Pollen Viability of Date Palm from Different Sources in Relation to its Chemical Composition

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ABSTRACT

This study was undertaken to determine pollen viability for nine pollen grain sources of date palm from three sites in Arab Republic of Egypt using staining and germination methods, in addition, to recording the differences in the chemical composition i.e., total carbohydrates, protein and amino acids, as well as, the relationship between them. This experiment was carried out to select the amino acids that can be used in the future to improve pollen viability and to increase productivity of date palm using suitable male palms by modern methods. Therefore, three selected males from three sites in three governorates i.e., El-Behira, El-Fayoum and Sohag were chosen for this study. Results showed that pollen grains brought from Rasheed , El Behera governorate (M1) produced the highest staining and germination percentages (91 and 84 %, respectively), as well as the highest protein content of 31.21% . Similarly M1 had the highest lysine and serine content (6.41 and 4.80, respectively), as compared to the other pollen sources. There was a highly positive correlation between both stained and germinated pollens with the arginine content (r = 0.693 and 0.700) respectively. The principal component analysis was performed to clarify the relationship between the evaluated variables i.e. chemical composition revealed that the first four components (PCA1, PCA2, PCA3 and PCA4) accounted for more than 83% of the total variations for the variables. The biplot showed that there was a high correlation between the stained, germinated pollen grains and the content of lysine and arginine.

Date palm*, Phoenix dactylifera*, Stained, Germination, Pollens, Chemical Composition.

INTRODUCTION

Date palm (Phoenix dactylifera L.) is dioecious and is naturally cross pollinated. Under commercial cultivation, female palms are artificially pollinated using compatible pollen source. However, artificial hand pollination represents a great task for the date growers, and such a practice should be done using the most viable pollens in order to get the highest fruit set and yield (El-Hammady *et al.,* 1977; Khalifa *et al*., 1980; El-Makhtoun, 1981; Hamdy, 1982 and Rabie, 2007). They reported that pollen sources vary in their influence on the physical and chemical characteristics of fruit. These variations are accounted for genetic or environmental factors or for the interaction between both. In the meantime, date growers use any pollen source that is readily available without paying any attention to the choice of proper type. However, to get best set and yield they should select the appropriate male type as a source of pollen.

Many studies have addressed the use of different pollen grain sources on yield and fruit characteristics of date palms (Iqbal *et al*., 2008 Mustafa *et al*., 2014, Merwad *et al*., 2015 and Nesiem *et al*., 2016). The different sources usually have different pollen viability (Bacha *et al*., 2000). The term viability refers to the capacity of the pollen to germinate and grow normally, high viable pollen will improve the fruit set and ultimately will produce higher yield. In vitro germination is a valuable technique to determine the viability of the pollen grains and to identify the best pollinators for the pollination process (Ibrahim *et al*., 2014). Pollen viability is determined in different fruit species using the two methods of germination and Acetocarmine staining (Bacha *et al*., 2000).

At present, very little is known about the impact of pollen source and its chemical composition on its viability. Thus, the present study aims to declare the relationship between the viability of pollen grains from different sources and their chemical composition to select the suitable source of pollen for increasing the production of palm trees as well as accumulation of information about the reasons to modify this character (pollen viability).

MATERIALS AND METHODS

This investigation was carried out during the 2014 using nine date palm males grown in different Egyptian governorates (Table, 1). Four mature spathes were randomly collected from each male tree. The strands were dried out at room temperature for seven days. The pollen grains were separated from the strands using a sieve, and then pollen grains were transferred to stopper bottles and stored in a dry place at room temperature.

Viability determination

Two general methods assaying pollen viability were used:

1- Staining test

The dehiscent pollens were lightly tapped on a drop of 1% acetocamine stain on a slide. Six slides were made for each pollen source, for each slide ten microscopic fields were randomly chosen and pollen grains were counted and graded as viable and aborted in two classes. The pollen grains which darkly stained and round pollen grains were considered viable, while those lightly stained or shrunk were considered aborted. This technique was previously described by Ibrahim (1989) and El-Makhtoun and Abd El-Kader (1990).

2- Germination test

A mixture of pollen grains from each pollen source was collected in clean Petri dishes to minimize variations that might exist between pollen grains of the same spathe of a given source. The basic medium in Petri dishes was prepared by adding 2 g sucrose, 1 g agar and 15 ppm boric acid per 100 ml water, and then was boiled. Ten milliliters of the mixture was poured into every sterile Petri dish. Five dishes from each pollen source were used as replications. Pollens were carefully sown on the medium of each dish, and then all dishes were incubated at 25º C for 10 hrs. Randomly count of 100 pollen grains was made in each replication under $600 \times$ magnifications. This technique has been previously reported by Hamdy (1982).

Chemical composition

Total carbohydrates and sugars were determined according to Malik and Singh (1980), while crude protein was determined by multiplying nitrogen determined by the micro Kjeldahl method (A.O.A.C, 1995) by 6.25. The results were expressed as g / 100 g or percent on dry weight basis. In addition, amino acids content in protein hydrolyzate was detected using Amino Acid Analyzer according to Moore *et al*., (1958). The concentration of amino acids were calculated as grams per 100 grams of protein on dry weight basis. Backman Amino Acid Analyzer Model 119CL (according to the methods of Block *et al*., 1958) was used for amino acid determination in pollen grains materials.

Statistical analysis

The data obtained throughout this study were separated and compared using least significant difference (L.S.D.) at 0.05 level of probability according to Snedecor and Cochran (1980).The statistical analysis was performed using SAS (Statistical Analysis System) version 9.13, (2008). In addition correlation analysis among the pollen viability and their chemical composition was performed to determine the best indicator for the pollen viability. Principal component analysis (PCA) was performed based on the observations. Both correlation and PCA were performed using Microsoft R Excel 2013/XLSTATc-Pro by Fahmy, 2015 (Version, 2015.6.01.23953, Addinsoft, Inc., Brooklyn, NY, USA).

Table 1: Pollen grain sources

No. Male	Source
M1	Rasheed, EL-Behira governorate
M2	Edko, EL-Behira governorate
M3	Hosh-Isa, EL-Behira governorate
M4	Sinnuris, El-Fayoum governorate
M5	Tamiyyah, El-Fayoum governorate
M6	Ibshaway, El-Fayoum governorate
M7	El-Maragha, Sohage governorate
M8	Tema, Sohage governorate,
M9	Tahta, Sohage governorate

RESULTS AND DISCUSSION

Viability determination

The date presented in Table (2) cleared that, the male source number M1 had the highest staining percentage value of 91 % as compared to all other pollen sources having no significant difference with M2, M4, M6 and M8. On the other hand, male M7 recorded the lowest significant staining percentage as compared to the other pollen sources except with M3 and M9. In the meantime, M2, M4 and M6 did not significantly differ among each other and had higher staining percentage than M7 and M9. Additionally, almost similar trend was followed by conducting the germination test for all the male sources. M1 had significantly the highest germination percentage value i.e. 84 % as compared to all other pollen source, while M7 had the lowest values of germination percentage. These results are inagreement with previous findings of other investigators who studied some physical characteristics of the inflorescence of different date palm males (Bacha *et. al*., 1988; Rabie, 2007 and Nesiem *et al.,* 2016). They all indicated that the different pollen grains of different sources showed a wide variation in both staining and germination percentages.

Chemical composition

Data in Table (2) show that M5 had significantly the highest total sugars percentage (8.61 %) as compared to all other sources, while both M7 and M9 had the lowest sugar content. In addition, M8 recorded the highest significant total carbohydrates of 18.75 % as compared to the other pollen sources. However, M3 had the lowest total carbohydrates percentage. Furthermore, M1 recorded the highest protein percentage of 31.21 % in comparison to all other sources, whereas, M9 had significantly lower protein percentage than all other sources.

The genotypic differences and the environmental factors highly influenced the chemical composition of the pollen grains during maturation and after dehiscence (Stanley, 1971).

Table 2: The percentage of viability and total sugars, total carbohydrate and protein content of different date palm pollen sources

No. Male	Pollen viability		Chemical composition		
	Germination	Staining	Total sugars	Total carbohydrates	Protein
M1	91.00	84.00	6.52	14.31	31.21
M2	88.00	70.25	6.09	15.29	25.75
M3	84.00	67.00	7.71	11.81	24.22
M4	89.00	74.00	6.05	15.15	27.20
M5	85.00	68.00	8.61	16.18	23.79
M6	89.00	75.00	5.00	13.47	20.89
M7	80.00	62.00	4.46	14.02	23.81
M8	87.00	70.00	5.95	18.75	22.85
M9	83.00	65.00	4.90	14.41	18.44
L.S.D.$_{.0.05}$	5.00	3.16	0.56	0.93	1.52

It was reported that, during pollen development and maturation, high temperatures tend to reduce the pollen carbohydrates as a result of high respiration rate and also decreased the viability of the pollen grains (Pressman *et al*., 2002). Also, all factors that influence the photosynthetic rate would result in less carbohydrate accumulation in the mature pollen (Al-Tahir *et al*., 1982).

As for the amino acids content in the different pollen grains, the data presented in Tables (3 & 4) showed that the nine date palm male sources contained 8 essential amino acids and 9 nonessential ones (Hassan, 2011). Concerning the essential amino acids, the results in Table 3 indicated that M3 had a higher isolucine content followed by M1 and M5 as compared to all other male sources, while M7 had the lowest isolucine value. It was also noticed that M6 had significantly the higher leucine content followed by M9 as compared to all other male source, while M5 had the lowest leucine value. In the Meantime, M1 had significantly the higher lysine content followed by M6, M5 and M2 in comparison to all other male sources, while both M8 and M9 contained the lowest values.

Table 3: Essential amino acid contents of different date palm pollen sources (g / 100 g protein).

No. Male	Essential amino acids*							
	Ile	Leu	Lys	Phe	Thr	Val	His	Met
M1	6.14	6.83	6.41	4.23	4.24	4.95	2.98	1.25
M2	5.42	6.40	6.12	3.96	4.32	4.84	2.58	1.22
M3	6.33	4.65	5.76	4.84	3.68	5.02	2.61	1.14
M4	5.65	6.30	5.58	4.52	4.12	5.00	2.56	0.99
M5	6.13	3.75	6.17	3.69	3.65	5.62	2.70	1.21
M6	4.33	8.19	6.20	3.08	3.25	4.64	3.44	1.53
M7	4.06	6.29	5.55	3.39	3.00	3.35	4.24	1.35
M8	4.86	6.35	5.16	3.72	3.07	3.19	2.27	1.36
M9	4.28	7.64	5.31	3.23	3.73	3.85	2.75	1.45
L.S.D.$_{.0.05}$	0.16	1.21	0.18	0.20	1.02	0.24	0.16	0.10

*: Ile: (Isolucine), Leu: (Leucine), Lys: (Lysine), Phe: (Phenylalanine), Thr: (Threonine), Val: (Valine), His: (Histidine), Met: (Methionine).

Table 4: Nonessential amino acid contents of pollens of the different date palm sources (g /100g protein).

No. Male	Nonessential amino acids*								
	Ala	Arg	Asp	Glu	Gly	Ser	Cys	Tyr	Pro
M1	6.10	5.17	11.25	10.83	4.23	4.80	0.32	3.52	3.95
M2	6.26	4.60	11.35	11.18	3.74	3.77	0.23	3.52	4.14
M3	6.13	5.05	9.41	10.53	4.79	4.46	0.38	3.37	3.90
M4	5.80	4.84	12.00	11.31	3.65	4.19	0.41	3.22	3.09
M5	5.44	4.41	13.08	12.51	4.17	4.73	0.32	2.96	3.75
M6	4.59	5.80	8.50	8.61	3.10	4.06	0.29	4.20	1.37
M7	4.50	4.15	8.99	9.32	4.1	3.28	0.30	3.22	1.69
M8	4.39	4.85	9.23	7.75	4.42	3.42	0.39	3.32	1.61
M9	4.63	4.23	9.42	9.91	4.20	2.55	0.29	3.16	1.44
L.S.D. $_{0.05}$	0.20	0.15	2.81	0.30	0.17	0.28	0.02	0.23	0.18

*: Ala: (Alanine), Arg: (Arginine), Asp: (Aspartic acid), Glu: (Glutamic acid), Gly: (Glycine), Ser: (Serine), Cys: (Cystine), Tyr: (Tyrosine), Pro: (Proline)

Meanwhile, M3 had significantly higher phenylalanine content followed by M4 as compared to all other male sources, while M6 and M9 had the lowest value of phenylalanine. In the meantime, M1, M2 and M4 had significantly higher values of threonine content than the other male sources, while M6, M7 and M8 had the lowest threonine content. In addition, M5 had a significant higher valine content followed by M3, M4 and M1, while both M7 and M8 had the lowest significant valine content. Furthermore, M7 had a higher histidine content followed by M6 than the other male sources, while M8 contained the lowest histidine value. Furthermore, M6 and M9 had a significant higher methonine content as compared with that of the other male sources, while M4 contained the lowest methionine value. The values of essential amino acids contents of the other sources of pollen grains were inbetween these two extremes

In accordance to the nonessential amino acids, the results in Table (4) indicated that M1, M2 and M3 had the highest significant alanine content in comparison with the other male sources, while M8 had the lowest alanine content. It was also noticed that M6 had the higher significant arginine content followed by M1 and M3 than the other male source, while M7 had the lowest value. In the Meantime, M1, M2, M4 and M5 had significantly the higher aspartic acid content, while the remained males had the lowest aspartic acid content.

Furthermore, M5 had significantly the higher glutamic acid content followed by M4 and M2 than the other male sources, while M8 had the lowest significant value. In the meantime, M3 had the higher significant glycine content followed by M8 than the other male sources, while M6 had the lowest glycine content. In addition, M1, M3 and M5 had significantly the higher serine content, while M9 contained the lowest value. Furthermore, M3, M4 and M8 had significantly the higher cystine content than the other male sources, while M2 had the lowest value. Furthermore, M6 had significantly the higher tyrosine content followed by M1, M2, M3 and M8, while M5 and M9 had the lowest significant tyrosine content. In the meantime, M2 had significantly the higher proline content followed by M1, M3 and M5, while M6, M8 and M9 had the lowest significant values of proline content. The values of nonessential amino acids contents of the other sources of pollen grains were inbetween these two extremes.

Different sources for date palm pollens showed a different combination of amino acids, similar results were recorded by Bishr and Desoukey (2012). Many studies have shown that 2-5 amino acids constitutes almost 50% of the protein weight in the pollen and the remaining amino acids (15 to 18) constitutes to the other 50% (Szczêsna, 2006). Amino acids content of pollen grains varied according to the climatic and nutritional conditions of the plants (Stanley, 1971).

Correlation analysis

Correlation coefficients among the staining and germinating percentage of pollen grains on one side and their chemical composition on the other one are presented in Table (5). The data showed that there was a positive significant correlation between stained and germinated pollen grains (r = 0.939). These results indicated that high staining percentage of pollen grains positively correlated to the germination percentage of the pollen grains.

Furthermore, both stained and germinated pollen grains were positively correlated with all the chemical constitutes in the pollen except for histidine, methionine and glycine which showed a negative correlation. The highest positive significant correlation was found between both stained and germinated pollen grains on one side and their content of arginine on the other one. This indicates that arginine can be used as an indicator of the viability of the pollen grain as compared with that of all other male sources.

Table 5: Correlation coefficient among stained and germinated pollen grains on one side and their chemical composition on the other one.

Variables	Stained	Germinated
Stained	1	
Germinated	0.939	1
Total sugars	0.180	0.132
carbohydrates	0.183	0.019
Protein	0.567	0.646
Isolusine	0.418	0.402
Leucine	0.261	0.296
Lysine	0.532	0.606
Phenylalanine	0.274	0.248
Threonine	0.551	0.495
Valine	0.451	0.423
Histidine	-0.416	-0.198
Methionine	-0.195	-0.123
Alanine	0.436	0.397
Arginine	0.693	0.700
Aspartic acid	0.352	0.280
Glutamic acid	0.124	0.110
Glycine	-0.403	-0.320
Serine	0.541	0.593
Cystine	0.088	0.090
Tyrosine	0.488	0.489
Proline	0.354	0.319

**Principal component analysis**

When analyzing a large number of multivariate variables, it is desirable to reduce their dimensionality using the PCA technique. The original variables are replaced with a smaller number of derived variables and most of the variability is retained Jolliffe (2002). A principal component analysis (PCA) was performed to clarify the relationship between the different evaluated variables. The Eigen value of each principle component is presented in Table (6). The PCA1,

PCA2, PCA3 and PCA4 explained 44.914 %, 20.805 %, 10.440 % and 7.147 % of the total variance, and all of them contributed to 83.68 % (Table, 6). In the plot (Figure 1) the weight of the different variables are presented with vectors and are shown to vary from the origin. The longer are the vectors, the greater are the loadings. Carbohydrates, histidine and cystine had lower weights as compared to the other amino acids. Thus, they contributed less to explain the data variance.

The biplot enabled the evaluation among the quantified variables, with lines presenting the same direction being more closely correlated. Serine, valine, protein content and are highly correlated also glutamic, aspartic acid, phenylaalaine and total sugars are highly correlated. There was a high correlation between the stained, germinated pollen grains and the content of lysine and arginine. Males number 7, 8 and 9 were similar in their chemical content having the lowest level of the studied chemical content.

Table 6: Principle component analysis

	PCA1	PCA2	PCA3	PCA4
Eigenvalue	9.881	4.577	2.088	1.429
Variability (%)	44.914	20.805	10.440	7.147
Cumulative %	44.914	65.719	76.528	83.675
Stained	0.157	0.328	0.064	-0.149
Germinated	0.148	0.346	-0.043	0.533
Total sugars	0.256	-0.137	0.090	0.112
Carbohydrates	-0.033	-0.119	0.137	-0.033
Protein	0.234	0.097	0.030	0.335
Isolusine	0.306	-0.049	-0.134	-0.123
Leucine	-0.189	0.301	0.296	-0.012
Lysine	0.180	0.290	-0.178	0.331
Phenylalanine	0.250	-0.092	-0.078	-0.049
Threonine	0.239	0.090	-0.169	-0.485
Valine	0.270	0.105	-0.065	-0.086
Histidine	-0.150	0.099	-0.015	0.037
Methionine	-0.256	0.156	0.465	0.099
Alanine	0.290	0.041	-0.284	0.231
Arginine	0.046	0.375	-0.340	-0.056
Aspartic acid	0.253	-0.080	0.151	-0.317
Glutamic acid	0.257	-0.092	0.189	-0.143
Glycine	0.061	-0.333	0.503	0.054
Serine	0.264	0.129	0.245	0.072
Cystine	0.068	-0.154	-0.071	-0.036
Tyrosine	-0.067	0.425	0.064	-0.149
Proline	0.301	-0.024	-0.043	0.533
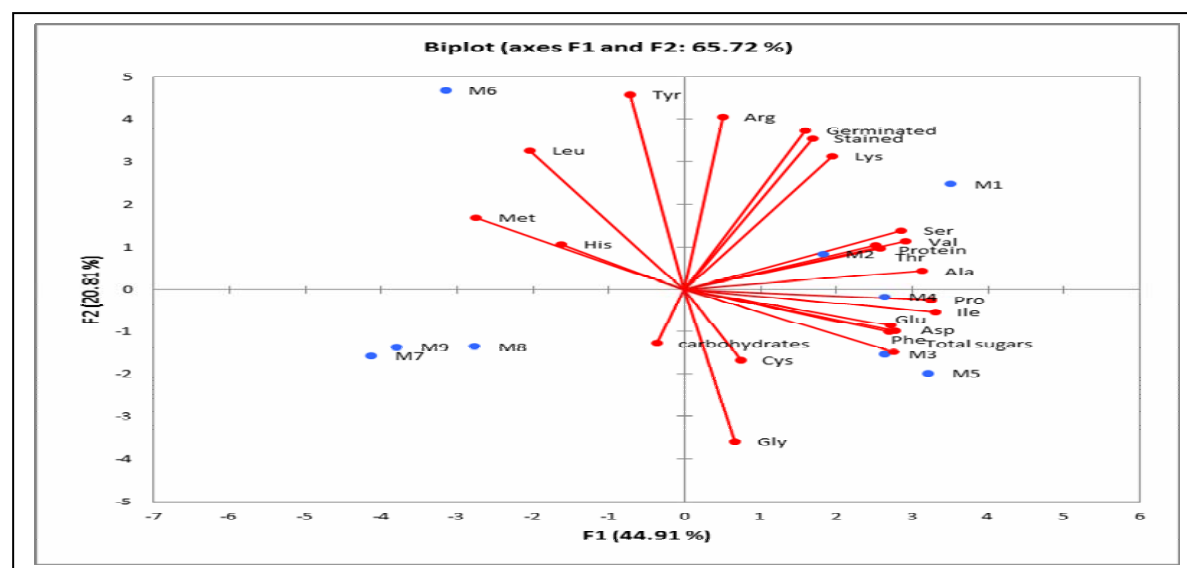
Figure 1: Biplot of the first two principle component axes for the chemical content of the pollen grains

The figure also illustrated that carbohydrate content located on the left side of the PCA1. Major chemicals defining the first principle component were total sugars, isolusine, valine, alanine, glutamic acid, serine and proline. The second principle component was mainly characterized by arginine and tyrosine. Males 3 and 5 were characterized by large amount of total sugar.

CONCLUSION

From the above mentioned results, it is generally noticed that pollen viability and chemical composition differed extremely in accordance to the different male source studied. This cleared that it (pollen viability) affected by both environmental factors in addition to genetic constituents of pollen grains of each source. It can be concluded that both arginine and lysine of pollen grains are more correlated to pollen viability. Thus, this study needs to how to increase the concentration of these two amino acids in pollen grains using some genetic markers to suitable male palm trees to increase the productivity of the female ones.

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