

Trials for prolonging pollen grains storability in pollination Saidy date palms grown under new valley conditions

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Abstract

This study was undertaken during 2017 and 2018 as a trial to extend the storability of pollen grains in date palm cv. Saidy grown under New Valley environmental conditions. To overcome the problem of decreased pollen grains in the new cultivation areas with high female density without giving attention to male cultivation in addition to solve pollen grains of each good male varieties. The treatments included storing of pollen grains under room temperature (25-30 °C), cooling at 5-7 °C for one year and freezing at -18°C for two, four or eight years. Percentages of pollen viability and germination, fruit setting %, yield, bunch weight and fruit quality in response to the present treatments were investigated.

Pollination with fresh, refrigerated, cooled and room temperature stored pollen grains, in descending order succeeded in improving the percentages of pollen viability and germination, fruit setting %, yield, bunch weight and fruit quality. Increasing storage durations was followed by decreasing all the studied parameters. The best method of storage was freezing process at -18°C followed by cooling at 5-7 °C. Storing at room temperature occupied the last position in this respect.

Pollination of Saidy date palms at 2 days after cracking with fresh pollens or freezed pollens at -18 °C for two years gave the best results with regard to yield and fruit quality. Hence, it could be concluded that freezing pollen grains for two years to used them for pollination.

Keywords: Pollen viability, Pollen germinations, freezed pollen grains, cooled pollen grains, fruit setting %.

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Introduction

Artificial pollination of date palms is considered as important horticultural practice essential for producing an economical yield. For succeeding of pollination both male and female flowers should be bloom at the same time (Simon, 1987, Ashour *et al.*, 2004; El-salhy *et al.*, 2020 and El-Salhy *et al.*, 2012). Hence there is a never shortage to pollination processes that are very important recently for economic fruits production Therefore good storage for keeping higher variability and germination as well as transmit them from one place to an there. Pollen subjected to damage under higher temperature and relative humidity conditions. For prolonging storage duration of pollens, they are dried, placed in air tight conditions and held at lower temperature i.e. cooling or freezing (Simon, 1987, Shaheen *et al.*, 1986 and Wrigley, 1995).

Previous studies showed that pollination in different date palm cvs. just after spathes cracking with fresh pollen grains occupied the top method in improving yield followed by using pollens stored under cooling and freezing status. Storing pollen grains at room temperature (25-30 °C) for more than one year gave unfavorable effects on percentages of pollen viability and germination, fruit setting, yield, bunch and fruit quality characteristics. Extending storage durations of pollens especially when they held at room temperature caused a gradual reduction on all the investigate-d parameters in different date palm cvs. (Rahim, 1975; Shaheen *et al.*, 1986; El-Kassas *et al.*, 1995; Ashour *et al.*, 2004; El-Agamy *et al.*,

2008; Saad, 2008; El-Sese *et al.*, 2010, El-salhy *et al.*, 2012 and Mohammedi and Aboutaleb, 2014).

The merit of this study was evaluating the percentages of pollen viability and germination, fruit setting, yield, bunch weight and fruit characteristics of Saidu date palms grown under El-Kharga Oasis, New Valley region conditions in response to pollination with pollen grains stored at different temperatures.

Materials and methods

This study was conducted during 2017 and 2018 seasons in a private date palm orchard situated at El-Kharga Oasis, New Valley Governorate on 26 years old Saidu (as semi dry date palm cv). These palms were produced through conventional propagation by offshoots as well as characterized by regular bearing. Also, they are uniform in vigor, healthy, good physical conditions, free from insects, diseases and damages. They planted at 7x7 meters a part and irrigated with well water through surface irrigation system.

All the selected palms (18 palms) received the common and usual horticultural practices that already applied in the orchard except those dealing with hand pollination, Bunches /leaf was adjusted to 1:6 according to (Sayed, 2002).

For pollen extraction, the spathes on male of Saidu date palm trees were cut and all strands were cut off and spread in paper sheets till dry. The dried pollen grains were separated by using fine sieves (40 meshes). Pollen grains were used immediately or stored under the different temperature conditions. The pollens were stored in vials supplied with calcium chloride for planting the increase in the relative humidity and kept under storing conditions for one, two, four and eight years until the next pollination seasons.

Fresh pollen was used just after preparation, while stored pollens through room temperature (25-30 °C) and via cooling (5-7 °C) were used after one year from preparation. Pollen grains held at -18°C was left for two, four and eight years before using in hand pollination.

The study included the following fresh and stored pollen grains treatments:

- 1- Hand pollination with fresh pollens.
- 2- Hand pollination with stored pollens at (25-30 °C) for one year.
- 3- Hand pollination with cooling stored pollens at (5-7 °C) for one year.
- 4- Hand pollination with freezed pollens at -18°C for two years.
- 5- Hand pollination with freezed stored pollens at -18 °C for four years.
- 6- Hand pollination with freezed stored pollens at -18 °C for eight years.

Each treatment was replicated three times, one palm per each. Hand pollination was carried out two days after female spathe cracking. The female spathes before cracking were covered by white paper sacks to avoid any mixed pollinations from strange pollens. Pollination mixture was used at fixed rate for the six treatments (4 g/palm). It consists from one gram fresh or stored pollens as previously mentioned plus 3 g wheat flour carrier (according to El-Sese *et al.*, 2010). Hand pollination was conducted by inserting a piece of white cotton in the sacks containing the previous pollination mixture and then it shielded on the female spathes and put it in the center of the spathe. The spathes were tied and covered with a marked paper sacks and the paper sacks were removed after one month of hand pollination. Generally, during both seasons the following measurements were taken:

Percentages of viability and germination of pollen grains were recorded before-hand pollinations as follows:

Pollen stainability % was tested with acetocarmine staining. One drop of 1% acetocarmine was placed in a microscope slide, then a small amount of pollens was dispersed. Pollens were

microscopically examined. Colorless or unstained pollen grains were considered nonviable (according to Moreira and Gurgel, 1941 and Al- Tahir and Asif, 1981).

Percentage of pollen germination was calculated for vitro germination of fresh pollen grains:

It was estimated by culturing the pollens on a medium containing 1% agar, 20% sucrose and 100 mg/ L boric acid. After 24 hours at room temperature (20 – 25 °C) pollen grains were microscopically examined for germination from each petri dish. An initiation of pollen tube was considered as evidence of germination according to (Furr and Enriquez, 1966).

Percentage of initial fruit setting:

It was determined 6 weeks after hand pollination by labeling five strands / spathe and the percentage of initial fruit setting was calculated by dividing number of setted fruits by total number of flowers and multiplying the product x 100. Total number of flowers equals number of fruits plus all scars on the strand.

Bunch weight, yield/palm and fruit quality

At ripening bunch weight was recorded. 30 fruits from each bunch were randomly selected to determine fruit weight (g.); flesh %; T.S.S. % by hand refractometer; total reducing and non-reducing sugars (Lane and Eynon, 1965 and A.O.A.C., 2000),

Statistical analysis:

This experiment was set up in a randomized complete block design (RCBD). The analysis of variant (ANOVA) was used according to Mead *et al.*, (1993). Treatment means were compared using L.S.D test at 5 % level.

Results and discussion

Percentage of viability and germination of pollen grains

It is clear from the obtained data in Table (1) that storing pollen grains via room temperature (25-30 °C) and cooling at 5-7 °C for one year as well as freezing at -18 °C for two, four and eight years significantly reduced the percentages of viability and germination of pollen grains relative to the use of fresh pollens. There was a gradual and significant reduction on such two parameters with prolonging the storage duration from two to eight years at -18 °C. Freezing pollen grains at -18 °C significantly superior than cooling pollens grains at 5-7 °C or storing them at room temperature (25-30 °C) in enhancing the percentages of viability and germination of pollen grains. The lowest values in both seasons were recorded on the pollen grains stored at room temperature (25-30 °C) for one year. Percentage of pollen viability reached 55.5 & 56.2 % and percentage of germination were 53.5 & 54.1 % in the stored pollens at room temperature, during both seasons, respectively. The highest percentages of pollen viability (79.9 & 80.5 %) and pollen germination (78 & 78.5 %) were recorded due to use fresh pollens during 2016 & 2017 seasons, respectively. It can be stated from the present data that using fresh pollens or stored pollen via freezing at -18 °C for two years for pollination of Saidu date palms is essential for promoting fruit setting and yield. These results were true during both seasons.

1. Fruit setting, bunch weight and yield/palm

It is evident from the data in Tables (1 & 2) that pollination using pollens stored via cooling at 5-7 °C for one year or freezing at -18 °C for two to eight years significantly increased the percentages of initial fruit setting %, bunch weight (kg) and yield/palm (kg) compared to use stored pollens for one year at room temperature (25-30 °C). Pollination of the palms with fresh pollens significantly increased these parameters than the other pollen storage treatments. Cooling or freezing pollen grains were significantly preferable than storing at

room temperature in improving these traits. The reduction on these parameters was significantly related to the increase in the storage durations from two to eight years at -18°C . Storing the pollen via freezing at -18°C for two and four years was significantly favorable than storing the pollens via cooling at $5-7^{\circ}\text{C}$ for one year. The lowest results were recorded when pollination was carried out by using stored pollen at room temperature ($25-30^{\circ}\text{C}$). Using fresh pollens, stored pollens via freezing at -18°C for two and four years, in descending order gave the best results

The maximum values of initial fruit setting % (77.3 & 78.9 %), bunch weight (11.3 & 11.5 kg) and yield/palm (113 & 115 kg), were recorded on the palms that pollinated with fresh pollens during both seasons, respectively. No significant differences were recorded due to use frozen pollens for two years or use fresh ones. The lowest values were recorded on the palms pollinated with stored pollens at room temperature ($25-30^{\circ}\text{C}$) for one year. These results were true during both seasons.

Fruit characteristics

A. Physical characteristics:

Data presented in Table (2) showed the effect of different pollination with fresh pollens and those stored at room temperature ($25-30^{\circ}\text{C}$), at $5-7^{\circ}\text{C}$ for one year or at -18°C for two to eight years on fruit weight, and fruit flesh % of Saidu date palms during 2017 and 2018 seasons

Generally, the results took an opposite trend of fruit set or bunch weight in both seasons. The least values of fruit weight and fruit flesh % were produced from fresh pollens. It reached (8.7 & 8.5 g) and (84.8 & 84.5%). during the first and second season, respectively

The maximum values of fruit weight (9.5 & 9.4 g) and fruit flesh % (88.2 & 88.1 kg) during both seasons, respectively were observed with palms pollinated with fresh pollens.

These results could be due to the reduction on the fruit set percentage. Such reduction in fruit set percentage cause a shortage in the number of fruits per bunch without changing the number of leaves that may induce the better supply of carbohydrates that are manufactured in the leaves (El-salhy *et al.*, 2012 and Samouni *et al.*, 2016)

B. chemical characteristics:

Data presented in Table (3) cleared the effect of pollination with different pollen grains on the percentages of T.S.S total reducing and non-reducing sugars in the fruits of Saidu date palms during 2017 & 2018 seasons.

There was a negative correlation between the percentage of T.S.S% and the fruit set %, meaning that the higher fruit set %, and the lower T.S.S%.

On the other hand the results of total sugars took the same trend of the T.S.S % where the lowest value was obtained from the Hand pollination with fresh pollens where it reached (74.5 & 74.0%) during the first and second season, respectively.

Total sugars % reached the highest values when the palm trees pollinated with stored pollens at room temperature ($25-30^{\circ}\text{C}$) for one year. as the results were (70.8 & 70.3 %) during the first and the second season, respectively

Unfavorable effects on fruit quality were recorded with pollination of the palms with fresh pollens followed by pollen stored via freezing at -18°C for two years. Percentage of non-

reducing sugars was insignificantly affected with the present treatments. Similar trend was noticed during both seasons.

Table (1) Effect of pollination with fresh or different stored pollen grains on the percentages of viability and germination of pollens as well as initial fruit setting of Saidu date palms during 2017 & 2018 seasons.

Storage pollen treatments	Pollen viability %		Pollen germination %		Initial fruit setting %	
	2017	2018	2017	2018	2017	2018
Hand pollination with fresh pollens.	79.9	80.5	78.0	78.5	77.3	78.9
Hand pollination with stored pollens at (25-30 °C) for one year.	55.5	56.2	53.5	54.1	64.5	66.0
Hand pollination with stored pollens at (5-7 °C) cooling for one year.	68.0	68.7	66.0	66.6	69.3	70.6
Hand pollination with stored pollens -18 °C (freezing) for two years.	72.9	73.9	71.0	72.0	75.0	76.4
Hand pollination with stored pollens -18 °C (freezing) for four years	70.0	71.0	68.0	69.0	72.2	73.6
Hand pollination with stored pollens -18 °C (freezing) for eight years.	66.1	66.8	64.2	64.7	66.6	67.7
L.S.D. at 5 %	0.9	0.9	1.0	1.1	1.9	2.0

Table (2) Effect of pollination with fresh or different stored pollen grains on bunch weight, yield, palm, fruit weight and percentage of fruit flesh of Saidu date palms during 2017 & 2018 seasons.

Storage pollen treatments	Bunch weight		Yield/palm (kg)		Fruit weight (g)		Fruit flesh %	
	2017	2018	2017	2018	2017	2018	2017	2018
Hand pollination with fresh pollens.	11.3	11.5	113.0	115.0	8.7	8.5	84.8	84.5
Hand pollination with stored pollens at (25-30 °C) for one year.	9.2	9.4	92.0	94.0	9.5	9.4	88.2	88.1
Hand pollination with stored pollens at (5-7 °C) cooling for one year.	9.8	10.0	98.0	100.0	8.9	8.8	86.7	86.5
Hand pollination with stored pollens -18 °C (freezing) for two years.	10.7	10.8	107.0	108.0	9.0	8.9	85.0	85.1
Hand pollination with stored pollens -18 °C (freezing) for four years.	10.2	10.5	102.0	105.0	9.1	9.1	86.2	86.1
Hand pollination with stored pollens -18 °C (freezing) for eight years.	9.3	9.4	93.0	94.0	9.3	9.2	86.8	86.6
L.S.D. at 5 %	1.1	1.2	11.9	12.3	0.2	0.3	1.5	1.5

Table (3) Effect of pollination with fresh or different stored pollen grains on the percentages of T.S.S., total reducing and non-reducing sugars in the fruits of Saidy date palms during 2017 & 2018 seasons.

Storage pollen treatments	T.S.S%		Total sugars %		Reducing sugars %		Non-reducing sugars %	
	2017	2018	2017	2018	2017	2018	2017	2018
Hand pollination with fresh pollens.	74.5	74.0	68.2	67.8	58.9	58.5	9.3	9.3
Hand pollination with stored pollens at (25-30 °C) for one year.	77.8	77.3	70.8	70.3	61.6	61.2	9.2	9.1
Hand pollination with stored pollens at (5-7 °C) cooling for one year.	76.8	76.2	69.9	69.3	60.8	60.3	9.1	9.0
Hand pollination with stored pollens -18 °C (freezing) for two years.	75.5	75.1	68.8	68.4	59.9	59.58	8.9	8.82
Hand pollination with stored pollens -18 °C (freezing) for four years.	76.2	75.8	69.5	69.0	60.5	60.1	9	8.9
Hand pollination with stored pollens -18 °C (freezing) for eight years.	76.8	76.4	70.0	69.7	60.9	60.7	9.1	9
L.S.D. at 5 %	0.6	0.7	0.7	0.7	0.6	0.6	NS	NS

Discussion

The positive action of pollination with fresh pollen on the percentages of viability and germination of pollens surely reflected on enhancing the percentage of initial fruit setting % could explain the present results regarding the promotion on yield and fruit characteristics. (Abo- Hassan *et al.*, 1982; Simon, 1987; Shaheen *et al.*, 1986 and Wrigely, 1995).

These results are in harmony with those obtained by Rahim, (1975); Hussein (1983); Shaheen *et al.*, (1986); El-Kassas *et al.*, (1995); Ashour *et al.*, (2004); Abo- Hassan *et al* (1982) El-Agamy *et al.*, (2008); Saad, (2008); El-Sese *et al.*, (2010); El- salhy *et al.*, (2012) and Mohammedi and Aboutalebi, (2014).

Fruit characteristics took an opposite trend of fruit set or bunch weight these results could be due to the reduction on the fruit set percentage. Such reduction in fruit set percentage cause a shortage in the number of fruits per bunch without changing the number of leaves that may induce the better supply of carbohydrates that are manufactured in the leaves (El-salhy *et al.*, 2012 and Samouni *et al.*, 2016)

Conclusion

The objective of this experiment was to examine the effect of pollination with fresh or different stored pollen grains to find the best way to store pollen grains.

Our results indicated that stored pollen grains via freezing at -18 °C for 2 years was better than stored pollen grains at either room temperature or cooling at 5-7 °C.

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محاولات لإطالة القدرة التخزينية لحبوب اللقاح في تلقيح نخيل البلح الصعيدي النامي تحت ظروف

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الملخص العربي

أجريت هذه الدراسة خلال موسمي ٢٠١٧ و ٢٠١٨ في محاولة لإطالة القدرة التخزينية لحبوب اللقاح في تلقيح نخيل البلح صنف الصعيدي النامي تحت الظروف البيئية للوادي الجديد لحل مشكلة قلة عدد الذكور الجيدة أو النقص الحاد في حبوب اللقاح في مناطق الزراعات الجديدة وبالتالي فشل عملية التلقيح اللازمة لانتاج محصول عالي جيد الصفات وقد اشتملت التجربة علي تخزين حبوب اللقاح علي درجة حرارة الغرفة (٢٥ درجة مئوية) والتبريد (٥-٧ درجة مئوية) لمدة عام واحد اما التجميد (-١٨ درجة مئوية) فقد تم لمدة ٢ و ٤ و ٨ سنوات. تم دراسة تأثير هذه المعاملات علي النسبة المئوية لحيوية وانبات حبوب اللقاح، النسبة المئوية لعقد الثمار، كمية محصول النخلة، وزن السوباطة وخصائص جودة الثمار. ولقد نجح التلقيح باستخدام حبوب اللقاح الطازجة، حبوب اللقاح المجمدة، حبوب اللقاح المبردة، حبوب اللقاح المخزنة تحت درجة الغرفة مرتبة ترتيبا تنازليا في تحسين النسبة المئوية لعقد الثمار، كمية محصول النخلة، وزن السوباطة وخصائص الجودة. اوضحت النتائج ان زيادة فترة التخزين يتبعها نقص جميع القياسات السابقة وكانت افضل طريقة للتخزين هي عملية التجميد (-١٨ درجة مئوية) يليها التبريد (٥-٧ درجة مئوية) اما التخزين علي درجة حرارة الغرفة فقد احتل المرتبة الاخيرة في هذا الصدد. ولقد امكن الحصول علي افضل النتائج بخصوص افضل نسبة عقد للثمار وبالتالي كمية المحصول عند تلقيح نخيل البلح الصعيدي النامي تحت ظروف بيئية منطقة الوادي الجديد بحبوب اللقاح الطازجة او حبوب اللقاح المجمدة علي (-١٨ درجة مئوية) لمدة عامين. من نتائج هذه الدراسة يمكن التوصية بإمكانية تخزين حبوب اللقاح عدة سنين تحت ظروف التجميد دون نقص يذكر في حيويتها وبالتالي يمكن استخدامها في التلقيح.

الكلمات الدالة:- حيوية حبوب اللقاح – انبات حبوب اللقاح – حبوب اللقاح الطازجة- حبوب اللقاح المجمدة – عقد الثمار