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***In Vitro* Germination of Date Palm Pollen Grains Affected By Different Sugar Types.**

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ABSTRACT

This study clarified the effect of the sugar types on *in vitro* germination of some date palm cultivars. Three date palm (*Phoenix dactylifera* L.) pollen grains cultivars ('Barhee', 'Amhat' and 'Hayani') germinated *in vitro* on growth media were contained five types of sugars (fructose, glucose, lactose, maltose and sucrose). The results cleared that, the favorite sugar type of pollen germination (PG) medium of 'Hayani' and 'Amhat' was lactose medium 52.16% and 29.71 % respectively while 'Barhee' was sucrose medium 47.52%. The pollen germination of the sugar type was the highest mean on lactose 36.65 %, sucrose 35.83% and maltose media 33.87% insignificantly differences. The mean of pollen tube length ranged from 70.70 to 250.8 μm , on glucose and sucrose media respectively. Pollen tube length of 'Hayani' was higher than 'Barhee' and 'Amhat' 177.7, 151.8 and 151.2 μm , respectively, whereas PG% and Pollen tube length were the lowest on glucose media of all three cultivars. *In vitro* Pollen germination percentage and pollen growth were affected by the sugar type and the different cultivars.

Keywords: *Phoenix dactylifera* L., *in vitro* Pollen germination, sugar types and pollen tube growth.

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INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a dioecious fruit tree native to the hot arid regions of the world, mainly grown in the Middle East and North Africa. Since ancient time this majestic plant has been recognized as the tree of life because of its integration into human settlement, wellbeing, and food security in hot and barren parts of the world, where only a few plant species can flourish [1].

Date palm (*Phoenix dactylifera* L.) is a multipurpose tree, with a long history of cultivation and utilization in north Africa and Middle East for at least 5000 years. Date palm is a diploid ($2n=36$), perennial, monocotyledonous plant. Being a dioecious species in character, date palm has separate male and female individuals. Female trees are cultivated mainly for their nutritive fruits. Although the average economic life of a date palm tree is estimated to be up to 50 years, the tree may stay productive up to 150 years [2]. On the other hand, the average pollen bearing capacity of a good male palm would be sufficient to pollinate 50 female palms, determined by both the number of flowers and the pollen quantity per flower [3]. However, pollen of different males has been found to have different effects on the size of fruits and seeds (xenia) as well as time of fruit ripening (metaxenia). The importance of date palm culture for its high nutritive, economic and social values is well recognised, especially in arid and semi-arid areas where it plays an important role in affecting microclimate in a way that enhances the production of other agricultural crops. Worldwide production, utilisation and industrialisation of dates are increasing continuously [4]; the Egypt production of dates had 1270478 to 1373570 ton, planting area had 36925.56 – 41651.82 (ha.) during 2009-2011 respectively according to the statistic of Agriculture Directorates of Governorates.

Being a dioecious species in character, date palm sexes are borne by separate individuals. The unisexual flowers are pistillate and staminate in character, and so artificial pollination is an essential cultural practice to obtain satisfactory fruit set [5]. Pollen grains also have a direct effect on the size, shape, and weight, as well as time of ripening of the resulting fruit [6].

Pollen viability is generally considered to indicate the ability of the pollen grains to perform their function of delivering the sperm cells to the embryo sac following compatible pollination [7].

The pH of the in vitro germination medium is the important factor controlling pollen germination and pollen tube development in different plant species [8-15]. Once released from anthers, pollen grains act as independent functional units.

Pollen germination and tube growth are known to be among the more sensitive botanical indicators of atmospheric pollution [16]. The adverse effects of air pollutants on the reproductive processes have been recorded at concentrations lower than those at which foliar

effects have been detected [17]. Pollen germination and pollen tube growth differ in their response to air pollution, although pollen germination and tube growth are interdependent, because germination includes the formation of a tube [18].

This study investigated the effect of different sugar types (fructose, glucose, lactose, maltose, sucrose) on in vitro pollen germination and pollen tube growth of date palm 'Barhee', 'Hayani' and 'Amhat'.

MATERIALS AND METHODS

The experiment carried out in Horticultural Crop Technology Department, the National Research Center, Egypt, during 2012 and 2013 Seasons.

Plant material:

Date palm pollen used in this study was obtained from trees grown at private farm, El khatatba, El Behera governorate, Egypt. The spathes of three commercial male date palm cultivars, namely 'Barhee' 'Hayani' and 'Amhat' were obtained in February month from male palms of same age and had been subjected to the same agricultural practices. During 2012 and 2013 Seasons

Pollen grains collection and processing:

The spathes of each male cultivar were taken to the traditional drying room (25- 30°C and 30-40% RH) as soon as it cracked. After 48 hours, extraction of pollen grains was carried out by the traditional hand method, and the pollen grains were shaken out as the anther dehisced and spread on large sheets of paper. Pollen grains of 5-6 male spathes were mixed together to minimize variations that might have existed between the pollen grains of those spathes then transferred into lab.

***In vitro* Pollen Germination and Pollen Growth:**

The germination medium used was the [19]. prepared in distilled water (pH 7.0), 10% from one of the sugar types (fructose, glucose, lactose, maltose and sucrose), boric acid 150 ppm and agar 1%. Germination media pH was adjusted 6.0 with 0.1 N HCl or 0.1 N NaOH measuring by digital pH meter calibrated with standard buffers solution (HANNA instrument) at pH 4.01 and 10.01 ± 0.1/ 25 °C. 10 ml Media was poured in 6-cm diameter Petri dishes in triplicates then inoculated with pollen grain using a piece of cotton and incubated in (MELAG INCUBAT®80) at 25°C for 24 hours.

Pollen germination (PG) was determined by direct microscopic examination (OLYMPUS CX 31RTSF, Japan). Pollen grains which produced a tube equal to their own diameter were

counted as germinated [9]. Pollens germination percentage was determined according to equation (1)

$$\text{Pollens germination percentage (\%)} = \frac{\text{Number of germinated pollen grains per field}}{\text{Total number of pollen grains per field}} \times 100 \quad (1)$$

Pollen tube length (um) was measured after 24 hr by T.S View soft Ware version 6.2. The average length of 100 pollen tubes for each sugar type was calculated.

Statistical analysis:

The experiment was randomized incomplete block design with three replications. The data were subjected to ANOVA and were evaluated by MSTATC program. The differences between means were compared using LSD test at 5% level.

RESULTS AND DISCUSSION

The average data of the two seasons cleared in (Fig. 1) that, the highest pollen germination was sucrose medium of Barhee 47.52% and lactose medium of ‘Amhat’ and ‘Hayani’ 29.71 and 52.15 % respectively.

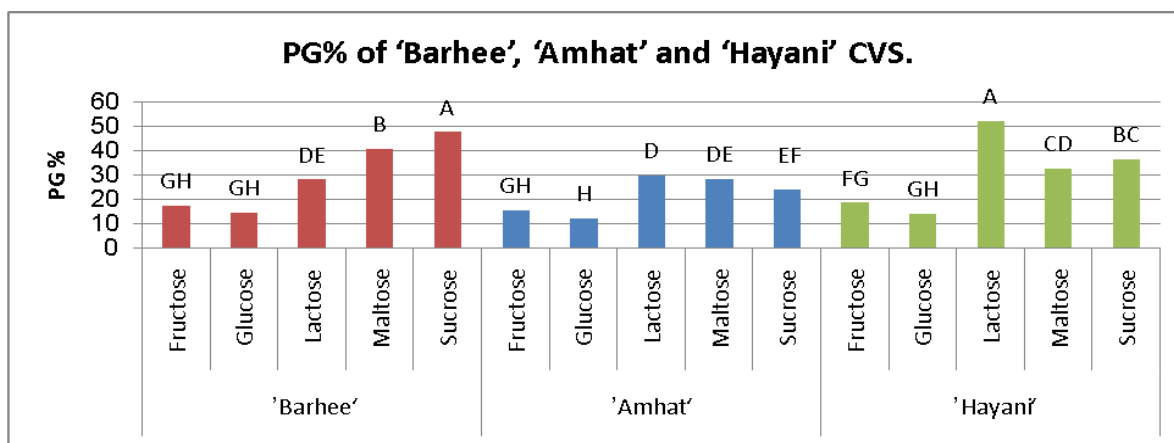


Fig 1: Pollen germination percentage of ‘Barhee’, ‘Amhat’ and ‘Hayani’ on different sugar media of fructose, glucose, lactose, maltose and sucrose. Differences in letters above the bars indicate lineages that differ significantly at p≤ 0.05.

The pollen germination mean percentage of the three cultivars was ‘Hayani’ 30.79 %, ‘Barhee’ 29.60 % insignificantly then ‘Amhat’ 21.77 %. The mean of pollen tubes length ranged from 250.8 to 70.70 μm, on sucrose and glucose media respectively. The pollen tubes length of ‘Barhee’ and ‘Hayani’ ranged from (290.5 to 65.07 μm) and from (273.8 to 62.24 μm) on sucrose and glucose media respectively, while for ‘Amhat’ ranged from (245.0 to 80.73 μm) on lactose and fructose respectively.

The mean of Pollen tubes length of ‘Hayani’ was higher than ‘Barhee’ and ‘Amhat’ 177.7, 151.8 and 151.2 μm , respectively. Then lactose media was the highest value of pollen tube length of ‘Amhat’ whereas the sucrose media was more optimal for pollen growth of ‘Barhee’ and ‘Hayani’.

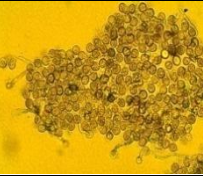
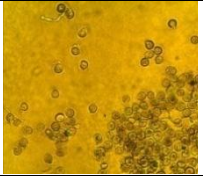
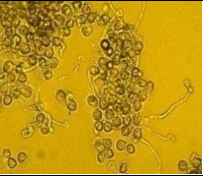
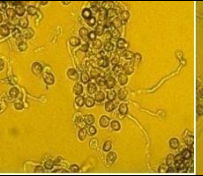
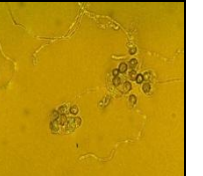
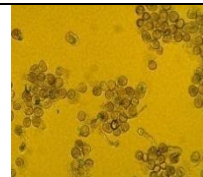

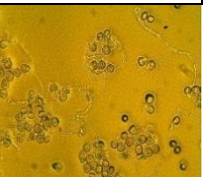
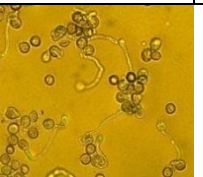
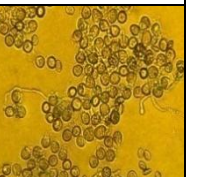
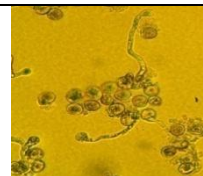
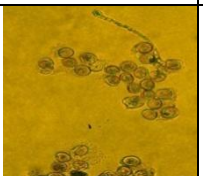
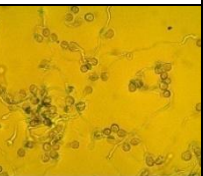
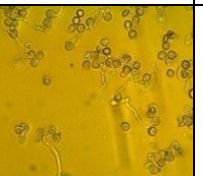
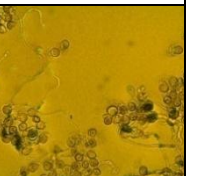
Sugar	Fructose	Glucose	Lactose	Maltose	Sucrose
Cv.					
‘Barhee’					
Pollen tube length (μm)	115.5 FG	65.07 H	129.9 EF	158.0 DE	290.5 A
‘Amhat’					
Pollen tube length (μm)	80.73 GH	84.80 GH	245.0 B	157.4 DE	188.3 CD
‘Hayani’					
Pollen tube length (μm)	112.8 FG	62.24 H	244.2 B	195.3 C	273.8 AB

Fig 2: Pollen tube length of ‘Barhee’, ‘Amhat’ and ‘Hayani’ on different sugar media of Fructose, glucose, lactose, maltose and sucrose. Note, long pollen tube length on sucrose media of ‘Barhee’ and ‘Hayani’, also on lactose media of ‘Amhat’, whereas short pollen tube length on glucose media.

The data proofed that, the pollen germination affected by the sugar type of the medium, where the highest values of pollen germination mean of the sugar types were lactose 36.65 %, sucrose 35.83% and maltose 33.87% insignificantly differences, whereas PG% and pollen tube length were the lowest on glucose medium of the three cultivars.

It is worthwhile to note there is no relationship between dominant sugar in the fruit of the three cultivars and the sugar type of the pollen germination media where the sucrose content of the fruit dry weight was ‘Amhat’ 4.1 % while 0.0% of ‘Barhee’ and ‘Hayani’ but the reduction sugars percentage was 75.7, 58.4 and 80.7% of ‘Amhat’, ‘Barhee’ and ‘Hayani’ respectively [19].

The pollen germination percentage varied according to the type of sugar and the cultivars. The soluble sugars, proteins and phenols have a positive role in pollen germination [20].

The PG% was the lowest on glucose medium of the three cultivars this result in agreement with [21] studied the monosaccharide composition of pollen and stigma surface macromolecule preparations, found that the pollen preparations contained galactose, mannose and rhamnose in approximately equal amounts, arabinose and glucose were present in lower concentrations. [22], sucrose enhanced pear pollen germination and pollen tube growth much more effectively than glucose, while fructose strongly inhibits germination on agar medium. [23], reported that, on glucose medium, the tube growth of *Camellia japonica* is inhibited competitively by maltose, whereas on sucrose medium is not. These results suggest that inhibition of pollen tube growth is due to binding of maltose to the glucose transporter, which consequently inhibits the uptake of exogenous glucose, but not that of sucrose, whereas [24] found that the promotion of lactose on pollen germination and pollen tube growth were most significant. Finally, the effect on sugar on pollen germination and pollen growth differ among species and cultivars.

CONCLUSION

Pollen germination percentage and pollen growth varied according to the sugar types in the medium and the different cultivars. The disaccharides (sucrose, maltose and lactose) were more enhanced than the monosaccharides (fructose and glucose) for pollen germination media and pollen growth of 'Barhee', 'Amhat' and 'Hayani' date palm cultivars. This study contributes of further studies to determine the sugar types on the stigma to select the appropriate pollinator.

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