

## Short Communication

## Evaluation of pollen quality and pollination method of date palm (*Phoenix dactylifera*)

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**ABSTRACT:**

The present study aimed to improve the cultural practices applied to the cultivation of the date palm, *Phoenix dactylifera*. The trial focused on the study of two pollen viability test methods and the comparison between two pollination methods, viz: a traditional manual method and a mechanical method. The pollen viability test by acetocarmin staining showed an average viability rate of 96% for the fresh pollen and the pollen resulting from the grinding of the entire male inflorescence. The pollen seed germination test on the modified Brewbaker and Kwack culture medium gave an average of 52% for fresh pollen and 4% for crushed pollen. One-way analysis of variance on the fruit set rate of the Deglet Nour variety showed a significant difference between mechanical pollination tested with three pollen carriers and manual pollination. The results of the present research encourage the mechanization of the pollination technique with ash as a support for the pollen.

**Keywords:**

Date palm, Pollination, Color test, Germination test, Deglet Nour.

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## INTRODUCTION

The pollination of the date palm, *Phoenix dactylifera* has specific aspects. It is a dioecious plant, which requires the intervention of the wind or man to pollinate the female flowers. Artificial pollination, which makes it possible to overcome the drawbacks of dichogamy, is known for a very long time (Munier, 1973). According to Toutain (1967) and Munier (1973), due to the considerable decrease in the number of male palms on farms for economic reasons, traditional pollination has become insufficient. Several studies have been carried out to mechanize pollination in view of its importance and its difficulty (Nourani *et al.*, 2017).

Male palms locally named "Dokkars" form heterogeneous populations that are rarely cloned in which each individual has its own characteristics (Boughdiri, 1994). Much research on the selection and evaluation of male palms showed that these palms were different in their physiological parameters.

According to Sedra (2003), the choice of male palms must present important characteristics, in particular the high production of pollen grains, its aptitude for conservation, its biological fertilization capacities (quantity and quality of pollen grains) as well as its metaxenic effects or xenics on the productivity, quality and maturity of the fruit. Appreciation of the germination, fertility and vitality of pollen is necessary for the successful operation of pollination.

The lack of qualified labor, the need for frequent climbs with the high risk of accident, the extension of the areas of the palm grove by the development of new land requires the mechanization of cultivation operations to encourage the cultivation of palm trees or date palm. There is always a scarcity of labor, the limits of mechanization and the interest of diversifying activities. In all the cases, with the possible exception of the Nile valley, the scarcity and aging of the labor force constitute a major problem (Ferry, 1996). The objective of the present study is to highlight the efficiency of cultural

practices used for date palm pollination by comparing between crushing the entire male inflorescence and pollen without second crushing and comparing between two pollination methods, *viz*: a traditional method applied manually and a second method requiring the use of a good quality pollen lightened with three types of carriers namely wheat flour, corn flour and ash.

## MATERIALS AND METHODS

### Location of the study site

The study is carried out on a new developed farm located in the Hassi Ben Abdellah wilaya of Ouargla region, with an area of 200 ha which presents 4000 vines of date palm including 90% variety of Deglat Nour aged between 12 years and 30 years old located 14 km from the capital of the wilaya of Ouargla having the geographic coordinates of 31°57'09.0" North and 5°29'22.7" East. The pollen was collected from the experimental exploitation of Kasdi Merbah Ouargla University having the geographic coordinates of 31°56'27.0" North 5°17'39.0" East located six km from the capital of the city of Ouargla. The climate of the region is a contrasting of desert climate, very little rainy and very irregular, with significant thermal differences strong and frequent winds.

### Pollen collection

For this step, two methods were adopted:

- For the first method after collecting the husks, it was put in shelter for two days to dry, the pollen was recovered by shaking the spathe and finally the whole is sieved to recover as much pollen as possible.

- In the second method consists in crushing the whole of the male inflorescence, the spathe and part of the spadix were crushed using an electric grinder, then the pollen were recovered by sieving.

### Methods for assessing pollen quality

In order to detect existing variations between the studied pollens coming from different plants, as well as to see the difference between a fresh pollen newly col-

lected and a pollen whose inflorescence is totally crushed, a practice often used to recover the maximum of pollen was used. To choose the best pollen (a germination rate exceeding 60%) for a mechanical pollination, two viability tests were opted.

#### Color test

In order to estimate the quality of the pollens, acetocarmin staining was done. The grains stained in red were considered viable and the unstained grains were considered non-viable (Tahir and Asif, 1983). The percentage of viability (PV) was determined using the following formulae:

$$P.V (\%) = \frac{\text{Number of coloured grains}}{\text{Total number of grains}} \times 100$$

#### Germination test

We used the culture medium of Brewbaker and Kwack (1963), which was modified and adapted for date palm pollen by Furr and Enriquez (1966). The sowing of pollen in the culture medium was carried out under the hood by gently shaking the pollen with a brush. After a 24 h incubation, in an oven set at 25° C, the counting was done under an optical microscope (x400). The Germination Percentage (PG) was evaluated by examining three microscope fields at a rate of 50 grains/field according to the following formula:

$$P.G (\%) = \frac{\text{Number of coloured grains}}{\text{Total number of grains}} \times 100$$

#### Pollination methods and experimental set-up

Two pollination methods were tested, namely the so-called traditional or manual method, in which the knowledgeable worker must climb the palm tree several times the pollination is done by introducing the male spikelets upside down in the female inflorescence after bursting of the spathes. Inflorescences were loosely tied up. Mechanical pollination consisted of diffusing the powder of pollen grains mixed with a mechanically inert support without the need to climb the palm trees. We adopted an experimental block device comparing the traditional method with the mechanical method with

three supports (wheat flour, corn flour and ash), each pollination method is repeated ten times making a total of 40 female feet of the Deglet Nour variety. The fruit setting rate as a variable measured was calculated after one month of pollination (Number of fruits at the fruit setting stage), the number of flowers fertilized compared to the total number of flowers was counted.

#### Pollination apparatus

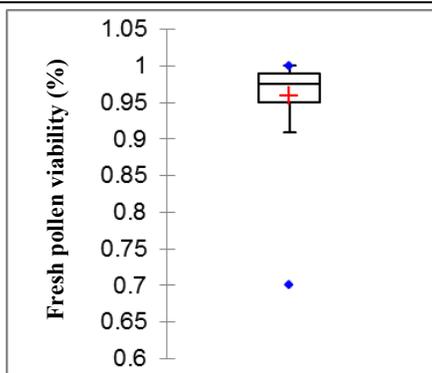
The apparatus composed of a motor and a turbine fitted with a light telescopic rigid peach, the end of which provided with a nozzle to ensure good dispersion of the pollen. The pollen and the support were placed in a glass jar attached to the side of the turbine, provided with a ventilator to stir the mixture (Figure 1).

#### Pollen preparation with support

Powder pollination requires 2 to 3 times more pollen than the traditional practice. In order to save the pollen, we have added three types of support, such as wheat flour, corn flour and the ash from the incineration of finely sieved palm grove waste. According to Babahani *et al.* (1997), on mixing the pollen with a diluent (talc, wheat flour, ash, etc.), this mixture saves the quantities of pollen and ensures its good distribution on the inflorescence.



Figure 1. Mechanical pollination apparatus



**Figure 2. Percentage of viable fresh pollen by staining test**

#### Percentage of pollen support

The percentage of pollen proposed according to the Belaroussi protocol (1994) was 20%, and Babahani *et al.* (1997) was 9% pollen only. But in our study we have used 14% pollen, with an average amount of 3 grams per palm tree, a total of 90 g for the 30 mechanically pollinated plants.

#### Number of passes

The number of passages is closely related to the receptivity of the cultivar, because beyond a certain time, the passage is no longer effective. The Deglet Nour date variety, the subject of our trial, according to Belguedj and Tirichine (2008) has a floral receptivity period of 14 days. We made two passes for each support, the interval between the two treatments was 6 days.

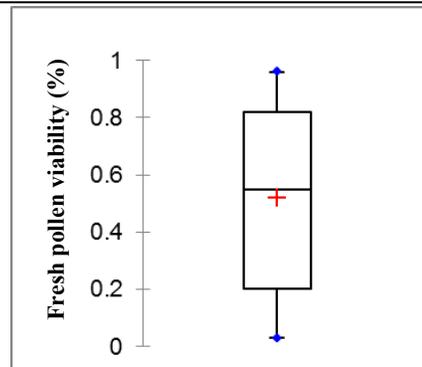
#### Statistical analysis

A box plot presentation of the results of pollen viability and an analysis of variance (one-way ANOVA) was performed on pollination methods with Fisher's test (LSD) for analysis of the differences between the modalities with a confidence interval of 95% by the XLSTAT software version 2009 1.02.

## RESULTS

#### Viability test by staining acetocarmin from fresh pollen

The viability of fresh pollen tested by staining was between 70% and 100%, the average being 96%



**Figure 3. Germination rate of fresh pollen on modified Brewbaker and Kwack culture medium**

(Figure 2). The pollen grains were easily colored by acetocarmin, a single sample showed a rate of 70%.

#### Viability test by germination of fresh pollen

There was a remarkable difference between the different percentages of germination of the pollen of the selected male plants. The interval of the rate of germination of the grains of pollen germinated on culture medium of Brewbaker and Kwack modified was between 3% and 96%, with an average of 52%, (Figure 3). 50% of the samples tested showed a germination rate greater than or equal to 60%. The pollen grain was considered alive after germination and appearance of the pollen tube.

#### Viability of pollen grains crushed with spathe

##### Acetocarmin stain viability test

The coloring test carried out on the ground pollen, revealed a very high percentage of viability, the viability interval was between 94% and 99%, with an average of 96% (Figure 4).

##### Germination viability test of crushed pollen

The pollens tested after grinding revealed a very low germination rate, a maximum of 10% and a minimum of 0% with an average of 4% (Figure 5). Crushing the entire inflorescence damaged the pollen. Viable pollen with a pollen tube was difficult to observe.

#### Influence of pollination methods on fruit set rate

The analysis of variance (Table 1), on the fruit set rate for the four pollination methods, traditional pollination and mechanical pollination with the three types

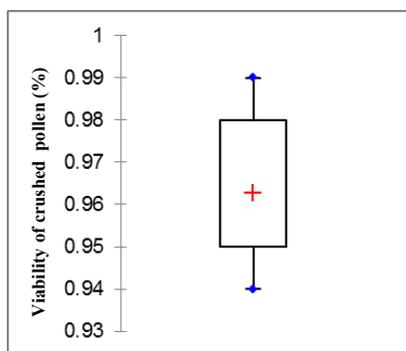


Figure 4. Percentage of viable crushed pollen by staining test

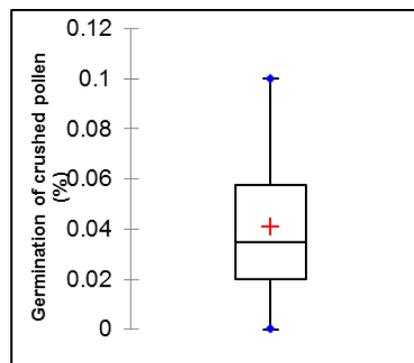


Figure 5. Germination rate of crushed pollen on modified Brewbaker and Kwack culture medium

of pollen supports, wheat flour, corn flour and ash showed significant differences ( $P=0.042$ ).

According to the Fisher test (LSD), analysis of the differences between the modalities with a confidence interval of 95%, presented three homogeneous groups (Figure 6). The traditional pollination method (Group A) presented the lowest fruit set had of 27.99%, mechanical pollination with corn support (intermediate group AB) a rate of 28.59% and a third group (Group B) which presents the two methods of mechanical pollination with ash support had of 30.54% and 31% for the wheat flour support.

### DISCUSSION

Pollination of the date palm is a limiting factor in the dates production, which is also a difficult operation and requires skilled labor. Phoeniculturists consider this to be the crucial phase of production, which must be carried out with precision to ensure good economic performance (Mostaan, 2006). Mechanical pollination must replace the know-how of phoeniculturists for this, and this modern practice requires the mastery of two important techniques:

### Determination of pollen quality

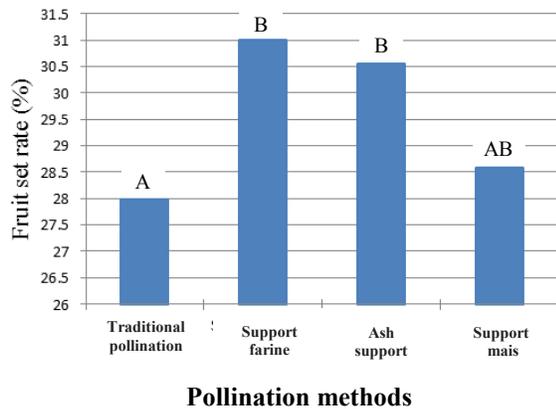
The quality of the pollen determined from the staining by acetocarmin gave all types of pollen a very high percentage of viability between 70% and 100%. Although this method is simple and fast, it is still used in many laboratories. Boughediri and Carbonnier (1993) observed that this test was not a viability test itself, since the carmine binds to the cytoplasm and the pollens which degenerate were also colored, since the cytoplasm was always present there.

The pollen germination viability test exhibited a significant variability between 0% and 96%. According to Peyron (2000), the pollen must germinate *in vitro* over 60% to ensure good fruit set. *In vitro* germination is an important technique for estimating pollen viability, but it is difficulty to choose the optimal culture medium (Khatun and Flowers, 1995).

Although the crushed pollen has a high percentage of viability by the method of staining with acetocarmin, the *in vitro* germination method gave very low rates between 0% and 3%, The operation of crushing all the male inflorescence deteriorated the quality of pollen. According to Boughediri (1994) germination

Table 1. Analysis of variance on the fruit set rate

S. No	Source	DDL	Sum of squares	Average of squares	F	Pr> F
1	Pollination methods	3	60.336	20.112	3.057	0.042
2	Mistake	33	217.126	6.580	-	-
3	Total corrected	36	277.461	-	-	-



**Figure 6. Graph of the average fruit set rate by pollination method**

can be influenced by incubation temperature, pH, osmotic pressure, water content, mineral composition, sowing technique and density as well as pollen related factors.

#### The pollination method

The comparison between the two methods of traditional and mechanical pollination showed that the medium used to lighten the pollen is important for this operation. The analysis of variance showed a significant difference between the two methods used for this experiment, namely traditional pollination and mechanical pollination. Several researchers working on the mechanization of the date palm are working to develop devices that help pollination (Hussein *et al.*, 1987; Mostaan, 2006; Kerhoas-Digonnet and Gay, 1989; Mortazavi *et al.*, 2010; Khalil and Al-Shawaan, 1983).

The comparison between the different supports used for mechanical pollination namely wheat flour, corn flour and the ash with a support/pollen percentage of 14% has shown that the highest fruit set rates were obtained by the mechanical method with the flour support and the ash support. According to Nourani *et al.* (2017), it turns out that the 20% pollen dose gave the best fruit set, while the 100% pollen dose produced a lower rate. This may be due to the effect of the wheat flour which easily transports the pollen grains to the flowers. According to Sedra (2003), mechanical pollina-

tion has a low cost of the operation requiring less labor load. This method has a higher yield compared to traditional manual pollination. Among these advantages, a saving in the quantity of pollen at ease and speed of carrying out the operation with a low risk of accidents due to frequent ascents of the palm tree by workers, in the case of traditional manual pollination, the ash support has shown a significant effect comparable to wheat flour.

#### CONCLUSION

Percent viability of pollen tested by acetocarmin staining method over estimated pollen viability, Brewbaker and Kwack's modified pollen germination test method showed a clear difference between different male plants. From a pollen quality point of view, this test may be a good method for choosing quality pollen for possible use by mechanical pollination. Mechanical pollination with the more economical ash medium and more available to phoeniculturists can replace traditional pollination, the means most currently used for date palm pollination. Mechanical pollination as a technique can be successful if a good means of extension is found.

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