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Investigations on the pollen morphology of some date palm males (*phoenix dactylifera* L.) in Saudi Arabia

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Abstract

Pollen grains of eleven date palm males (*phoenix dactylifera* L.) were examined and photographed, using Scanning Electron Microscopy (SEM) to find morphological difference between males. Scanning electron analysis of pollen grains revealed specific variation in some morphological properties. Pollen grains from all males were monad, elliptical, and fusiform. Exine pattern of pollen grains was reticulate and irregular. The pollen grains were also different in shape, size, pollen weight, germination percentage, length, width and number of pores. The Serry cultivar followed by Succary (males) showed the highest pollen grains weight compared with the other date palm males in the two seasons. The Safry cultivar followed by Succary male showed significantly higher germination percentage and dimensions of pollen grains compared to other date palm males in both seasons. Date palm males Sallag, Khalas and Kadary had little number of pores. The date palm cultivars Dikhiny, Nabout-Zamel, Schagra and Maktumi males possessed a medium number of pores and date palm males Succary, Menify, Serry and Safry high number of pores. A comparison between shape, pores frequency and exine patterns of pollen grains proved significant variation among studied palm males. Study of pollen morphological traits can help identification of date palm males.

Keywords: Date palm, male, pollen grains, morphology, SEM.

Abbreviations: SEM_ Scanning Electron Microscopy;

Introduction

Date palm (*Phoenix dactylifera* L.) represents a focal point in crop production within the oasis system in the Middle East. It is one of the most important horticultural crops In Saudi Arabia. It ranks top among fruit trees as far as numbers (23 Million trees), area (157074 hectares) and production (986409 tones) (Ministry of Agriculture, 2009). Date palm fruits represent an important source of food to local people, because they contain minerals, carbohydrate, organic, acid, total soluble solids, vitamins and high energy. Fruit of date palm can be preserved, stored and transported to long distances.

About 400 cultivars of date palm are grown in the Kingdom of Saudi Arabia. These cultivars differ in distribution and frequency, in all growing regions. In Saudi Arabia, each date palm cultivar is specific and characterized to a region. Each female cultivar has also appropriate male pollinator. The effect of pollinator source can influence the physical and chemical characteristics of fruit set. The available number of date palm males is insufficient for efficient pollinating of increasing number of female plants in Kingdome of Saudi Arabia. Most of available pollinating date palm males are mainly originated from seed propagation, resulting in many different local males that represent genetic diversity. Characterization and evaluation of available highly potent male palms is the first step to find superior ones to fertile female plants (Rizk et al, 2007). Therefore, it is important to conduct experiments to evaluate males parents, in terms of vegetative and flower characteristics, determination of biodiversity, opining early and late pollination, and also monitoring the similarities and differences. Evaluation of physical and chemical quality of fruits, fertilized with different pollen males, is critical since source of pollen is one of the most important factors to improve production and fruits quality of date palm cultivars (Elshibli et al 2007).

The pollen formation of date palm trees (the pollination period) is during the months of February–April in Saudi Arabian environment. Most attempts to distinguish date palm males are mainly based on morphological characteristics yet (Shaheen, 1983). Some studies were undertaken to determine interspecific and intraspecific relationships between fruit tree species. A number of specific pollen grain properties have been utilized to understand differentiation between closely related plant groups. Pollen grains are the male gametophytes and take part in the reproductive biology. Fruit set in the date palm mainly depends on the pollination and fertilization process. For this reason, the viability and/or sterility of the pollen grains is of great importance in the fruit set of palm cultivars (Mert and Soylu, 2006; Fogle, 1977 a, b) compared to pollen grains of some other fruit tree species such as peach, nectarine, apple, plum and sweet cherry through

Table 1. The analysis of variance (ANOVA) of weight, germination, length and width of the pollen grains for eleven date palm males.

SOV	df	Mean squer					
		Pollen grains weight	Germinate	Pollen grains weight	Germinate	Length Pollen grain	Width Pollen grain
Male	10	92.88^{ns}	325.06**	110.98*	363.66**	6.521**	4.420**
Error	22	50.28	2.29	35.98	1.728	0.400	0.182
Total	32						

*Significant at 0.05 level probability. **Significant at 0.01 level probability. Ns = Not significant.

exine details obtained by scanning electron microscopy. The selected pollinator trees grown at Aswan, Assiut and Kerdasa were the best to improve the productivity and fruit quality of Samani and Zaghloul date palm which grown at the Experimental Research Station, in Cairo University (El-Kosary and Soliman, 2003).

The pollen morphology is an expression that comes from genome and like any other characters to be it critical to understand differences of the genotypes. The macro-morphological properties may be useful in taxonomic studies of some species or plant groups. In many species valuable information can be derived from a full pollen study leading to understand both the morphology and where possible, the functional role of the characters of the pollen grain. An understanding of the ontogeny of the pollen grain and deposition and fundamental structure of sporopollenin are of significance for comparative pollen morphologists, contributing to understand and interpret some of the structures used as distinguishing features. The pollen grain may be studied not only for comparative morphological data alone but also as clue to the unexpected aspects of breeding systems and hybridization. In this way, a better understanding of the whole biology of the group under investigation may occur. Another aspect is the relating of morphological data to fossil pollens and the fossil record and where this is possible additional insight may be obtained into evolution. (Ferguson, 1985).

Several authors suggested that structural characteristics of the pollen grains of some fruit species and pollen morphology, including size, shape and exine striation patterns may be utilized for species identification (Maas, 1977; Westwood and Challice, 1978; Lanza et al., 1996; Currie et al., 1997; Martens and Fretz, 1980; Javady and Arzani, 2001; Arzani et al., 2005; Mert and Soylu, 2007; Mert, 2009).

So, the objectives of this investigation were to evaluate the surface morphology and ultrastructure of pollen grains from seedling date palm males used in the pollination of date palm cultivars in the Dyrab, Riyadh region of Saudi Arabia.

Results

Eleven date palm males were grown in the Research and Agriculture Experimental Station at Dirab, College of Food and Agricultural Sciences, King Saud University, Riyadh of Saudi Arabia. The scanning electron microscope (SEM) study of the pollen grains indicated that all males had a monad, elliptical-oblate pollen with one deep germinal furrow across the polar surface (Fig 1 and 2)**.** In Table 1, analysis of variance (ANOVA) of weight, germination, length and width of the pollen grains for eleven date palm males have been shown. In Table 2, the results of the pollen grains weight, germination percentage and dimensions of the date palm male in the two seasons are presented.

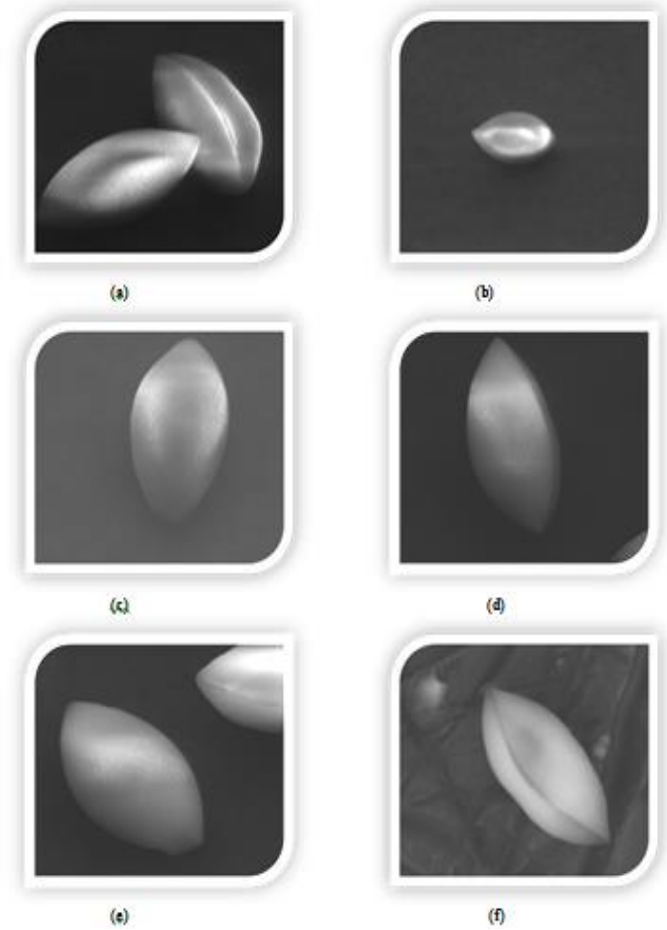

Fig 1. Scanning electron microscopy (SEM) images of pollen grains morphology of date palm males (*phoenix dactylifera* L.). (a) Succary male, (b) Menify male, (c) Sallag male, (d) Dikhiny male, (e) Nabout-Zamel male and, (f) Serry male.

We also presented the the results of morphological characteristics in Table 4 and 5.

Pollen grains weight

Analysis of variance (ANOVA) showed that pollen grains weight is not significantly different among the eleven date palm males. However, it noted that there are other significant differences among males. The result of ANOVA indicates that there is no significant difference in pollen grains weight between different date palm males in both seasons.

Table 2. Mean values for weight, germination, length and width of the pollen grains from eleven date palm males.

Male	2011-2012 seasons		2012-2013 seasons		2012-2013 seasons	
	Pollen grains weight (g)	Germinate (%)	Pollen grains Weight (g)	Germinate (%)	Length Pollen grain (µm)	Width Pollen grain (µm)
Succary	21.33^{a}	81.33^{b}	33.25^{ab}	82.80^{a}	21.40^{b}	10.20^{a}
Menify	16.05^{a}	76.40^{c}	15.27^{c}	77.43^{b}	19.33^{cde}	9.20^{b}
Sallag,	16.87^{a}	57.50^{g}	22.05^{c}	59.43^{e}	19.30^{cde}	9.60^{ab}
Dikhiny	18.58^{a}	61.33^{f}	24.52^{bc}	59.53^{e}	19.66^{cd}	7.43^{e}
NaboutZame	12.63^{a}	67.87^{d}	25.00^{bc}	66.27^{c}	19.10^{de}	7.67^{de}
Serry	21.42^{a}	75.67^{b}	36.62^{a}	76.83^{b}	20.30^{c}	8.10^{cd}
Khalas	15.33^{a}	61.63^{f}	21.30^{c}	59.40^{e}	18.40^{e}	7.22^{e}
Shagra	13.27^{a}	63.42^{ef}	21.46^{c}	62.30^{d}	19.57^{cd}	8.43^{c}
Safry	4.97^{a}	87.43^{a}	19.42^{c}	84.27^{a}	22.63^{a}	10.30^{a}
Maktumi	8.29^{a}	65.43^{de}	21.06^{c}	66.67^{c}	18.47^{e}	7.63^{de}
Kadary	7.08^{a}	54.10^{h}	23.28^{bc}	50.70^{f}	17.20^{f}	6.97^{e}

* Each value is an average of 3 specimens. Mean values with the same letters in the same column are not significantly different at 5% level of probability according to LSD test.

Date palm Serry followed by date palm Succary male showed the highest pollen grains weight compared to other date palm males in two seasons.

Germination percentage of pollen grains

Data in Table 2. clearly indicated that germination percentage was significantly variable in different date palm males in both seasons. Date palm Safry followed by Succary, Menify and date palm Serry male significantly increased germination percentage compared to other date palm males in both seasons. In addition, date palm Kadary male followed by Sallag and Khalas male showed significant decrease in germination percentage compared to others date palm males in the first and second seasons, respectively.

Morphological characteristics of pollen grains

Pollen grain dimensions (µm)

Table 2. clearly indicate that average pollen grain length and diameter (dimensions) are significantly different among cultivars in different seasons. Date palm Safry male followed by date palm Succary significantly showed bigger pollen grain dimensions compared to other date palm males. Date palm Kadary males and Khalas exhibited significant decrease in pollen grain dimensions compared to others date palm males.

Number of pores

The number of pores per µm^2 in pollen grains and average number of pores were significantly differed among cultviars. The results obtained indicate that males could be divided into three groups as follows: males having a little number of pores (under 10 pores), males having a medium number of pores (10-20 pores) and males having a many number of pores (more than 20 pores), named as first, second and third groups, respectively. Therefore, date palm males Sallag, Khalas and Kadary had little number of pores, date palm males Dikhiny, Nabout-Zamel, Schagra and Maktumi males medium number and date palm males Succary, Menify, Serry and Safry higher number number of pores.

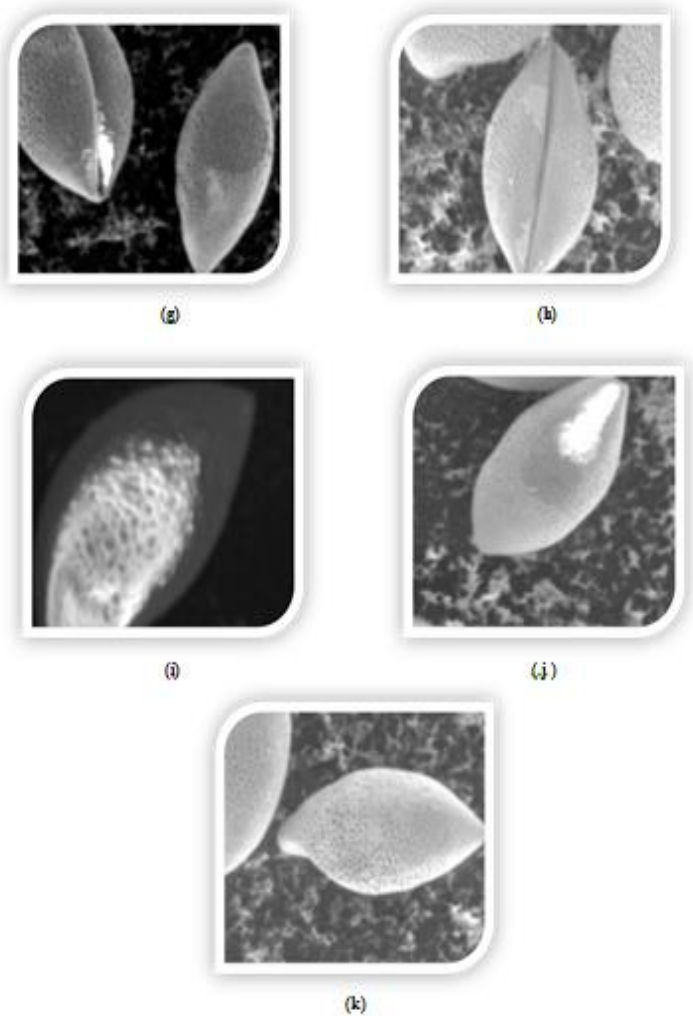

Fig 2. Scanning electron Microscopy (SEM) images of pollen grains morphology of date palm males (*phoenix dactylifera* L.). (g) Khalas male, (h) Schagra male, (i) Safry male, (j) Maktumi male and (k) Kadary male.

Table 3. Description characteristics of date palm males during 2011-2012.

Males	Palm top	Pinnae colour	Pinnae distribution	Pinnae end	Pinnae bent	L. basal colour	Spines
Succary	Open	Dark green	Single	Single	Bent	Light green	Double
Menify	Open	Dark green	Single	Single	Bent	Light green	Double
Sallag,	Open	Dark green	Single	Single	Bent	Light green	Single
Dikhiny	Open	Dark green	Single	Single	Bent	Light green	Single
Nabout-Zamel	Open	Dark green	Single	Single	Bent	Light green	Single
Serry	Open	Dark green	Single	Single	Bent	Light green	Single
Khalas	Open	Dark green	Single	Single	Bent	Light green	Single
Shagra	Open	Dark green	Single	Single	Bent	Light green	Double
Safry	Open	Dark green	Single	Single	Bent	Light green	Single
Maktumi	Open	Dark green	Single	Single	Bent	Dark green	Single
Kadary	Open	Dark green	Single	Single	Bent	Light green	Double

Therefore, these numbers of pores in the pollen grains could be used for the identification of such males (Table 2).

Discussion

The results of this study explained that the pollen grain weight is highest in Serry and lowest in Safry male, compared to others. The germination percentage was also significantly varied in different date palm males in both seasons of study. The differences in pollen germination among studied males cultivars could be due to the variability in their genetic background. It seems that pollen of males Safry and Succary have the highest compatibility with the main female cultivars in Saudi Arabia condition. These results are in line with those reported by (Tisserat et al., 1985; Al-Helal et al., 1988; Wang et al., 2004; Mortazavi et al., 2010; Beyhan and Serdar, 2009). Pollen grain length and diameter were significantly differed in both seasons which might be a genetic variability. These results are in agreement with other authors on different cultivars of date palm males (Tisserate and De Mason, 1982; Shaheen, 1983; Shaheen et al., 1986), Cornelian Cherry (*Cornus mas* L.) cultivars (Mert, 2009), some fruit species (Evrenosoglu and Misirli 2009), anemophilous trees (Molina, 1996), Walnut (*Juglans regia* L.) (Mert, 2010), and wild atles pistachio (*Pistacia atlantica* Desf., Anacardiaceae) (Belhadj et al., 2007). Similar results were reported in strawberries, stone fruits and pyrus species (Mass, 1977; Fogle, 1977 a, b; Westwood and Challice, 1978).

The number of pores divided into three groups, low, medium and high. Number of pores differed significantly in male pollen grains and this is due to the different genotypes characteristics of each male to be taken to distinguish different males. In addition, these numbers of pores in the pollen grains could be used for the identification of such males. These results are in agreement with those found by (Tisserat and De Mason, 1982; Shaheen, 1983; Shaheen et al., 1986) on date palm, (Fogle, 1977a, b; Mert and Soylu, 2007; Mert, 2009) on other fruit tree species.

Materials and methods

The present investigation was carried out at Research and Agriculture Experimental Station at Dirab, College of Food and Agricultural Sciences, King Saud University, Riyadh. Eleven 10-year-old date palm males: Succary, Menify, Sallag, Dikhiny, Nabout-Zamel, Serry, Khalas, Schagra, Safry, Maktumi and Kadary grown on sandy soil were selected. The experimental palms were healthy, uniform in growth, vigor and height. All cultural practices were carried out according to the normal schedule for experimental palms. Pollen samples were collected from flowers of eleven seedling date palm males. Three branches from various parts of each tree (three to each male) were isolated before anthesis with paper bags to avoid contamination with other pollen sources. The strands of each spathe were cut and left to dry at room temperature. Then, pollen grains were separated from the flowers using fine sieves (40 mesh) (Shaheen, 1986). After that, the weight of pollen grains per male (three for each male). Pollen was collected in small vials and stored in desiccators at 3-5 ˚C (Javady and Arzani, 2001) until used. Pollen grains observed at 20 KV with a Stereoscan (360 SEM - FEI / Inspect S50 model) were photographed at 10000 x for whole grain.

Germinate percentage

A little quantity of pollen grains was placed on sugar solution (at 8 % sucrose) in Petri-dish for 2-4 hours. After that, pollens were examined under Olympus compound microscope (Bx-51) to find pollen grains viability, which measured by ocular micrometer. The total number of pollen grains and number of germinated pollens was calculated as follows.

$$\text{Germinate \%} = \frac{\text{Germinate number of pollen grains}}{\text{Total number of pollen grains}} \times 100$$

Statistical analysis

The experimental design was complete randomize design (CRD) according to (Steel and Torrie, 1980). The results were analysis using statistical analysis system, SAS. The least significant differences of 0.05 probabilities (LSD 0.05) were applied to detect the differences between males.

Conclusion

The differences of pollen grains among eleven male date palms were studied. Studied parameters helped identification and of date palm males. The pollen of cultivar Safry and Succary had the highest compatibility with the main female cultivars. It could be concluded that pollination of females with the these males (Safry and Succary) are the best to improve the productivity and fruit quality (physical and

Table 4. Leaf morphological characteristics of date palm males during 2011-2012 seasons.

Males	Mean leaf Length (cm)	Mean pinnae part length (cm)	Mean pinnae number	Mean pinnae Length (cm)	Mean pinnae Width (cm)	Mean spines Part length (cm)	Mean spines number	Mean Sspines Length (cm)
Succary	414.00^{ab}	309.67^{a}	224.00^{a}	61.00^{a}	3.07^{de}	104.33^{abc}	21.67^{a}	9.23^{cd}
Menify	384.67^{abc}	256.33^{e}	175.33^{d}	48.33^{cd}	2.71^{e}	128.33^{a}	16.00^{bc}	12.10^{b}
Sallag,	418.67^{a}	301.67^{a}	194.00^{b}	53.33^{bc}	2.97^{de}	117.00^{ab}	13.00^{cd}	10.20^{bcd}
Dikhiny	369.33^{c}	268.33^{cde}	220.00^{a}	53.67^{bc}	4.30^{a}	101.00^{abc}	14.67^{cd}	9.00^{cd}
Nabout-Zamel	362.67^{cd}	277.00^{bc}	198.33^{b}	48.00^{cd}	3.73^{bc}	85.67^{bcd}	12.67^{cde}	8.53^{cd}
Serry	349.67^{cd}	262.00^{de}	214.00^{a}	51.67^{cd}	3.27^{cd}	87.67^{bcd}	15.33^{bcd}	9.10^{cd}
Khalas	379.33^{bc}	286.00^{bc}	176.00^{d}	59.00^{ab}	2.97^{de}	93.33^{abcd}	18.33^{ab}	15.33^{a}
Shagra	384.68^{abc}	307.68^{a}	180.67^{cd}	46.33^{d}	2.70^{e}	77.00^{cd}	11.67^{def}	11.67^{b}
Safry	374.33^{c}	279.33^{bc}	189.67^{bc}	50.00^{cd}	3.07^{de}	96.00^{abc}	12.33^{cdef}	9.67^{cd}
Maktumi	327.33^{d}	272.00^{bcd}	168.87^{d}	49.00^{cd}	3.77^{b}	55.33^{d}	8.67^{f}	11.00^{bc}
Kadary	380.00^{bc}	380.00^{a}	193.33^{b}	60.67^{a}	3.37^{bcd}	66.67^{cd}	9.00^{ef}	10.33^{bcd}

*Each value is an average of 3 specimens. Mean values with the same letters in the same column are not significantly different at 5% level of probability according to LSD test.

Table 5. Spathe morphological characteristics of date palm males during 2011-2012 seasons.

Male No.	Mean spathe length (cm)	Mean spathe diameter (cm)	Mean spathe Stand length (cm)	Mean spathe Weight (kg)	Mean strands Number/ spathe	Mean strands Length (cm)	Mean flowers Number/ strand	Mean total number of flowers
Succary	153.33^{a}	18.33^{abc}	76.00^{a}	4.133^{b}	233.3^{bc}	35.67^{a}	56.47^{bc}	13174.45^{cd}
Menify	117.33^{bcd}	18.67^{a}	47.33^{bc}	3.717^{bc}	283.3^{ab}	32.47^{a}	50.87^{c}	14411.47^{c}
Sallag,	94.00^{def}	20.33^{a}	52.67^{ab}	3.167^{c}	246.7^{abc}	28.67^{ab}	49.57^{c}	12228.92^{cd}
Dikhiny	144.00^{ab}	19.67^{a}	62.00^{ab}	5.100^{a}	291.0^{ab}	33.30^{a}	69.80^{b}	20311.8^{b}
Nabout-Zamel	123.33^{bc}	15.67^{bcd}	51.00^{ab}	2.933^{cd}	286.0^{ab}	31.13^{a}	61.13^{bc}	17483.18^{bc}
Serry	97.33^{cde}	13.00^{d}	38.33^{bcd}	1.600^{ef}	150.7^{d}	27.67^{ab}	57.10^{bc}	8604.97^{d}
Khalas	72.00^{efg}	14.67^{d}	26.33^{cd}	2.000^{e}	293.3^{a}	30.57^{a}	99.10^{a}	29066.03^{a}
Shagra	60.33^{g}	17.67^{abc}	23.67^{cd}	1.633^{ef}	242.7^{abc}	21.87^{ab}	65.10^{bc}	15799.77^{bc}
Safry	108.00^{cd}	13.33^{d}	48.33^{bc}	2.133^{de}	154.0^{d}	25.80^{ab}	55.10^{bc}	8485.4^{d}
Maktumi	71.67^{efg}	18.00^{abc}	16.67^{d}	0.770^{f}	200.0^{cd}	15.90^{b}	59.10^{bc}	11820^{d}
Kadary	70.33^{fgbc}	17.67^{abc}	21.67^{d}	2.267^{de}	220.0^{cd}	26.90^{ab}	59.57^{bc}	13105.4^{cd}

*Each value is an average of 3 specimens. Mean values with the same letters in the same column are not significantly different at 5% level of probability according to LSD test.

chemical properties of fruits) under Saudi Arabia's condition.

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