

Evaluation of pollen germination of some palm males and pollination impact on bunch weight and fruit quality in Kadary date palm cultivar (*Phoenix dactylifera* L.)

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Abstract

The present study was carried out during two successive seasons (2013-2014) at the Research and Agriculture Experiment Station, Dirab, College of Food and Agricultural Sciences, King Saud University, Riyadh. Pollen grains, used in this trial, were collected from eleven male, namely Succary, Menify, Sallag, Dikhiny, Nabout Zamel, Serry, Khalas, Shagra, Safry, Maktumi and Kadary in order to pollinate female Kadary date palm cultivar. Results indicated that pollens of Safry male followed by Succary, Menify and Serry increased the physical and biochemical characteristics of fruits during both seasons. Also, all the studied characters were significantly affected with different pollen males used in this trial. Pollens of Safry male gave highest fruit retention and bunch weight and best fruit quality like fruit and flesh weight, fruit size and fruit length and fruit biochemical properties as compared with other male. *In vitro* pollen germination of eleven males was studied using various concentration of sucrose (4, 6, 8 and 10 %). The results showed that best germination was achieved with 8 % sucrose concentration; however, 10 % sucrose resulted in reduced percentage of pollen germination. In addition, pollen tube growth of five males (Succary, Sallag, Nabout-Zamel, Khalas and Safry) at 8% sucrose was also studied at 2, 4, 6 and 24 hr time interval. Elongation rate of pollen tube increased significantly after 24 hrs and it was found that pollens of Safry male gave highest pollen tube growth after 24 hrs than pollens of other male.

Key words

Date palm, Fruit quality, Pollen germination, Pollen tube growth

Introduction

Date palm (*Phoenix dactylifera* L.) is one of the most important fruit species grown in the Kingdom of Saudi Arabia. It ranks top among fruit trees as far as number (25104161 million trees), area (156901 ha) and production (1095158 tones) (Ministry of Agriculture, 2013) is concerned. Dates are rich source of minerals, carbohydrate, organic, acid, total soluble solids, vitamins with high calorific value. Fruits of date palm can be preserved, stored and transported to long distances. It can grow well under adverse environmental conditions, which is otherwise not suitable for other fruit species. It is important to conduct an

evaluation to males and females in terms of vegetative and flowering characteristics. This suggests that these characteristics are important features for studying the diversity of date palm cultivars and fruit quality (Elshibili *et al.*, 2007).

Unisexual flowers are either pistillate and staminate in character, so artificial pollination is an essential cultural practice to obtain satisfactory fruit set (Zaid, 1999). Farmers are forced to use pollens of unknown sources which are characterized by low fertility and incompatibility due to emergence of early inflorescence on female date palms before opening of adequate number of male inflorescences.

The ability of pollens to perform its function is determined by delivering sperm cells to embryo sac following compatible pollination (Shivanna *et al.*, 1991). On the other hand, pollens' capacity to fertilize ovule and fruit set is considered as a measure of natural aptitude (Boughediri and Bounaga, 1987), and identifying a suitable method is a key to more accurate estimation of pollen viability (Rodriguez-Riano and Dafni, 2000). *In vitro* germination has been used as an important technique for testing pollen viability (Khatun and Flowers, 1995). Ibrahim *et al.* (2014) found that germination of pollen grains *in vitro* is very important and useful to determine the best pollinator for pollination process. Dioecious plant, unisexual flowers are either pistillate or staminate (Ashour *et al.*, 2008) and natural pollination occurs by wind and insects. If the pollination process is dependent on these two agents, fruits may be of inferior quality due to inadequate pollination. Therefore, artificial hand pollination becomes a necessity as a mean to ensure good yield. Pollen grains have direct effect on physical properties (length, diameter, fruit weight and seed weight) of fruits and time required for its ripening (Soliman and Osman, 2001). The available number of date palm males is insufficient for efficiently pollinating the increasing number of female plants in Kingdom of Saudi Arabia.

In light of the above, the present study was carried out to investigate *in vitro* pollen germination of eleven date palm cultivars of Saudi Arabia. Further, physical and biochemical quality of fruits obtained from fertilization of selected male cultivars were also studied.

Materials and Methods

Experimental set-up : The present study was carried out at the Agriculture Research and Experiment Station, Dirab, College of Food and Agricultural Sciences, King Saud University, Riyadh during two successive seasons 2013 and 2014 on female Kadary date palm. Five date palm trees uniform vigorous 15-year-old, growing on sandy soil, were selected according to their capacity of bearing approximately same number of spathes and leaves. Only eleven bunch (spathe) were left on each experimental tree. All the cultural practices were carried out according to the traditional schedule for experimental palms. Pollination was achieved by using pollen grains from different male namely Succary, Menify, Sallag, Dikhiny, Nabout Zamel, Serry, Khalas, Shagra, Safry, Maktumi and Kadary. Female spathe was pollinated manually by inserting 5 male strands in the middle of female inflorescence in all the treatments. Pollination was carried out in last week of February and first week of March during 2013 and 2014 seasons, respectively. After spathe pollination, inflorescence was individually wrapped in paper bags to prevent natural contamination by

strange pollens from the surrounding males. Bags were removed after 3 weeks of pollination. The same procedure was applied to all the male type cultivars on a single female palm.

Experimental treatments (eleven) were arranged in randomized complete block statistical design with five replications (one palm tree for each replication). The number of eleven treatments applied to all five palms, so that repeated eleven treatments on each palm separately.

The yield of experimental trees was harvested in first half of August during both the seasons. Average bunch weight was recorded in kg per palm. Samples of 10 date fruits were picked randomly for determination of physical (fruit weight, flesh weight, seed weight, fruit volume, fruit length, fruit diameter, initial fruit set, fruit retention and bunch weight) and chemical (moisture content, total soluble solid, fruit acidity and reducing, non-reducing and total sugar fruit characteristics according to the method of AOAC (1995).

***In-vitro* pollen germination :** Pollen samples were collected from flowers of each date palm male. Three male spathes were selected from various parts of each tree (three to each male) and collected in paper bags to avoid contamination from other pollen sources. Strands of each spathe were cut and dried at room temperature. Pollen grains separated from male flowers, by fine sieve (40 mesh) were collected in small vials and stored in desiccator (Javady and Arzani, 2001) until further use.

Germination test of pollen grain was carried out by hanging drop technique using a wide range (4, 6, 8 and 10 %) of sucrose solution. Pollens were dispersed using a brush to distribute it uniformly on the dish. This uniformity is important to avoid the influence of "mass effect" as agglomeration of pollen grains results in higher pollen germination (Giulivo and Ramina, 1974). A small quantity of pollen grain was put on sugar solution in Petri-dish and left for a period of 2, 4, 6 and 24 hrs and examined under Olympus compound microscope (Bx-51) to assess the viability of pollen grains. Additionally, pollen grains of Succary, Sallag, Nabout Zamel, Khalas and Safry males were selected to measure growth rate of pollen tube at 8 % sugar solution at 2, 4, 6 and 24 hr time interval. Pollen germination of different male cultivar was calculated by the following formula:

$$\text{Pollen germination (\%)} = \frac{\text{Number of pollens germinated}}{\text{Total number of pollen grains}} \times 100$$

Statistical analysis : All the data obtained were processed by Microsoft Excel (Microsoft, 2000). Statistical analysis such as simple correlation (Snedecor and Cochran, 1980), as well

as, stepwise multiple regression analysis were performed using statistical program (Stat Soft Inc., 1995).

Results and Discussion

All the four concentration of sugar enhanced germination of pollen grains (Table 1). Data showed significant difference in germination of pollen grains of different males. With increasing sugar concentration germination percent increased up to 8 %, however, at 10 % a clear reduction in germination percentage was noted. Germination of pollens was significant at 8 % sugar concentration and this concentration was considered ideal for pollen germination as compared to other sugar concentrations. Pollens of Safry male followed by Nabout Zamel, Khalas and Sallag males showed highest pollen germination (86.0, 82.6, 77.0 and 76.4 %) than other male type. These results are in line with the results obtained of (Tisserat *et al.*, 1985), Al-Helal *et al.* (1988), Wang *et al.* (2004) and Mortazavi *et al.* (2010). They found that the pollen germination and pollen tube growth needs favorable conditions for germination such as temperature and humidity and some nutrients. Ismail (2014) reported the effect of fructose, glucose, lactose, maltose and sucrose in germination of pollen grains of Barhee, Amhat and Hayani date palm cultivar and concluded that effect of different sugar on pollen germination and pollen growth differed among

species and cultivars. Highest pollen germination in Hayani and Amhat cultivar was found in lactose medium, while Barhee cultivar showed highest pollen germination in sucrose medium. Perusal of data indicated significant difference in growth of pollen tube. Pollen tube growth considerably increased with time duration at 8 % sucrose level (Table 2). However, the level of effect varied with duration. As compared to other time duration, maximum pollen tube growth was found at 24 hr at 8 % sucrose level. Pollen tube growth was observed in the following order: Safry, Nabout Zamel, Khalas, Sallag and Succary males, respectively after 24 hrs of treatment (244.3, 227.3, 231.7, 222.0 and 207.0 μm). Fig. 1,2,3 and 4 show the effect of time on pollen tube growth of Safry male at 8 % sucrose concentration. Pollen tube growth was found to be 66, 94, 143 and 244.3 μm after 2, 4, 6 and 24 hrs, respectively as compared to other males. Safry male pollinator good affected strongly and positively on the physical and chemical characteristics in the both seasons. These results are in line with the reports of Asif *et al.* (1984) and Al-Helal (1994). Beyhan and Serdar (2009) found that 10% sucrose concentration was best for high germination rate and pollen tube growth in *Castanea* genotypes. A linear relationship between sucrose concentrations and pollen tube length was found to be significant in most genotypes. Germination of pollen grains has great importance in flower-flower and

Table 1 : Effect of different concentrations of sugar on germination of pollen grains

Males	Concentration				LSD at 0.05%
	4%	6%	8%	10%	
Succary	55.40	64.63	66.86	63.07	3.0
Menify	49.33	53.33	55.67	52.43	3.5
Sallag,	65.27	74.67	76.37	71.20	7.3
Dikhiny	49.6	56.67	61.37	53.33	3.6
Nabout Zamel	68.00	78.00	82.57	72.33	3.6
Serry	49.50	53.67	57.23	52.80	4.2
Khalas	64.30	73.67	77.00	69.0	1.8
Shagra	50.90	57.90	60.40	54.60	2.2
Safry	71.2	80.53	86.03	76.90	5.1
Maktumi	51.30	61.23	65.97	57.47	1.7
Kadary	48.27	58.07	62.57	57.43	5.2
LSD at 0.05%	3.0	3.1	2.8	2.4	

Table 2 : Effect of time on pollen tube growth (μm) at 8% sucrose concentration

Males	2 hr	4 hr	6 hr	24 hr	LSD at 0.05%
Succary	55.3	74.0	119.0	207.0	10.4
Sallag,	59.0	80.3	132.0	222.0	3.9
Nabout Zamel	64.0	87.7	136.0	231.7	5.7
Khalas	62.3	84.0	134.0	227.3	4.0
Safry	66.0	94.0	143.0	244.3	5.9
LSD at 0.05%	2.4	4.9	9.2	6.2	

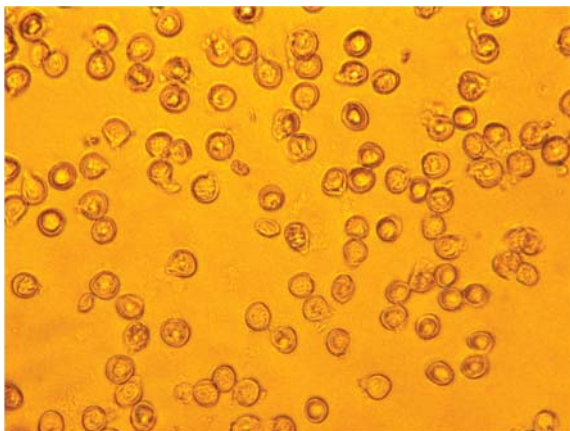


Fig. 1 : Pollen tube growth of Safry male after 2 hrs of 8 % sucrose treatment

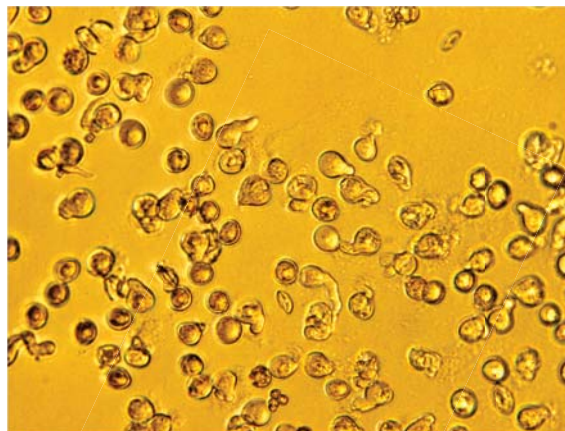


Fig. 2 : Pollen tube growth of Safry male after 4 hrs after 8% sucrose treatment

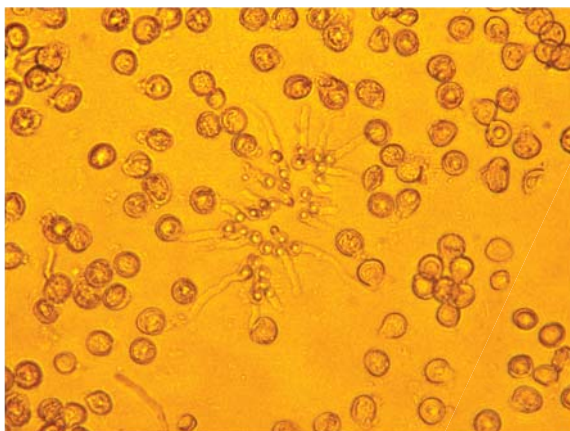


Fig. 3 : Pollen tube growth of Safry male after 6 hrs of sucrose treatment



Fig. 4 : Pollen tube growth of Safry male after 24 hrs of sucrose treatment

flower-pollinator interaction and fruit setting. Germination requirement of pollens vary from species to species (Patel and Mankad, 2012). Pollens generally require moisture, sucrose, boron and calcium for satisfactory germination and tube growth. Sucrose is a prerequisite for pollen germination as it serves as energy source and an osmoticum. It maintains the osmotic pressure and acts as a substrate for pollen metabolism (Shivanna and Jhori, 1989). Several reports are available on different level of sucrose requirement for optimum germination of pollens of different plant species. It was found that 5 % sucrose in *Selix* spp. (Kopp *et al.*, 2002); 11-15% in *Asclepias syrica* (Kevan *et al.*, 1989) proved best for optimum germination of pollen grains.

Data presented in Table (3) indicate that fruit retention was significantly affected by pollens of all the male cultivar selected during both the seasons (2013-2014). A positive relationship between male type and fruit retention

was observed. Highest fruit retention was observed in pollination of Safry male (71.3, 66.3 %) followed by Succary male (64.8, 59.4 %) as compared to other males during both the seasons, while pollination by Serry males (49.8, 45.8 %) followed by Nabout Zamel male (51.7, 46.7 %) gave lowest fruit retention during both the seasons.

Improvement in fruit retention could be explained as a result of increase in pollen grain germination and pollen tube elongation due to male type and metaxenia. The above mentioned results are in agreement with those obtained by Ashour *et al.* (2004) on date palm where boron spraying treatments improved yield, retained fruits and physical and chemical characteristics. Similarly highest bunch weight of dates was obtained from pollination of Safry male (16.00, 15.67 kg) followed by Succary male (15.50, 14.67 kg) during both the seasons, respectively. These results are in agreement with the reports of Kosary and Soliman (2003) and Soliman

Table 3 : Effect of pollination by pollen grain from different males on bunch weight, fruit retained and physical Properties

Males	Fruit weight (g)	Flesh weight (g)	Seed weight (g)	Fruit volume (cm)	Fruit length (cm)	Fruit diameter (cm)	Initial fruit set (%)	Fruit retention (%)	Bunch weight (kg)
2013-2014 seasons									
Succary	11.96	11.01	0.95	11.67	4.15	2.35	82.6	64.8	15.50
Menify	11.56	10.54	1.02	11.67	4.10	2.33	79.8	57.6	14.69
Sallag,	9.69	8.83	0.86	10.00	3.80	2.25	85.6	60.5	13.45
Dikhiny	10.10	9.16	0.94	10.33	4.00	2.32	65.5	55.2	11.00
NaboutZamel	11.30	10.43	0.87	11.67	4.13	2.35	78.0	51.7	14.33
Serry	11.55	10.61	0.94	11.50	4.17	2.50	58.6	49.8	9.20
Khalas	10.27	9.37	0.90	11.17	4.03	2.47	60.0	57.7	11.20
Shagra	10.49	9.48	1.01	10.67	4.10	2.47	66.1	58.4	14.40
Safry	12.10	11.15	0.95	12.17	4.30	2.47	75.9	71.3	16.00
Maktumi	10.53	9.59	0.94	11.00	4.10	2.40	80.8	54.3	13.20
Kadary	9.49	8.61	0.88	9.67	3.97	2.60	80.8	59.9	10.00
LSD at 0.05	2.31	2.30	0.11	2.28	0.33	0.10	15.3	4.7	5.33
2013-2014 seasons									
Succary	11.65	10.6	1.05	12.20	4.2	2.43	79.6	59.4	14.67
Menify	10.34	9.4	0.94	10.70	4.0	2.43	74.8	51.6	14.33
Sallag,	10.22	9.2	1.02	10.20	3.8	2.37	80.0	55.5	14.00
Dikhiny	10.04	9.2	0.84	10.20	3.9	2.43	60.5	50.2	10.33
NaboutZamel	10.52	9.6	0.92	11.00	4.0	2.33	74.0	46.7	14.00
Serry	9.77	8.8	0.97	9.70	3.8	2.33	63.6	45.8	10.33
Khalas	9.58	8.7	0.88	10.30	4.0	2.40	65.0	53.7	12.33
Shagra	10.94	10.0	0.94	11.80	4.2	2.40	64.1	54.4	14.00
Safry	12.09	11.1	0.99	12.70	4.3	2.50	74.9	66.3	15.67
Maktumi	10.43	9.5	0.93	10.80	4.0	2.37	77.2	50.3	14.00
Kadary	9.58	8.61	0.97	9.30	3.9	2.37	76.5	53.9	10.33
LSD at 0.05	3.8	3.7	0.17	4.11	0.72	0.29	12.7	3.4	6.59

and Al-Obeed (2013) on several date cultivars. Perusal of data revealed that highest fruit weight (12.10, 12.09 and 11.96, 11.65 g), fruit flesh weight (11.15, 11.10 and 11.01, 10.60 g), fruit volume (12.17, 12.70 and 11.67, 12.20 cm) and largest fruit length (4.30, 4.30 and 4.15, 4.20 cm) was found in fruits obtained from pollens of Safry male followed by Succary male during first and second season, respectively. Regarding fruit diameter, pollens of Kadary male followed by Serry male gave largest fruit diameter during 2013, while Safry male followed by Succary male gave largest fruit diameter during 2014. These results are in line with the study of Ashour *et al.* (2008), Abd El-Zaher (2008) and Ahmed *et al.* (2014). They reported that fruit physical quality varied according to type of males used in pollination of date palm. Biochemical characteristics of fruits showed that total soluble solids, sugar content, moisture content and total acidity percentage in different cultivars were significantly different during both the seasons (Table 4). During first season highest TSS content in fruits was obtained from pollens of Safry male followed by Succary male (73.2, 70.8 %); while during second season highest TSS was obtained from pollens of Safry male followed by Menify male (76.4, 74.0 %), respectively. Highest reducing sugar in date palm fruits was

obtained from pollens of Safry male followed by pollens of Menify male cultivar during first season; and in fruits obtained from pollens of Menify male followed by Maktumi during second season, respectively. Highest non-reducing sugar in date palm fruit was obtained from pollens of Khalas male followed by Safry male during the first season, and during the second season from pollens of Safry followed by Succary male, respectively. Highest total sugar in date palm fruit was obtained from pollens of Safry male (70.9, 71.6 %) followed by Succary male (68.8, 68.9 %) during first and second season, respectively. Fruits obtained by pollination of Shagra male followed by Safry male cultivar showed highest acidity (when fruit harvest) during first season, while reverse trend was seen during second season. Similarly, highest moisture content was noted in fruits obtained from pollens of Safry male followed by Serry male (24.1, 22.6 %) during first season, while reverse trend was noted for moisture content (19.1, 18.1 %) during the second season, respectively. The present data is in agreement with the Ahmed *et al.* (2014). Pollens of date palm directly influence the shape, size, development of fruit and time of ripening of fruit obtained from vegetatively propagated female varieties. The effect of male parent on the development of date palm fruits is definite

Table 4: Effect of pollination by pollen grain from different males on bio chemical properties

Males	Acidity %	TSS %	Reducing sugars	Non-Reducing sugars	Total sugars	Moisture content %
2013-2014 seasons						
Succary	1.305	70.8	45.5	23.3	68.8	19.9
Menify	1.229	68.6	45.8	20.5	66.3	19.9
Sallag,	0.845	56.4	35.7	18.8	54.5	20.3
Dikhiny	0.845	55.6	37.2	15.5	52.7	21.2
NaboutZamel	0.729	61.8	37.8	21.3	59.1	18.9
Serry	1.229	67.2	45.4	19.6	65.0	22.6
Khalas	0.960	65.0	34.1	25.5	59.6	18.5
Shagra	1.459	59.2	42.6	13.5	56.1	19.9
Safry	1.44	73.2	46.4	24.5	70.9	24.1
Maktumi	1.198	66.4	37.7	21.8	59.5	20.2
Kadary	1.00	67.2	45.7	18.1	63.8	19.2
LSD at 0.05	0.322	7.4	6.1	10.4	7.1	3.7
2013-2014 seasons						
Succary	0.1536	70.8	46.5	22.4	68.9	16.0
Menify	0.1165	74.0	47.3	21.1	68.4	12.3
Sallag,	0.1241	62.4	45.7	13.5	59.2	15.8
Dikhiny	0.1408	61.8	42.6	16.6	59.2	16.8
NaboutZamel	0.1536	62.0	42.0	15.3	57.3	17.8
Serry	0.1472	70.8	44.8	22.3	67.1	19.1
Khalas	0.1152	61.8	46.0	12.8	58.8	17.7
Shagra	0.1664	58.2	43.8	11.3	55.1	16.8
Safry	0.1728	76.4	45.8	25.8	71.6	18.1
Maktumi	0.1413	66.2	47.1	16.5	63.6	16.7
Kadary	0.1344	63.2	46.5	13.7	60.2	15.9
LSD at 0.05	0.022	2.29	2.76	4.77	3.07	4.82

and varies with particular male used to fertilize female flowers (Ibrahim *et al.*, 2014). Swingle, (1928) proposed a term 'metaxenia', a phenomena in which pollens directly affect all the parts of seed and fruit lying outside and endosperm. Effect of metaxenia has been reported on fruits size, fruit and seed weight (Abd El-Zaher, 2008) and fruit quality (Ibrahim *et al.*, 2014).

The results of the present study suggests that germination of pollen grains is very important and useful to determine the best pollinator for pollination process as a some good pollinator strongly affects the physical and chemical characteristics of fruits of date palm.

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