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Date Palm Pollen Germination and Growth Susceptibility to Different pH Medium

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ABSTRACT

This study investigated the effect of different pH levels on *in vitro* pollen germination and pollen tube growth in 'Barhee' and 'Amhat' date palm. The susceptibility of date palm pollen to acidity or alkalinity of germination medium (pH 4.0, 5.5, 6.0, 6.5, 7.0, 8.0 and 8.5) were tested. The results cleared that the pollen germination percentage (PG%) of 'Barhee' ranged from 22.05 to 60.84% at pH 4.0 and 6.5 respectively and the PG% of 'Amhat' ranged from 12.05 to 62.44% at pH 4.0 and 5.5 respectively. The mean of pollen tube length ranged from 42.7 to 275.8 µm, at pH values 4.0and 8.0, respectively. Generally, the suitable range of pH for pollen germination of 'Barhee' and 'Amhat' was pH 6.0 to 6.5 while the PG% decreased at pH 4.0. The pH 8.0 was the highest value of pollen tube length of both cultivars. The pollen germination percentage affected by acidity or alkalinity of the medium and it was insignificant differences between cultivars.

KEYWORDS: *Phoenix dactylifera* L., pH levels, *in vitro* pollen germination and pollen tube growth.

1. 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 5 000 years. 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). Worldwide production, utilization and industrialization of dates are increasing continuously [2]; the Egypt production of dates had 1270478 to 1373570 ton; planting area has 36925.56 – 41651.82 (ha.) during 2009-2011 respectively according to the statistic of Agriculture Directorates of Governorates.

Pollen grains also have a direct effect on the size, shape, and weight, as well as time of ripening of the resulting fruit [3]. 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 [4].

The pH of the in vitro germination medium is the important factor controlling pollen germination and pollen tube development in different plant species [5-13] 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 [14]. 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 [15]. 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 [16].

This study investigated the effect of different pH levels on in vitro pollen germination and pollen tube growth of datepalm 'Barhee' and 'Amhat'. The susceptibility of different date palm pollen grains to pH medium.

2. MATERIALS AND METHODS

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

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2.1 Plant material

Date palm pollen used in this study was obtained from trees 7 years old, grown at private farm, El khatatba, El Behera governorate, Egypt. The spathes of two commercial male date cultivars, namely 'Barhee' and 'Amhat' were obtained in February month from male palms had been subjected to the same agricultural practices.

2.2 Pollen grains collection and processing

The spathes of each male cultivar were taken to the traditional drying room (25- 30ºC 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 to lab for cultivation on the germination media.

2.3 *In vitro* Pollen Germination (PG) and Pollen Tube Growth

The germination medium used was the [17] prepared in distilled water (pH 7.0), sucrose 10%, boric acid 150 ppm and agar 1%. pH was adjusted with 0.1 N HCl or 0.1 N NaOH. A series of germination media solutions with pH 4.0, 5.5, 6.0, 6.5, 7.0, 8.0 and 8.5 were prepared. The pH was measured using a digital pH meter calibrated with standard buffers solution (HANNA instrument) at pH 4.01 and 10.01 t 0.1/ 25 ºC. 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 at 25°C for 24 hours. MELAG INCUBAT®80.

Pollen germination (PG) was counted after 24 h by direct microscopic observation (OLYMPUS CX 31RTSF, Japan) Pollen grains which produced a tube equal to their own diameter were counted as germinated [6].The examination of PG were calculated at 10 fields/Petri dish and Pollens germination percentage was determined according equation (1)

Pollens germination percentage (%) = (Number of germinated pollen grains per field)/(Total number of pollen grains per field) ×100 (1)

Pollen tube length (um) was measured after 24 h. by TSView soft Ware version 6.2. The average length of 30 pollen tubes /replicate and the two cultivars represented by three replicates of each pH level were calculated.

2.4 Statistical analysis

The experiment was randomized complete 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 & DISCUSSION

The average data of the two seasons showed in (Fig. 1). It was found that pollen germination percentage of 'Barhee' was the highest values 60.84 and 59.68 % at pH 6.5 and 6.0 respectively whereas, the lowest values of PG% were 22.05 and 29.75 % at pH 4.0 and 8.5 respectively. The PG% data of 'Amhat' cv. cleared that the highest values were 62.44 and 59.52 % at pH 5.5 and 6.0 respectively followed by 52.18 % at pH 6.5, whereas the lowest value of PG% was 12.05 at pH 4.0. In general could be noticed that insignificant difference of PG% between 'Barhee' and 'Amhat'. The best level of pH for the highest PG% was at pH 6.5 and 6.0 also noticed that at pH 7.0 neutralized medium the PG% decreased that means the slight alkalinity or acidity in the germination medium stimulated the pollen germination. This results may be back to an electrical signal is able to cause the biochemical response [18] reported that local changes in ion concentration can lead to modified activities of enzymes in the cell wall (e.g. pectinase), the plasmalemma (e.g. cellulose synthetase) [19,20] found that Ions play a crucial role in the control of pollen tube growth. pH is also essential for growth. H+ form an intracellular gradient consisting of a slightly acidic domain at the extreme apex and an alkaline band located along the clear zone. H+ also exhibit an apical influx.

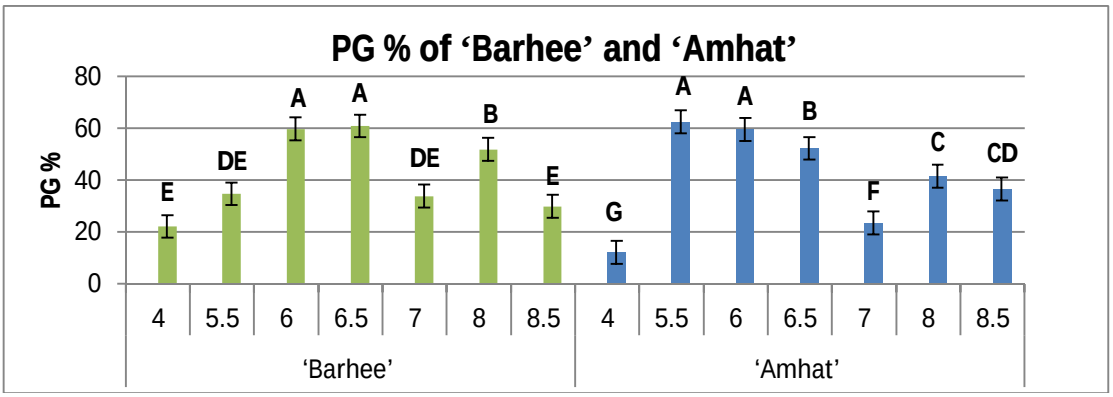


Fig (1) Pollen germination percentage of 'Barhee' and 'Amhat' at different pH levels. Differences in letters above the bars indicate lineages that differ significantly at $p \leq 0.05$.

The microscopic examination 10x of pollen germination and pollen tube length of 'Barhee' and 'Amhat' at different pH levels after 24 hours showed in (Fig. 2).

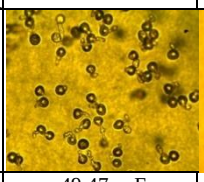
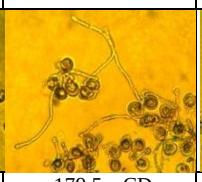
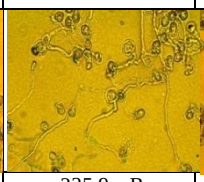
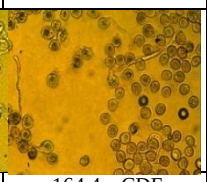
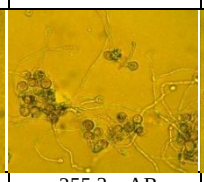
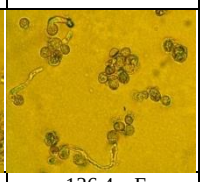
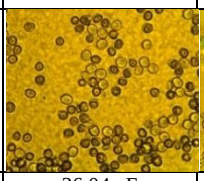
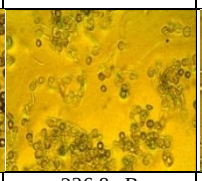
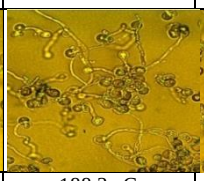
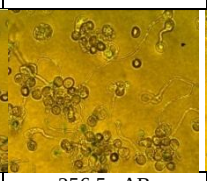
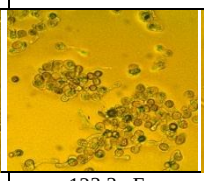
pH / Cv.	4.0	5.5	6.0	6.5	7.0	8.0	8.5
'Barhee'							
Pollen tube length (um)	49.47 F	179.5 CD	235.9 B	164.4 CDE	142.3 DE	255.3 AB	136.4 E
'Amhat'							
Pollen tube length (um)	36.04 F	236.8 B	188.3 C	256.5 AB	123.3 E	296.3 A	271.8 AB

Fig (2) Pollen germination and pollen tube length (um) of 'Barhee' and 'Amhat' at different pH levels under microscope 10x after 24 hours. Note short pollen tube length at pH 4.0 and long pollen tube length at pH 8.0.

The pollen tube length of 'Barhee' was ranged from (49.47 and 255.3 µm), while for 'Amhat' ranged from (36.04and 296.3 µm) at pH values 4.0 and 8.0 respectively. The mean of Pollen tube length of 'Amhat' was higher than 'Barhee' 201.3 and 166.2 µm, respectively. The pH 8.0 was the highest value of pollen tube length of both cultivars.

Pollen viability depends not only on its quality, but is also related to temperature, mineral nutrient and different plant growth regulators etc. in the germination environment [11, 13].

This results in agreement with [21] found that inhibition of germination and tube length with increasing acidity also with [13] reported that the highest germination percentage of *Pistacia* species at pH 6.0 while pH 3.0 had the lowest value, there were correlation between pH and pollen tube length and pH and pollen germination.

This study demonstrate the role of pH on pollen germination where in recent years, it has become more clear that chemo attractant gradients in the pistil play an important role in guiding pollen tubes to the ovules. pH can have an effect of the state of ionization of acidic or basic amino acids, pH is also a factor in the stability of enzymes. The invasion of the pollen tube through the stigmatic cuticle and cell wall would be predicted to require enzyme modification of these layers, to allow further pollen tube growth [22], the proteins and lipids in the pollen coat and on the surface of the stigmatic papillae intermix (termed foot formation) in a process that is essential for the acceptance of compatible pollen [23,24]. So that it is important to know the behaviour of date palm pollen grains at the different pH levels, also this information can be used to detect the optimum pH level which gives the pest pollen germination and may be used at the field level to increase the fruit set.

Conclusion

The suitable range for pollen germination was pH 6.0 to 6.5 of 'Barhee' and pH 5.5 to 6.0 of 'Amhat', while the PG% and the pollen tube length decreased at pH 4.0 of both cultivars. Those date palm cultivars their pollen had adaptable to a wide range of pH levels and the slight acidity or alkalinity stimulated pollen germination. The results show that the pH level of in vitro germination media is the important factor controlling of date palm pollen germination and pollen tube growth.

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