

## Commercial Production of Tissue Culture Date Palm (*Phoenix dactylifera* L.) by Inflorescence Technique

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### ABSTRACT

Date Palm (*Phoenix dactylifera* L.) is a salt and drought tolerant fruit crop mainly cultivated in Arabian countries. Shoot tip explants of offshoots were used traditionally for various micropropagation protocols on research or commercial levels. However, its main disadvantage was the scarification of the entire plant. Subsequently, this hinders the micropropagation of male and female recalcitrant individuals with no offshoots or the interesting cultivars with a limited population. Consequently, current study is investigating the factors affecting commercial production of tissue cultured palms with cost-effectively and short-production cycle, which may also strongly enhance the transformation protocols. The innovative way by which the 15 cm long immature inflorescence was excised before emergence between fronds is reported herewith this study for the first time. Established spikelet explants were able within 2-3 months only without any callus phase to produce shining globular structures instead of immature florets. These embryogenic structures couldn't develop further without maturation process for 1-2 months under full darkness as well. Subsequently, well-matured embryogenic structures were shifted to the subsequent differentiation medium under illumination conditions. After then, green shoots and multiple somatic embryos have been subjected to the multiplication stage, then rooting and eventually successfully transplanted in the greenhouse. All used nutrient media and their sequential usage is reported in this study by which it became possible from one inflorescence to produce 10000 plantlets in rooting stage right now of Gulistan Pakistani cultivar without any bad consequences on the mother tree.

**Key Words:** Date palm, inflorescence, micropropagation.

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### INTRODUCTION

Date Palm (*Phoenix dactylifera* L.) is a dioecious, perennial monocot plant species of the Arecaceae family. It is one of the oldest fruit crops mainly cultivated in North Africa and Middle East countries. Shoot tip explants of the offshoots traditionally used for various micropropagation protocols of date palm on the research and commercial levels together (Abul-Soad et al. 2002a,b, Abul-Soad et al. 1999, 2004, El-Hadrami and Baaziz 1995 and Tisserat 1984b). Furthermore, other possible explants were tested and proved un-successful (Tisserat 1984a). However, the inflorescence explants proved promising and the required alternative explant for micropropagation of elite cultivars and rare male and female individuals of date palm (Abahmane et al. 1999, Abul-Soad 2003, 07a, Abul-Soad et al. 2004, 05, 07, 08, Bhaskaran and Smith 1992, El-Korchi 2007 and Feki and Drira 2007). Nevertheless, no any data was reported for commercial production of date palm based on the inflorescence explant. Handling pilot production is not easy task and many factors should be taken. On the practical side, the excision of immature inflorescence from the mother

tree, composition of starting medium during initial stage, sequential handling of different nutrient media remained the most decisive three factors could interfere the real success of inflorescence technique. Current study is investigating the above mentioned factors and discussing the commercial production business. A unique commercial trial has been applied at Date Palm Research Institute, Khairpur, Pakistan. The success have been done by Arab scientist is strongly recommended to be applied in one or more of Arab countries. It is expected that by which a huge number of elite Arabian cultivars that fit good for processing industry (Abul-Soad 2007b) in addition to recalcitrant male and female individuals can be produced in short time and less effort. Furthermore, an example of how and why micropropagation by inflorescence technique is being used will be presented.

### MATERIALS AND METHODS

This work was carried out in the Biotechnology Lab. of Date Palm Research Institute, Shah Abdul Latif

University, Khairpur, Sindh, Pakistan in 2008 - 2010. The protocol was done as under:

1. The immature inflorescence was excised from the mother tree of Gulistan cv. at D. I. Khan area, Pakistan in early spring.
2. The excised inflorescence was kept in clean plastic cover and handled carefully from an open field to the laboratory. In current case of far away places of source materials (spathes), it could be conserved for 1-2 days in ice box. It is observed that this process hasn't adverse impact on the survivability of transferred spathes from D. I. Khan area which is far 600 Kilometers away from Khairpur.
3. Once the whole inflorescence (spathe) has been brought to the lab, the real work was quickly started and the whole inflorescence was subjected to the surface sterilization procedure.
4. The intact spathe was dipped into fungicide solution for 30 seconds only without any shaking. The 2 grams l<sup>-1</sup> Topsin M 70 (the systematic fungicide) solution was used.
5. The spathe was carefully handled while washing it under current tap water for 30-60 seconds only. Water stream should be focused on the basal part of the spathe to remove the little dust.
6. 30 % Sodium Hypochlorite (NaOCl) solution (16%) was used for only 1-2 minutes.
7. The spathe was washed with sterilized distilled water for 30-60 seconds one time without shaking.
8. Once the spathe is surface sterilized, the outer protective sheath was longitudinally cut from the middle like T letter from one side only. Cut may be done in the central-swelled portion of the spathe due to its softness.
9. Spikelet explants were cut from their base and cultured intact if they are 3-4 cm in length. Longer spikelet explants were cut to pieces, each 2-3 cm and laid in such a way that the entire explant is in contact with the surface of nutrient medium. Each of which possessed 2-4 immature florets.
10. All cultured explants were incubated in a controlled growth room at 25 ± 2 °C under full darkness. Incubated explants were re-cultured 1-2 times, each was about 3-4 weeks on same starting medium as described in Table (1).
11. Well-responded explants were transferred into maturation medium for 1-2 re-cultures.
12. Matured and early-differentiated explants under darkness were shifted onto differentiation medium under illumination conditions for 1-2 re-cultures.
13. Subsequently the differentiated cultures were shifted to the multiplication stage or may be the rooting stage directly.
14. Well-rooted plantlets were subjected to the *in vitro* hardening by culturing onto low-nutrients medium along with more ventilation.
15. Date palm plantlets were successfully transplanted in the greenhouse.

## RESULTS AND DISCUSSION

The decisive factors and basic steps involved in date palm micropropagation using inflorescence explants could be three factors. These are the method of immature inflorescence excision, the composition of starting-induction medium of direct organs and the sequence of different nutrient media during entire protocol. This study has explained these three factors.

Current innovative protocol recommended excision process of the spathe while it remains beneath the frond's base in such a way that the location of young spathe was successfully estimated. Since decades and every date-palm tissue culturist in the world was trying to be away from touching the tree's head, otherwise it may be fallen down. The principal is the flower buds that are going to develop to inflorescences which are emerging in reciprocal position around the head of the tree mostly above the older 4-5 frond whorls. Then the spathes are emerging between the next 3-4 frond whorls (*Abul-Soad 2003*). Two innovative methods that are not done before were tested in current study. First method is based on a preliminary estimation of a frond which covers the spathe and select it. Then, the adjacent 4-5 fronds were technically peeled away. In case of wrong estimation, operation should be aborted to avoid any risk on the tree's head. Only the skilled person can carry out a second estimation for same tree to get maximum 1-2 spathes. Trial to get more than 2 spathes wasn't tested.

The second method proved successful as well, through pruning of outer mature fronds until to reach the first outer spathe. In latter method, the number of excised fronds was about 36-40 mature fronds which lost the tree a paramount source of food for ongoing season of emerging spathes compared to first method. In this regard, bunch thinning has obligatory been done to reduce the weight on the tree's head. The number of fruit bunches which were reduced are from 15-20 bunches to be 4 bunches only. However, in the subsequent season the mother tree entirely recovered and place of excision was almost disappeared. In both methods, time of excision, cultivar and climatic conditions particularly temperature are the variable factors that control the appropriate excision time, i.e. age of the spathe.

In the first method, the mother tree from which the spathe was excised lost only one bunch at that year and entirely

recovered in the next year. It is worth mentioning that the wound must be subjected to a post treatment with fungicide and pesticide to avoid any further infection with diseases or/and pests particularly Red Palm Weevil (Palm Cancer). Also, this experiment was tested for 20 trees at different heights and repeated for 3 consecutive years with no even a single failure trial (fallen head).

The second factor is the starting medium to induce the direct organs Table (1). The entire step-wise protocol was mentioned above in the materials and methods part. Therefore, one of the worth benefits of using the inflorescence technique is the simple sterilization protocol to get free contamination explants. Spikelet was the only explant type of whole inflorescence that responded well to the starting nutrient medium Figure (1a). Other tissue explants failed even to produce callus when cultured onto callus induction medium (Abul-Soad 2009). This is why current protocol is using only the spikelets explants.

The sequence of required nutrient media (Third factor) along with the time schedule of organogenesis from the well established initial explants till successfully *ex vitro* acclimatized plants was as below:

1. Shining globular creamy structures formation was about 2 months through 1-2 re-cultures (Figure 1a).
2. Maturation of initial structures for 2-3 months through 2-3 re-cultures.
3. Differentiation process for 1-2 months through 1-2 subcultures.
4. Proliferation phase of the individual shoots and the multiple somatic embryos extended up to 13 subcultures after differentiation (Figure 1b).
5. Rooting stage which can be extended from few months to 1-2 years depending on the required number of plantlets simultaneously its health (Figure 2a).
6. Acclimatization stage (Figure 2b).

As a live example, the cultures of inflorescence were multiplying well throughout 13 subcultures done at Date Palm Research Institute, Khairpur, Pakistan during the period from 2008-2010. The size of production during this period is shown in Table 2. After the differentiation process, three types of cultures were obtained which are embryogenic callus, somatic embryos and green shoots. Somatic embryos can be generally divided into two categories. First category is the individual somatic embryos and second is a cluster of embryos (multiple embryos). The growth behavior of the individual embryo is to grow vertically to produce more leaves and roots while the multiple embryo is usually proliferating to additional shoots and somatic embryos which suits the multiplication stage. Some of

these were transferred to the multiplication stage. In the first subculture of multiplication stage, 10 jars were having embryogenic callus, 7 jars with multiple embryos and 4 jars of shoots. All of these were transferred onto the forth medium of proliferation (Table 1). The embryogenic callus exposed high morphogenetic potentiality to differentiate to intact somatic embryos. During this process extra callus formation was occurred till subculture 7 where the callus jars increased to 16.

<sup>2</sup>Each culture vessel (350 ml jar or 250×25 mm long tube) contained a gram of callus, or 10 embryos or shoots in average. Each long tube contained an intact plantlet with shoot-root system.

But gradually the new clones of embryogenic callus decreased and eventually all callus differentiated to somatic embryos. This high ability of differentiation can make from this type of callus the typical targeted tissue in transformation protocols. Hence the all transgenic cells will have a big chance to proceed to intact viable plantlets. The long and complicated regeneration protocol of date palm is the big hinder of date palm transformation of date palm (Hassan 2007; Saker and Ghareeb 2007). Furthermore, this organogenetic potentiality can be used as ideal source for long-term Cryo-storage. It is reported that Cryo-storage has an advantage of long-term storage without going through frequent subcultures and somaclonal variation in compared to *in vitro* conservation techniques, cryopreservation and cold-storage for Gene Bank purposes (Jain 2010).

During multiplication stage some shoots were growing up and reached to an appropriate height for rooting stage (Abul-Soad *et al.* 2006). These were subsequently subjected to the fifth medium Table (1). The shoot jars increased from 4 in subculture 1, then 40 jars and eventually became 1644 jars in subculture 7. Each jar maintained 20-30 shoots, 5 of them at least in the size of rooting stage while 1212 plantlets were in rooting stage during the period from subculture 7 to 13. It was achieved from a single inflorescence of cv. Gulistan, 10000 healthy plantlets with root-shoot system and partially gradually transplanted into the greenhouse Figure (2b).

It is quite important to mention that during one century the population of this recalcitrant Gulistan cv. at D. I. Khan area was 1-2 thousand trees only due to the limitation of the new offshoots through the traditional propagation method. Notwithstanding within only 2 years became possible to produce that number from one inflorescence. This is telling us the feasibility of using the promising inflorescence technique.

**Table 1:** Nutrient media composition for inflorescence protocol and its sequence.

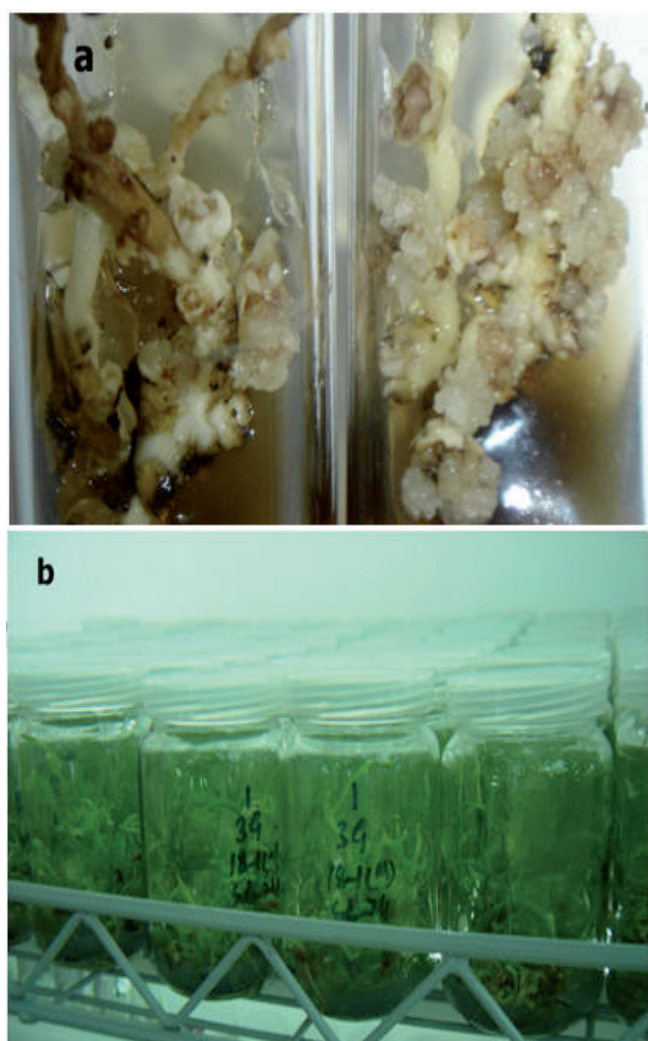
Medium	Composition (mg l <sup>-1</sup> )			
	Salts	Additives	Auxins	Cytokinins
1. Starting	Macro of B5 <sup>z</sup> + Micro of MS <sup>y</sup>	30000 Suc. <sup>x</sup> + 2200 Agar + 1400 Gel + Vit. <sup>v</sup> of MS + 170 KH <sub>2</sub> PO <sub>4</sub> + 100 Glutamine + 40 Ad. <sup>u</sup>	0.1 2,4-D + 0.1 IAA + 5.0 NAA	-
2. Maturation	Macro of B5 + Micro of MS	30000 Suc. + 2200 Agar + 1400 Gel + Vit. of MS + 170 KH <sub>2</sub> PO <sub>4</sub> + 100 Glutamine + 40 Ad. + 1500.0 AC <sup>w</sup>	5.0 2,4-D	1.0 2iP
3. Differentiation	MS	30000 Suc. + 2200 Agar + 1400 Gel + Vit. of MS +	0.1 NAA	0.1 Kinetin
4. Proliferation	MS	30000 Suc.+ 2200 Agar + 1400 Gel + Vit. of MS +	0.1 NAA	0.05 BA
5. Rooting	¾ MS	50000 Suc. + 2200 Agar + 1400 Gel + 0.1 Ca-panthothianate + Vit. of MS + with and without 3000.0 AC	0.1 NAA	

<sup>z</sup>B5: Gamborg et al. (1968) Nutrient Medium. <sup>y</sup>MS: Murashige and Skoog Medium (1962). <sup>x</sup>Suc.: Sucrose. <sup>v</sup>Vit.: Vitamins. <sup>w</sup>AC: Activated Charcoal. <sup>u</sup>Ad.: Adenine Sulfate.

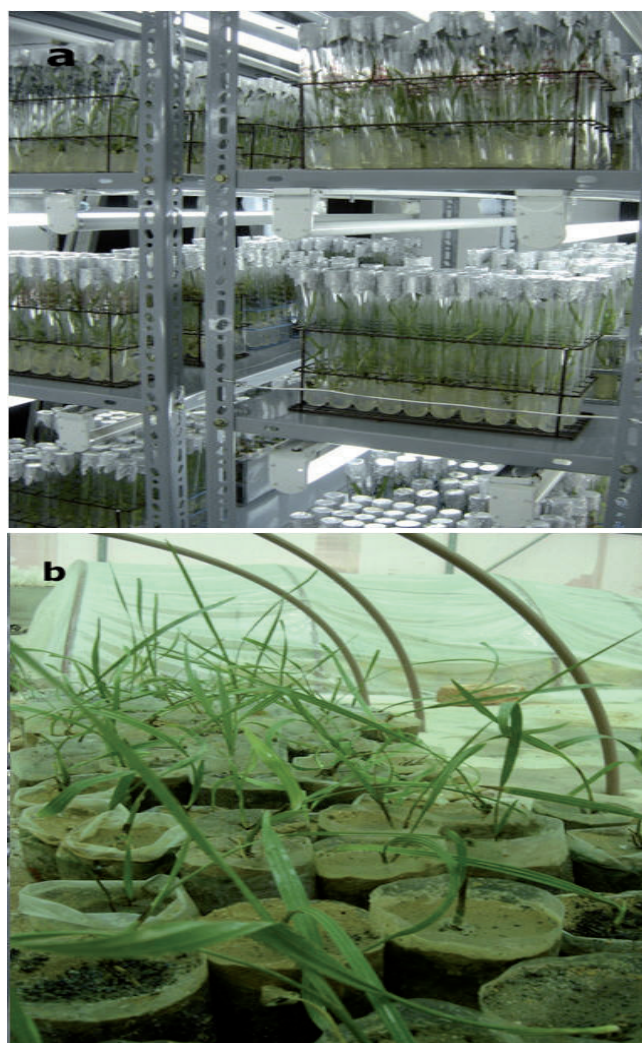
**Table 2:** Production capacity of a single inflorescence Gulistan cv. after 1, 7, 13 subcultures during the multiplication and rooting stages

Production (culture vessel <sup>z</sup> )											
Sub 1				Sub 7				Sub 13			
Callus	Embryo	Shoot	Total	Callus	Embryo	Shoot	Total	Shoot	Plantlet	Total	
10	7	4	21	16	70	40	126	1644	1212	2856	

<sup>z</sup>Each culture vessel (350 ml jar or 250×25 mm long tube) contained 1 gram of callus, or 10 embryos or shoots in average. Each long tube contained 1 intact plantlet with shoot-root system.



**Figure 1:** In A) The initial inflorescence explants of 2 months after culture on the starting medium. Notice direct initiation of the shining globular structures (pro-embryos) instead of the small florets. In B) Healthy shoots in the multiplication stage. Each culture vessel (350 ml) may contain 20-30 shoots. Note the growth vigor and length of the proliferated shoots is expressing readiness for rooting.



**Figure 2:** In A) 40 tubes-racks of *in vitro* rooting date palm are reflecting mega production. In B) One-month old date palm plantlets of female inflorescence technique were successfully acclimatized. Note thrive, tall and wide leaves.

## REFERENCES

- Abahmane, L., Bougerfaoui, M. and Anjarne, M. 1999.** 9-11 Nov; Use of tissue culture techniques for date palm propagation and rehabilitation of palm groves devastated by bayoud disease. Proceeding of International Symposium on Date Palm. pp. 385-388. Assiut University, Assiut, Egypt.
- Abul-Soad, A. A. 2003a.** Biotechnological studies of date palm: Micropropagation of inflorescence, molecular biology and secondary metabolites. Ph.D. diss., Pomology Department, Faculty of Agriculture, Cairo University.
- Abul-Soad, A. A., El-Sherbini, N. R. and Bakr, E. E. 2004a.** 6-8 Oct; Organogenesis in female inflorescence of date palm (*Phoenix dactylifera* L. cv. Zaghloul). The Second International Conference on Date Palm. pp. 139-163. El-Arish, Egypt.
- Abul-Soad, A. A., El-Sherbini, N. R. and Bakr, E. E. 2005a.** 14-16 Nov; Date palm (*Phoenix dactylifera* L. cv. Zaghloul) propagation using somatic embryogenesis of female inflorescence. 3rd Conference on Recent Technologies in Agriculture. pp. 3423-441. Cairo University, Egypt.
- Abul-Soad, A. A., El-Sherbini, N. R. and Bakr, E. E. 2007b.** Effect of basal salts and sucrose concentrations on morphogenesis in test tubes of female inflorescence of date palm (*Phoenix dactylifera* L.) Zaghloul c.v. Egyptian Journal of Agricultural Research **85**(n1B):385-394.
- Abul-Soad, A. A., Ibrahim, I. A., El-Sherbini, N. R. and Bakr, E. E. 1999b.** 12-14 Sep; In vitro and ex vitro optimization for rooting and acclimatization of date palm. The First International Conference, in Egypt, on Plant Tissue Culture and its Application. pp. 227-241. Egypt.
- Abul-Soad, A. A., Ibrahim, I. A., El-Sherbini, N. R. and Bakr, E. E. 2002a.** In vitro optimization for plant regeneration of date palm (*Phoenix dactylifera* L.). Minia Journal of Agricultural Research and Development (MCAES 1<sup>st</sup>), Egypt **22**(2D):2265-2282.
- Abul-Soad, A. A., Ibrahim, I. A., El-Sherbini, N. R. and Bakr, E. E. 2004b.** 8-11 Apr; Improvement and characterization of somatic embryogenesis in date palm (*Phoenix dactylifera* L.). Proceedings of The International Conference of Genetic Engineering & its Applications. pp. 359-373. The Egyptian Society of Genetics and Suez Canal University, Sharm El Sheikh City, South Sinai, Egypt.
- Abul-Soad, A. A., Markhand, G. S. and Shah, S. A. 2008.** 25-27 Apr; Effect of Naphthaleneacetic acid and Indole-3-acetic acid on somatic embryogenesis of female inflorescence explants of date palm (*Phoenix dactylifera* L.) cv. Aseel. The Third International Conference on Date Palm. pp. 222-231. Faculty of Agriculture and Environmental Science, Suez Canal University, North Sinai, Egypt.
- Abul-Soad, A. A., Zayed, Z. E., Salah, A. and Sedqi, R. A. 2002.** 15-17 Oct; Tissue culture of date palm (*Phoenix dactylifera* L.). The 3rd Scientific Conference of Agricultural Science. pp. 327-341. Assiut, Egypt.
- Abul-Soad, A. A., Zayed, Z. E. and Sedqi, R. A. 2006.** Improved method for the micropropagation of date palm (*Phoenix dactylifera* L.) through elongation and rooting stages. Bulletin of Faculty of Agriculture, Cairo University **57**(4):789-802.
- Abul-Soad, A. A. 2007a.** 5-8 May; Inflorescence tissue culture utilization for date palm (*Phoenix dactylifera* L.) micropropagation. Fourth Symposium on Date Palm. King Faisal University, Al-Hassa, Saudi Arabia.
- Abul-Soad, A. A. 2007b.** 5-8 May; Study of processing development problems of dates. Fourth Symposium on Date Palm. King Faisal University, Al-Hassa, Saudi Arabia.
- Abul-Soad, A. A. 2009.** 28 Jul; Inflorescence utilization in tissue culture for date palm (*Phoenix dactylifera* L.) micropropagation. Proceedings of the International Dates Seminar. pp. 33-43. Date Palm Research Institute, Shah Abdul Latif Univ., Khairpur, Pakistan.
- Bhaskaran, S. H. and Smith, R. H. 1992.** Somatic embryogenesis from shoot tip and immature inflorescence of *Phoenix dactylifera* L. cv. Barhee. Plant Cell Reports **12**:22-25.
- El Hadrami, I. and Baaziz, M. 1995.** Somatic embryogenesis and analysis of peroxidases in *Phoenix dactylifera* L. Biologia Plantarum **37**(2):197-203.
- Feki, L. and Drira, N. 2007.** 5-8 May; Development of an efficient plant regeneration system for date palm. Fourth Symposium on Date Palm. King Faisal University, Al-Hassa, Saudi Arabia.
- Gamborg, O. L., Miller, R. A. and Ojima, K. 1968.** Nutrient requirements of suspension cultures of soybean root cells. Experimental Cell Research **50**:148-151.
- Hassan, L. 2007.** 5-8 May; Agrobacterium-mediated genetic transformation of date palm (*Phoenix dactylifera*). Fourth Symposium on Date Palm. pp. 182. King Faisal University, Al-Hassa, Saudi Arabia.
- Jain, S. M. 2010.** 15-17 Mar; Prospects of in vitro conservation of date palm induced and natural genetic diversity. 4th International Date Palm Conference. Abu Dhabi, UAE.

**Murashige, T. and Skoog, F. 1962.** A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**:473-497.

**Saker, M. M. and Ghareeb, H. A. 2007.** 5-8 May; Factors influencing transient expression of Agrobacterium-mediated transformation of GUS gene in embryogenic callus of date palm. The Forurth Symposium on the Date Palm. pp. 183. King Faisal University, Al-Hassa, Saudi Arabia.

**Tisserat, B. 1984.** Date palm. In Handbook of plant cell culture,

edited by W. R. Sharp, D. A. Evans, P. V. Ammirato and Y. Yamada. New York: Macmillan Publishing Company. pp. 505-545.

**Tisserat, B. 1984.** Propagation of date palm by shoot tip cultures. *HortScience* **19**:230-231.

**Zaid, A., Al Kaabi, H. H. and El Korechi, B. 2007.** Large scale in vitro propagation of a rare and unique male date palm (*Phoenix dactylifera* L.) using inflorescences technique. *ISHS Acta Horticulturae* **736**:243-254.