SUST
Journal of Agricultural and Veterinary Sciences
Journal homepage:
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Pollination and Fertilization Capacity of *in vitro* Treated Male Date Palm (*Phoenix dactylifera* L.) Strands of Spathes Collected Before and After Cracking

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Article history: Received: 16/07/2013
Accepted: 10/12/2013

Abstract
This study was conducted to investigate the reproductive attributes that affect pollination and fertilization capacity of male date palm (*Phoenix dactylifera* L.) strands of spathes collected after and before cracking, and treated in *vivo*. Spathes were collected from three trees, one, two, three and four days before, and just after, cracking. Strands from each spathe were examined fresh and after drying and after incubated in distilled water (DW) or in warm acidified water (WAW) (42 ±2°C, pH5 ± 0.5). The results revealed that the strands from the spathes collected after, and one day before, cracking and incubated in WAW exhibited 100% open flowers and dehiscent anthers. Significantly less percent open flowers and dehiscent anthers were on the strands from the spathes collected two days, than those induced by the corresponding *in vitro* treatments of the strands from the spathes collected one day, before cracking. Some strands from the spathes collected three days before cracking were with open flowers and dehiscent anthers when incubated in WAW. The strands from the spathes collected four days before cracking were devoid of open flowers and dehiscent anthers. More than 80% of pollen grains released from dehiscent anthers were viable. Thus, collecting male date palm spathes within 24, and probably 48, hours, before cracking and incubating them in WAW would improve their pollination and fertilization capacity, reducing the number of times needed to climb male date palm trees and saving the otherwise dispersed pollen grains.

Keywords: Flowers, Anthers, Pollen, Dehiscence, Viability

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Introduction
Date palm is an important fruit tree in North Africa and Arabian peninsula (Alkiedy, 2000; Hussain, 2005). It is grown extensively as a major cash crop in northern Sudan. Unlike other dioecious fruit crops, the female trees need to be artificially pollinated to secure economic fruit set (Attalla *et al*., 1998). Traditionally, this is done by inserting few male strands into the female strands of each spathe. However, recently pollen grains are often mechanically dusted on the female bunches (Hamood and Mawlood, 1986; Osman, 2009). This, together with the low percentage of male, to spare the space for the female, trees, makes it important to save as much pollen grains as possible. The male spathes are usually collected after cracking, which could result in a loss of a considerable percentage of pollen grains, or when it is just about to (Hussain, 2005). In

either case, the laborers need to climb each male tree several times because the spathes on the tree do not reach these stages simultaneously. This makes the pollination process the most expensive operation in date production (Brown and Bahgat, 1982; Shabana and Shafaat, 1982). It has been observed that the male spathe cracked when its base was immersed in water (Warrag, M.O.A., 2011, unpublished data) . Hence, it seems that the male date palm flowers could probably continue its development *in vitro*. Should this happen, it would result in a remarkable reduction in the number of times needed to climb the male trees, as well as in saving of pollen grains. Hence, this study was conducted to investigate the effects of collection, and *in vitro* treatment of male date palm strands, before and after spathe cracking on some reproductive attributes of male date palm.

Materials and Methods

Easy to reach (*Phoenix dactylifera* L.) male date palm trees in Khartoum area were observed closely at flowering time during 2012. Only three male trees were used due to the scarcity of well developed ones and each tree was considered as a replicate. Spathes were collected from each tree, one, two, three and four days before, and just after, cracking. The day of cracking was judged by the shape and size of the spathe compared to the just cracked one. The sheath was removed from each spathe to expose the strands. Twenty strands were randomly cut from each spathe for immediate sampling and then were spread on a paper sheet for drying. Ten strands were cut from each spathe and their bases were immersed in distilled water (DW) in a glass beaker. Another ten strands were cut and their bases were inserted in holes made in a disc of Styrofoam fitted in a thermos flask filled with warm distilled water (42 ± $^{\circ}$2C) acidified (pH 5 ± 0.5) with citric acid (WAW). All strands were kept under shade at room temperature for three days. Warm acidified water would dissolve any formed air bubbles in the xylem vessels, increase the rate of water influx and would retard microbial growth (Gast, 1997; Edinger, 2003).

Data collection and analysis

The number of strands with open flowers, the number of open flowers using a sample of 100 flowers and the number of dehiscent anthers using a sample of five open flowers per time of collection per *in vitro* treatment per replicate were recorded. These data were collected from the strands used for after drying sampling and from the DW and WAW incubated strands after three days, the time for maximum *in vitro* flower opening (Hussain, 2005). Open flowers were identified by a hand lens, whereas a dissecting microscope was used for dehiscent anthers. The percent strands with open flowers, the percent open flowers and dehiscent anthers were calculated. Pollen viability was performed by spreading some white powder dispersed from dehiscent anthers on a glass slide in a few drops of acetocarmine stain (Al-Tahir and Asif, 1981; Bacha *et al*., 2000). Twenty minutes later a cover slip was placed on the slide and then three microscopic fields were examined with a light microscope. The viable pollen grains would stain deep red, whereas the non- viable ones would not.

The percent strands with open flowers, and percent open flowers, data were arc sine transformed, whereas the percent dehiscent anthers and percent viable pollen grains data were square root transformed, for statistical analysis (Gomez and Gomez, 1984). All data were subjected to analysis of variance with two factors arranged in a randomized complete block design. Mean separation was done using Duncan's Multiple Range Test at the 5% level.

Results

Percent strands with open flowers

The strands from the spathes collected after cracking and examined fresh exhibited 100% strands with open flowers (Table 1). Few strands with open flowers were on the spathes collected one day before cracking and examined fresh. Most of these strands

were on the abaxial side of the spathes. The strands with open flowers were almost doubled after three days drying at room temperature. Statistically significant increase was brought about by distilled water (DW) incubation. All strands were induced to have open flowers by incubation in warm acidified water (WAW). Fewer strands with flowers were on the spathes collected two days before cracking and examined fresh. Again this was almost doubled by drying at room temperature. Further significant increase was induced by DW, whereas more than ten times increase was induced by WAW. No strands with open flowers were detected on the spathes collected three and four days, with the exception of WAW incubation which induced flower opening on few strands of the spathes collected three days, before cracking.

Open flowers

All flowers on the strands from the spathes collected after cracking were open (Table 2). Collecting the spathes one day before cracking resulted in a significant reduction in the percentage of open flowers on the freshly examined strands. Drying the strands at room temperature for three days approximately doubled the percentage of open flowers, whereas it was tripled by DW incubation. All flowers were induced to open by WAW incubation. The percent open flowers on the strands from the spathes collected two days before cracking was significantly less in comparison with the open flowers induced by the corresponding *in vitro* treatments of the strands from the spathes collected one day before cracking. Similar trend as percent strands with open flowers was followed by open flowers on the strands from the spathes collected three and four days before cracking (Tables1 and 2).

Dehiscent anthers

As with strands with open flowers, and with open flowers, 100% of anthers of randomly selected open flowers on the strands from the spathes collected after cracking were dehiscent (Table 3). Likewise, were those on the strands from the spathes collected one day before cracking and incubated in DW or WAW and from the spathes collected two and three days before cracking and incubated in WAW . More than 60% of anthers on the strands from the spathes collected two days before opening and examined fresh and after drying were dehiscent, whereas about 80% of the anthers on the spathes collected two days before cracking and incubated in DW were dehiscent. With the exception of the anthers on the strands from the spathes collected three days before cracking and incubated in WAW, all the anthers on the strands from the spathes collected three and four days before cracking were indehiscent.

Viable pollen grains

More than 80% pollen grains released from dehiscent anthers were viable based on acetocarmine stain test, irrespective of the collection time or *in vitro* treatments (Table 4). It was observed that the amount of free viable pollen grains released was positively associated with the percentage of the strands with open flowers, open flowers and dehiscent anthers. Thus, the largest amount of viable pollen grains was released by the strands from the spathes collected after cracking followed by those from the spathes collected one day, and then two days, before cracking, whereas for the one and the two days before cracking collection time, the largest amount of viable pollen grains was released by the strands incubated in WAW followed by those incubated in DW (Tables1, 2, 3 and 4).

Discussion

Collecting male date palm spathes after cracking resulted in the largest amount of free viable pollen grains (Tables1, 2, 3 and 4). However, under natural condition if the spathes crack before collection a considerable amount of pollen grains, depending on the degree of spathes cracking and the method of collection, would be lost by wind dispersion. Thus to save all pollen grains, the spathes should be

collected before cracking. Collecting the spathes when a cracking sound is heard by pressing the sheath with the thumb, is one way of trying to do so (Hussain, 1985), but some flowers would still be closed. From this study it could be deduced that all flowers would open and all anthers would be dehiscent and more than 80% pollen grains would be viable if spathes were collected within 24 and probably 48 hours before cracking and incubated in WAW (Tables1,2,3 and4), resulting in a significant increase in the pollination and fertilization capacity. The spathes collected two and three days before cracking would contain fewer dehiscent anthers and free viable pollen grain if incubated in WAW. Hence, such spathes could be incubated in WAW and used when there are no other means of obtaining viable pollen grains at the time of pollination. It is clear that flower opening and anther dehiscence in this study was due to the influx of water resulting from the difference in water potential which in turn resulted from the difference in osmotic potential. However, since the flowers and the anthers on the strands from the spathes collected four days before cracking did not respond to any *in vitro* treatment, it seems that water influx would not lead to flower opening and anther dehiscence unless a certain stage of floral development is reached. Probably this stage could be reached *in vitro* by using a preservative solution comprising warm distilled water, a source of energy such as sucrose (Mackay *et al*., 2003) and a biocide such as silver nitrate to prevent microbial growth (Jones and Hill, 1993; Meyer, 2005) and an acidifying agent such as citric acid to enhance the rate of water influx and retard microbial growth (Van Doorn, 1995) and an ethylene production inhibitor such silver thiosulfate to retard flower abscission (Gast,1997) and keep it at low air temperature and high relative humidity. Such a preservative solution is very successful for extending vase-life of cut floral spikes, resulting in progressive opening of flowers and /or floral buds (Edinger, 2003). However, with cut flowers, it is the colored parts such as petals or tepals, which would be needed to open, whereas in male date palm it is both the flowers and the anthers, to obtain free viable pollen grains. Should this treatment is successful, spathes could be collected early before *in vivo* cracking which would reduce the number of times the laborers need to climb male date palm trees and would save all pollen grains.

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Table 1: Percent male date palm strands with open flowers of spathes collected after and before cracking (Aft.crk and bef.crk, respectively) and examined fresh and after drying or after distilled water (DW) or warm acidified water (WAW) incubation for three days.

Collection time	Fresh	Dry	DW	WAW	Mean
Aft.crk.	100Aa	100Aa	100Aa	100Aa	100 A
1 day Bef.crk.	13.3Bd	25.0Bc	50.0Bb	100Aa	47.1B
2 days Bef.crk.	6.7Cd	15.0Cc	26.7Cb	71.7Ba	30.0C
3 days Bef.crk.	0Db	0Db	0Db	18.3Ca	4.6D
4 days Bef.crk.	0Da	0Db	0Da	0Da	0 E
Mean	24.0d	28.0c	35.4b	58.0a	

- Data were arc sine transformed for statistical analysis.
- Means with same upper case letters for columns and lower case letters for rows are not significantly different ($P \leq 0.05$) using Duncan's Multiple Range Test.

Table 2. Percent open flowers of male date palm (*Phoenix dactylifera*) strands from spathes collected after and before cracking (Aft.crk and Bef.crk, respectively) and examined fresh and after drying or after distilled water (DW) or worm acidified water (WAW) incubation for 3 days at room temperature.

Collection time	Fresh	Dry	DW	WAW	Mean
Aft.crk	100Aa	100Aa	100Aa	100Aa	100 A
1 day Bef.crk.	15.3Bd	28.3Bc	45.0Bb	100Aa	47.0B
2 days Bef.crk.	3.3Cc	3.7Cc	15.0Cb	78.3Ba	25.0C
3 days Bef.crk.	0Db	0Db	0Db	21.7Ca	5.4D
4 days Bef.crk.	0Da	0Db	0Da	0Da	0 E
Mean	23.7c	26.4c	40.0b	60.0a	

- Data were arc sine transformed for statistical analysis.
- Means with same upper case letters for columns and lower case letters for rows are not significantly different ($p \leq 0.05$) using Duncan's Multiple Range Test.

Table 3. Percent dehiscent anthers of open flowers of male date palm (*Phoenix dactylifera*) strands from spathes collected after cracking (Aft.crk.) and before cracking (Bef.crk.) and examined fresh and after drying or after distilled water (DW) or warm acidified water (WAW) incubation for 3 days at room temperature.

Collection time	Fresh	Dry	DW	WAW	Mean
Aft.crk.	100Aa	100Aa	100Aa	100Aa	100 A
1 day Bef.crk.	84.4Bb	89.9Bb	100Aa	100Aa	93.6B
2 days Bef.crk .	65.5Cc	65.5Cc	79.9Bb	100Aa	77.7C
3 days Bef.crk.	-	-	-	100	-
4 days Bef.crk.	-	-	-	-	-

- Data were square root transformed for statistical analysis.
- Means with same upper case letters for columns and lower case letters for rows are not significantly different ($p \leq 0.05$) using Duncan's Multiple Range Test.

Table 4. Percent viable pollen grains dispersed from dehiscent anthers of male date palm (*Phoenix dactylifera*) strands from the spathes collected after cracking (Aft.crk.) and before cracking (Bef.crk.) and examined fresh and after drying or after distilled water (DW) or warm acidified water (WAW) incubation for 3 days at room temperature.

Collection time	Fresh	Dry	DW	WAW	Mean
Aft.crk.	82.7	89.1	83.4	80.6	84.0
1 day Bef.crk.	87.5	80.3	85.0	84.7	84.4
2 days Bef.crk.	81.4	81.2	83.3	82.8	82.2
3 days Bef.crk.	-	-	-	86.7	-
4 days Bef.crk.	-	-	-	-	-

- Data were square root transformed for statistical analysis. Means are not significantly different at the 5% level using Duncan's Multiple Range Test.

كفاءة التلقيح والإخصاب بإستخدام شماريخ فحول نخيل البلح المعاملة معملياً قبل وبعد انشقاق الأغاريض

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المستخلص:

أجريت هذه الدراسة لفحص المكونات الزهرية التي تؤثر على كفاءة التلقيح والاخصاب باستخدام شماريخ من أغاريض فحول نخيل البلح المجموعة قبل وبعد انشقاقها ومعاملتها معمليا . جمعت الأغاريض من ثلاث أشجار عقب انشقاقها وبعد يوم ويومين وثلاثة وأربعة أيام قبل انشقاقها . فحصت بعض الشماريخ من كل اغريض غضة وبعد تجفيفها أو بعد وضعها في ماء مقطر أو ماء دافئ حمضى (pH 5±0.5,42±2°C).أوضحت النتائج أن الشماريخ التى من الأغاريض التي جمعت بعيد وقبل يوم من انشقاقها ووضعت فى الماء الدافئ الحمضى قد أعطت 100% أزهار متفتحة ومتوك منشقة. نسبة الأزهار المتفتحة والمتوك المنشقة فى الأغاريض التي جمعت قبل يومين من انشقاقها أقل معنويا من التي أحدثتها المعاملات المعملية المقابلة على الأغاريض التي جمعت قبل يوم من انشقاقها. بعض الشماريخ من الأغاريض التى جمعت قبل ثلاثة أيام من انشقاقها ووضعت قواعدها في الماء الدافئ الحمضي قد وجدت عليها أزهار متفتحة و متوك منشقة . لم توجد أزهار متفتحة ومتوك منشقة على الشماريخ فى الأغاريض التي جمعت قبل أربعة أيام من انشقاقها. أكثر من 80% من حبوب اللقاح التي إنتثرت من المتوك المنشقة حية . لذا فإن جمع أغاريض فحول نخيل البلح خلال 24 وربما 48 ساعة قبل انشقاقها ووضع قواعد شماريخها في ماء دافئ حمضي يحسن من كفاءتها التلقيحية والإخصابية مما ينتج عنه خفض عدد مرات تسلق فحول نخيل البلح وحفظ حبوب اللقاح التي كانت سوف تنتثر.