranate pollen grains that germinated was lower than the percentage of viable pollen assessed by acetocarmine technique. Similar results were obtained by Pearson and Harney [10] on roses.

Germinating pollen grains in sucrose solution was found to be better indicator of viability than staining with acetocarmine because many morphological normal pollen grains lacked the ability to germinate (Table 1). The absolute pollen viability percentage in the 11 cultivars ranged from 41.75 to 89.74 %. De Jativa and Molar cultivars (Spanish cultivars) had the lowest absolute pollen viability of the cultivars tested. Banaty and Khob El-jamil cultivars had the highest absolute pollen viability percentage. These results are in agreement with those obtained by Pearson and Harney [10].

Length, diameter and shape of pollen grains

Mean length, diameter and lenght to diameter ratio for viable and nonviable pollen grains in the different pomegranate cultivars is presented in Table 2. Mean length of viable pollen grains varied from 2.02 to 2.90 microns, while the diameter ranged from 2.00 to 2.75 microns. The length and diameter of the nonviable pollen grains differed from one cultivar to another and showed significantly higher length in Manfaluti and Hamid abiod than that of other cultivars.

Table 2. Length, diameter and length to diameter ratio [z] of viable and nonviable pollen grains in 11 pomegranate cultivars[y]

Cultivars	Viable pollen			Nonviable pollen		
	Length (L) μ	Diameter (D) μ	L/D ratio	Length (L) μ	Diameter (D) μ	L/D ratio
Balady red	2.19 fg ¶	2.16 ef	1.01 e	1.92 g	1.39 cd	1.38 cd
Al- Madina	2.29 e	2.19 de	1.05 a	1.98 ef	1.51 b	1.31 e
Khob El jamil	2.26 ef	2.17 ef	1.04 b	1.94 fg	1.47 bc	1.32 de
Taeifi	2.15 g	2.09 f	1.03 c	1.92 g	1.28 e	1.50 b
Hamid abiod	2.90 a	2.75 a	1.05 a	2.14 c	1.93 a	1.11 f
De Jativa	2.64 b	2.58 b	1.02 d	1.91 g	1.37 d	1.39 c
Molar	2.38 d	2.27 d	1.05 a	2.32 a	1.26 e	1.84 a
Banaty	2.02 h	2.00 g	1.01 e	2.01 de	1.49 b	1.35 cde
Manfaluti	2.64 b	2.54 b	1.04 b	2.29 a	1.97 a	1.16 f
Mellasy	2.28 e	2.18 e	1.05 a	2.01 de	1.44 bcd	1.40 c
Succary	2.48 c	2.41 c	1.03 c	2.05 cd	1.51 b	1.36 cde

* Means of 10 replications, each containing 200 pollen grains.
¶ = Values have the same letters are not significantly different at 5% level.
[z] = Length / diameter of pollen grains determined by measuring under a microscope with an eyepiece micrometer.
[y] = Means seperated by L.S.D. test

Although the pollen grains of Banaty cultivar exhibited a high value and germinated pollen percentage, it was found to have a smaller length and diameter among its viable pollen grains than among the other cultivars. Meanwhile, the viable pollen grains of Hamid abiod had large size pollen in comparison with that in the other cultivars.

The ratio between length and diameter (L/D ratio), which indicated the shape of the pollen grains (Table 2) ranged from 1.01 to 1.05 in viable pollen grains and 1.11 to 1.84 in nonviable pollen grains. The value of 1 to 1.25 indicate the circular shape. All viable pollen of the 11 cultivars and the nonviable pollen grains of Manfaluti and Hamid abiod cultivars had L/D ratios between 1 and 1.25. Value of 1.25 to 1.50 represent an oval

shape and this include all nonviable pollen grains except in Molar, Manfaluti and Hamid abiod cultivars. Values of more than 1.5 are the elongated ones as Molar cultivar. Many investigators reported pollen size and shape variation among the different cultivars (Stott [5] in apple, Al-Tahir and Asif [22] in date palm , Mamedov [17] and Sharma and Gaur [3] in pomegranate and Cruzan [1] in *Erythronium grandiflorum.*

Rates of pollen tube growth

Pollen tube length after cultured in sucrose hanging solution grew more rapidly in Banaty and De Jativa than those of other cultivars (Fig.1). Al-madina, Balady red and Mellasy cultivars gave the lowest pollen tube length. In general, the same trend was found in sampling carried out after 48 and 72 hr. from cultured in sucrose media. After 72 hr. from cultured, pollen tube length was less than 10 μ in Mellasy, Balady red, Taeifi, Al-madina and Succary cultivars and larger than 10 μ in Banaty, Hamid abiod, Molar,De Jativa, Manfaluti and Khob El-jamil cultivars. These results suggested that the growth of pollen tubes is probably controlled by the cultivars [4, 18], and suggest the presence of genetical differences among the 11 pomegranate cultivars because they were grown under similar conditions and the same location

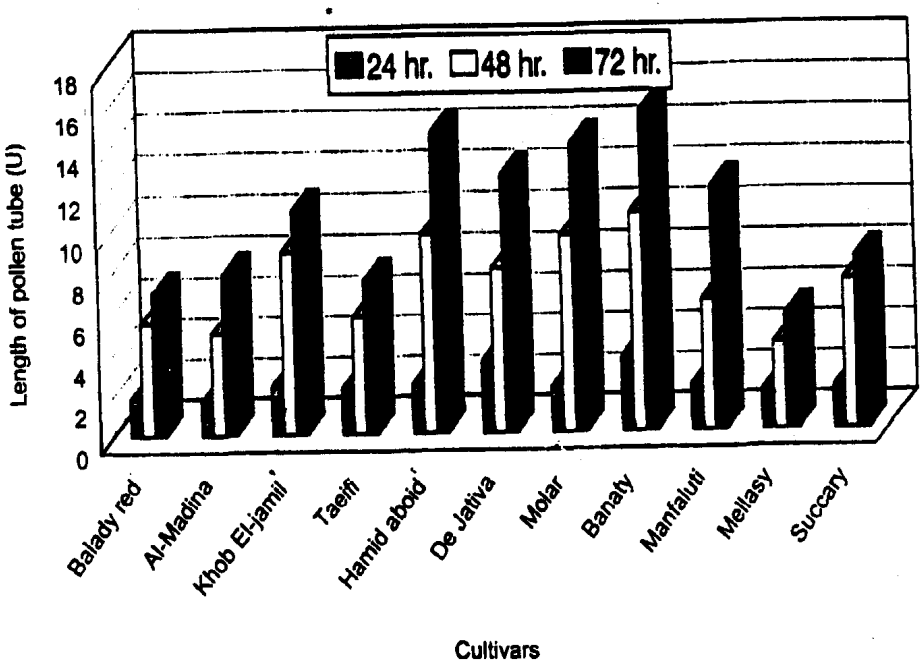

Fig. 1. Length of pollen tube after 24,48 and 72 hr. of culture in sucrose media for 11 cultivars of pomegranates.

A positive and significant correlation was found between the absolute pollen viability and pollen tube length in Al-madina cultivar (Table 3). There was a positive correlation in absolute pollen viability and pollen tube length among all tested cultivars, except in Hamid abiod, De Jativa and Succary cultivars. Pearson and Harney [10] reported that, correlation between pollen staining and percentage of pollen germination was positive and significant, but absolute pollen viability was found to be a better indicator of viability than staining. Absolute pollen viability calculation within genotypes is an improved method for estimating of actual pollen viability because many morphological normal pollen grains lacked the ability to germinate. On the other hand, Cruzan[1] reported that in *Erythronium grandiflorum* there was no correlation between pollen tube growth in media and the mean size of pollen grains.

Table 3. Correlation coefficients of absolute pollen viability (APV) with pollen tube length (PTL) in 11 pomegranate cultivars

Cultivar	Correlation coefficient (r)
Balady red	+0.738
Al- Madina	+ 0.974 **
Khob El jamil	+ 0.579
Taeifi	+ 0.547
Hamid abiod	- 0.234
De Jativa	- 0.315
Molar	+ 0.735
Banaty	+ 0.560
Manfaluti	- 0.512
Mellasy	+ 0.553
Succary	- 0.251

** Significant at 0.01 probability

It can be concluded that there is conformity correlation between the data obtained with the use of acetocarmine coloration and the use of sucrose hanging drop technique. Banaty, Khob El-jamil and Manfaluti cultivars were considered to be the proper pollen parents from the genotypes sampled and to have the highest potential to be more effective pollinators through a selective breeding program. Banaty, Khob El-jamil and Manfaluti have the greatest potential for crop improvement through improved pollination.

References

[1] Cruzan,M.B. "Variation in Pollen Size, Fertilization Ability, and Postfertilization Siring Ability in *Erythroniu grandiflorum*." *Evolution*, 44, 4 (1990), 843-856.

[2] Facteau,T.J.,Wang,S.Y. and Rowe,K.E." The Effect of Hydrogen Fluoride on Pollen Germination and Pollen Tube Growth in *Prunus avium*, L. cv. Royal Ann." *J. Amer. Soc. Hort. Sci..*, 98, 3 (1973), 234-236.

[3] Sharma, C.M. and Gaur, R.D. " Studies on Morphology, Germination and Viability of Pomegranate (*Punica granatum*,L.) Pollen". *J. Palynol.*, 20, 2 (1984), 87-92.

[4] Soliman, A.S., Al-Ani, B.A., Al-Salih, A.A. and Saadawi, I.S. "Viability Studies of Pollen Grains of Date Palm (*Phoenix dactylifera*,L.)." *Bull. Coll. Sci.*, 17, 1 (1976), 61-70.

[5] Stott, K.G. "Pollen Germination and Pollen Tube Characteristics in a Range of Apple Cultivars". *J. Hort. Sci.*,47 (1972), 191-198.

[6] Erlanson, E.W. "Sterility in Wild Roses and in Some Species Hybrides." *Genetica,* 16 (1931), 75-96.

[7] Visser,T., De Vries.D.P.,Scheurink,J.A.M. and Welles, G. W. "A Hybrid Tea Rose Pollen. II.Inheritance of Pollen Viability." *Euphytica,* 26 (1977), 729-732.

[8] Roberts, V.A. "Relationship Between Species in the Genus Rosa,Section Pimpinellifolia." *Bot. J. Linn. Soc.*,74 (1977), 309-328.

[9] Singh, J.P. and Dhuria, H.S. "Studies on Floral Biology of Sweet Lime". *Indian J. Hort.*, 17 (1960), 9-20.

[10] Pearson,H.M. and Harney,P.M. "Pollen Viability in Rosa." *Hort. Science,* 19, 5 (1984), 710-711.

[11] Visser, T.,De Vries, D.P., Welles,G.W.H. and Scheurink J. A. M. "Hybrid Tea Rose Pollen .I. Germination and Storage." *Euphytica,* 26 (1977), 721-728.

[12] Steel, R.G. and Torrie, J.H. *Principles and Procedures of Statistics.* New York:McGraw -Hill Book Co., (1981), 196 - 197.

[13] Randhawa, G.S. and Negi, S.S. "Further Studies on Flowering and Pollination in Grapes." *Indian J. Hort.*, 22, 3,4 (1965), 287.

[14] Prasade, A. "Investigation of Floral Biology, Pollination of Fruit Quality of Grapes." *Hort. Sci.,Calcuta,* (1969), 15-21 (Hort. Abst. 39, 6193).

[15] Randhawa, G.S. and Ramakrishnan,N.P.K. "Studies on Floral Biology of Plums Grown Under Tropical Conditions." *Indian J. Hort.*, 17, 2 (1960), 83-95.

[16] Soost, R.K. "Citrus Pollination". *Calif. Citrograph.*, 48, 12 (1963), 447- 452 .

[17] Mamedov, G.M. "Meiotic Abnormalities in Induced Forms of Pomegranate Following Treatment of Seed with Various Doses of Physical and Chemical Mutagens." *Veses. Knof. Po. S. Kh-radiol., Obninsk,* (1984) 16-20 , USSR (Plant Breed. Abst., 57, 4284).

[18] Al-Jibouri, A.A.M. , Kgazal, M. and Saadawi, I.S. "Effect of Gamma Irridiation on Pollen Germination and Pollen Tube Growth of Four Male Cultivars of Date Palm (*Phoenix dactylifera*,L.)." *Date Palm J.*, 5, 1 (1987), 9-18.

[19] Pfahler, P.L. "In-vitro Germination and Pollen Tube Growth of Maize (*Zea mays*, L.) Pollen V. Gamma Irridiation Effects." *Rad. Bot.*, 11 (1971), 233-237.

[20] Pfahler, P.L."In-vitro Germination and Pollen Tube Growth of Maize (*Zea mays*,L.) Pollen VII. Effects of Ultraviolet Irridiation". *Rad. Bot.*, 13 (1973),13-18.

[21] Agarwal, P.K. "Effect of Storage in Organic Solvents on the Germination of Grapevine Pollen ." *J. Hort. Sci.*, 58 (1983), 389-392.

[22] Al-Tahir,O.A. and Asif, M.I. "Study of Variations in Date Pollen Material." *First Symposium on Date Palm* .King Faisal Univ., Saudi Arabia. (1982), 62-66.

حيوية حبوب اللقاح وإنباتها ومعدل نمو الأنبوبة اللقاحية في بعض أصناف الرمان

محمود أحمد علي، محمد علي أحمد باشه، وفرحات الدسوقي فرحات

قسم الانتاج النباتي، كلية الزراعة ، جامعة الملك سعود، الرياض، المملكة العربية السعودية

(قدم للنشر في ١٤١٦/٨/٢٦هـ ؛ وقبل للنشر في ١٤١٧/٨/٢٠هـ)

ملخص البحث: استخدمت في هذا البحث حبوب لقاح الرمان من خمسة أصناف محلية هي أحمر بلدي، المدينة، خب الجميل، الطائفي، وحامض أبيض وصنفين مستوردين من أسبانيا هما ديجاتيفا ومولر، وأربعة أصناف مصرية هي البناتي، المنفلوطي، المليسي والسكري. وقد تم دراسة حيوية وإنبات وشكل حبوب اللقاح وكذلك معدل نمو الانبوبة اللقاحية بعد ٢٤، ٤٨، ٧٢ ساعة من زراعتها في بيئة السكروز.

وقد وجد أن أصناف الرمان الاسبانية وهي ديجاتيفا ومولار قد أعطت أقل نسبة مئوية لحيوية حبوب اللقاح والانسبات وكذلك النسبة المئوية لحيوية حبوب اللقاح المطلقة عند مقارنتها مع بقية الاصناف. وقد تراوحت نسبة طول حبوب اللقاح الحية الى عرضها من ١.٠١ الى ١١,٠٥ بينما تراوحت في حبوب اللقاح غير الحية من ١,١١ إلى ١٨٤.. كذلك وجد أن معدل نمو أنابيب اللقاح في أصناف البناتي والديجاتيفا والحامض الأبيض كان أسرع عن باقي الاصناف وذلك بعد ٧٢ ساعة من زراعة حبوب اللقاح في بيئة السكروز. وقد أظهرت النتائج وجود تلازم موجب بين حيوية حبوب اللقاح الفعلية أو المطلقة مع طول الأنبوبة اللقاحية في كل أصناف الرمان تحت الدراسة عدا في ثلاثة أصناف هي حامض أبيض وديجاتيفا والسكري .