AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

# Micropropagation of "KL1" date palm (*Phoenix dactylifera* L.)

Noppharat Intha[a], Peerasak Chaiprasart[a,b,c,*]

[a] *Faculty of Agriculture Natural Resources and Environment, Naresuan University, Phitsanulok 65000, Thailand*
[b] *Center of Excellence in Postharvest Technology, Naresuan University, Phitsanulok 65000, Thailand*
[c] *Postharvest Technology Innovation Center, Chiang Mai University, Chiang Mai 50200, Thailand*

**Article Info**

**Article history:**
Received 15 March 2018
Revised 18 October 2018
Accepted 24 December 2018
Available online 28 February 2020

**Keywords:**
1-Naphthaleneacetic acid (NAA),
2,4-Dichlorophenoxyacetic acid (2,4-D),
6-Benzylaminopurine (BAP),
Date palm,
Tissue culture

**Abstract**

Date palm (*Phoenix dactylifera* L.) is one of the most economically important fruit crops in many Arab countries. Recently, date palm cv. KL1 was developed and subsequently cultivated in many parts of Thailand. However, male and female trees could not be clearly distinguished until trees were flowering, and it should be propagated by tissue culture to obtain true-to-type plants. This study investigated the propagation of cv. KL1 female plants using a tissue culture technique. Offshoots aged 2 yr from mother plants were selected and surface sterilized in 0.1% mercuric chloride and 1% sodium hypochlorite. The dissected shoots were then cultured on modified Murashige and Skoog (MS) medium supplemented with 100 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 3 mg/L kinetin and 1 mg/L 6-benzylaminopurine (BAP), then cultured under dark conditions. Initiated callus formation was observed after 7 mth of culture. The embryogenic callus cultured in MS liquid medium supplemented with 6 mg/L 2,4-D produced the most somatic (2.59 g) growth at 1 mth. The somatic embryos were proliferated to new plantlets on MS medium, until 5 cm tall. In the final stage, root induction and elongation on MS medium supplemented with 0.9 mg/L 1-naphthaleneacetic acid induced the maximum number of roots (5.2) at 2 mth.

## Introduction

Date palm (*Phoenix dactylifera* L.) is a monocotyledonous, dioecious species belonging to the Arecaceae or Palmaceae family which is highly heterozygous and is considered the most important fruit tree in many Arab countries, such as Saudi Arabia and Iraq (Badawy et al., 2005). It is widely cultivated in arid regions of the Middle East and North Africa (Masmoudi-Allouche et al., 2011). Date palm is one of the most important cash crops in the Middle East and most of the total world production is sourced from the Arabian region (Gabr and Abd-Alla, 2010). In addition, it is one of the main trees used in this region for ornamental and landscape purposes because it has good drought tolerance and the tree's sap, seeds, leaves and trunk also find other uses that bring additional income to date palm growers (Al-Khalifah and Shanavaskhan, 2012). Recently, date palm has become very popular in Thailand with young plants from *in vitro* culture being imported from commercial laboratories in England, France, Israel and Namibia which have sources of date plant material (Zaid and de Wet, 2002). Most of these laboratories have focused on the Medjool and Barhi variety with an average sale price (FOB) of about USD 20–23 per plant, where the selling price depends on the variety, the number of plants ordered and the growth stage at delivery (Zaid and de Wet, 2002).

Gabr and Abd-Alla (2010) discussed the sexual propagation of date palm through seeds and vegetatively using offshoots. They noted that propagation using offshoots is limited because less than two per

* Corresponding author.
E-mail address: peerasakc@gmail.com (P. Chaiprasart)



https://doi.org/10.34044/j.anres.2020.54.1.11

tree are produced annually and that while seed propagation is easy and practical for farmers, it too has many limitations, such as seed dormancy resulting in a low rate of germination, a high percentage of male plants and progeny variation due to cross pollination among the highly heterozygous populations. To overcome these problems and to meet the demand for high numbers of plants, it is necessary to develop an alternative method of propagation, such as the use of the plant tissue culture technique (Mujib et al., 2004; Eshraghi et al., 2005).

The tissue culture technique has been used to propagate a wide range of important palms such as coconut, oil palm and date palm (Tisserat, 1983). Micropropagation of date palm is commercially practiced at Date Palm Developments Ltd. (Glastonbury, UK) and the Date Palm Tissue Culture Laboratory (Abu Dhabi, UAE). The Asia-Pacific Consortium on Agricultural Biotechnology (APCoAB), a program of the Asia-Pacific Association of Agricultural Institutions (APAARI), has been promoting the appropriate use of different biotechnological tools and techniques for the improvement of agriculture in the region (Al-Khalifah and Shanavaskhan, 2012). Micropropagation is one such option it is the true-to-type propagation of a selected genotype using *in vitro* culture techniques. The tissue culture technique has many advantages over conventional propagation; plants produced through tissue culture have proven to have wide acceptability by farmers (Al-Khalifah and Shanavaskhan, 2012).

The overall advantages of tissue culture techniques are: production of healthy disease-and pest-free cultivars of female or male plant with the desired qualities, (such as bayoud resistance in female plants), superior pollen production in male plants, large-scale multiplication of a large number of plantlets in any season of the year, production of genetically-uniform progeny, ability to propagate elite cultivars which lack offshoots and which produce seed-only derived plants, and facilitation of the exchange of plant materials among laboratories for research purposes without risk of spreading diseases or pests and so avoiding most plant quarantine regulations (Johnson, 2011).

Plant tissue culture techniques have been used to clone a wide range of date palm cultivars worldwide (Abahmane, 2013). There have been a number of previous reports of date palm micropropagation using the callus-somatic embryo pathway (Tisserat, 1979; Sharma et al., 1984; McCubbin et al., 2000; Eke et al., 2005; Fki et al., 2011; Al-Khayri, 2013) as well as organogenesis (Rhiss et al., 1979; Beauchesne, 1983). Apical meristematic tissues from axillary and lateral offshoots have given promising results and are now used as the widely accepted source of explants for date palm tissue culture; however, very young or mature offshoots are not recommended, with offshoots aged 2–3 yr being preferred for use in culture (Al-Khalifah et al., 2013; Hoffmann et al., 2013).

## Materials and Methods

### *Plant material, preparation and stylization of explants*

Offshoots from date palm (*Phoenix dactylifera* L.) cv. KL1, a well-known superior cultivar grown in Thailand, were collected from healthy, disease-free mother plants. Only offshoots aged 2–3 yr were selected and then cleaned, with the large outer leaves and fibers carefully and gradually removed using a sharp knife until the shoot tip zone was exposed. Explants were washed in running tap water for 1 hr and then immediately placed in a chilled antioxidant solution consisting of 150 mg/L ascorbic acid and 100 mg/L citric acid for 30 min to prevent browning. They were then surface sterilized in 0.1% mercuric chloride ($HgCl_2$) solution mixed with 2 droplets/100 mL of Tween 20 for 5 min followed by four rinses with sterilized water. After that, the sheathing leaf base was removed and the explants were re-sterilized using 10% Clorox® solution mixed with 2 droplets/100 mL of Tween 20 for 10 min followed by four rinses with sterilized water. The outer soft leaves were carefully removed to obtain a shoot tip comprised of the apical meristem and four primordial leaves. The shoot tips were cut longitudinally into four sections and re-immersed in antioxidant before culturing on an initiation medium.

### *Callus induction*

The sectioned pieces were cultured on Murashige and Skoog (MS) medium modified by supplementation with 100 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 3 mg/L kinetin and 1 mg/L 6-benzylaminopurine (BAP). For induction of callus and subsequent embryogenesis, culture had to be incubated in a growth chamber at 25 ± 1°C under completely dark conditions. It was also necessary to refresh the same medium every 2 mth until callus formation occurred.

### *Somatic embryogenesis*

The primary calli obtained after 7 mth of culture were used in the liquid medium and chopped with a scalpel blade and 0.1 g was transferred into a 200 mL round bottomed flasks containing 30 mL MS liquid medium (without agar) supplemented with 2,4-D at 0 mg/L, 3 mg/L, 6 mg/L, 9 mg/L, 12 mg/L and 15 mg/L, five replications each. The flasks were placed on an orbital shaker at 120 rpm, under a 16 hr photoperiod (2,000 Lux) at 25 ± 1°C for 1 mth until embryo formation. Somatic embryos were weighed and the data recorded.

### *Elongation and root induction*

Somatic embryos were cultured on MS medium without any added plant growth regulator until new plantlets had formed and the leaves were about 5 cm long. Then, the roots of the plantlets were cut off and the plantlets were transferred to new MS medium supplemented with NAA at 0 mg/L, 0.3 mg/L, 0.6 mg/L, 0.9 mg/L and 1.3 mg/L, each with five replications. The plantlets were cultured under a 16 hr photoperiod (2,500 Lux) at 27 ± 1°C for 2 mth.

### *Statistical analysis*

The recorded data, the weight of each formed embryo and the number of roots,were subjected to analysis of variance in a completely randomized design using ready-made statistical software. Mean values of treatments were differentiated using Duncan's new multiple range test at the 95% test level.

## Results

*Sterilization and callus induction*

The explants were placed in a chilled antioxidant solution consisting of 150 mg/L ascorbic acid and 100 mg/L citric acid for 30 min to could prevent browning on the explants. The sterilized technique with $HgCl_2$ and Clorox® solution destroyed microorganisms. Then, the sterilized explants were sectioned and cultured on an initiation medium (Fig 1A). Selected contamination-free explants were used subsequently for callus induction. Bacterial contamination of some explants was noticed after 4 d in culture because of internal contamination of date palm tissues, but fungal contamination was absent. The sterilized shoot tips were cultured on modified MS medium until initial callus formation was observed (Fig 1B.) after 7 mth of culture under dark conditions.

*Somatic embryogenesis*

The embryogenic callus (Fig 4A.) obtained was cultured on the different media to stimulate embryo formation. The six treatments used 2,4-D at 0 mg/L (control), 3 mg/L, 6 mg/L, 9 mg/L, 12 mg/L and 15 mg/L produced embryo weights of 0.27 g, 2.02 g, 2.59 g, 2.13 g, 1.97 g and 1.65 g, respectively (Table 1, Fig 2). The embryo had two parts: the primary leaf which was the first true leaf that emerged on a plantlet, and it is first or primary root, called the radicle (Fig 3), having a length of approximately 1–2 cm. After this stage, the embryo could be cultured on solid medium for shoot elongation.

*Elongation and root induction*

The somatic embryos were proliferated to new plantlets, until they had three leaves and were 5 cm long for 2 mth (Fig 4B). In the root induction stage, the MS medium was supplemented with NAA at 0.9 mg/L, 1.2 mg/L, 0.6 mg/L, 0.3 mg/L and 0 mg/L induced the highest numbers of roots of 5.20, 4.50, 2.70, 1.85 and 0.25, respectively. The MS medium was supplemented with NAA at 1.2 mg/L, 0.9 mg/L, 0.6 mg/L, 0.3 mg/L and 0 mg/L induced the longest root lengths of 5.04 cm, 4.92 cm, 4.55 cm, 3.17 cm and 0.27 cm, respectively (Fig 4C, Table 2).

## Discussion

Tissue culture techniques or micropropagation have proven to be effective methods for the propagation of dioecious plants like date palm in which seed propagation causes genetic variation and the sex produced is unpredictable. Knowledge of tissue culture of date palm is not widespread, with only a few private companies doing this on a commercial basis, so young plants from the *in vitro* technique are very expensive. The current study investigated many techniques which have not been mentioned by the private companies. Many researchers want to micropropagate date palms because there are many advantages such as disease free, improved quality of date fruit production using elite cultivars, uniform product and facilitated safe germplasm exchange (Ferry, 2011).

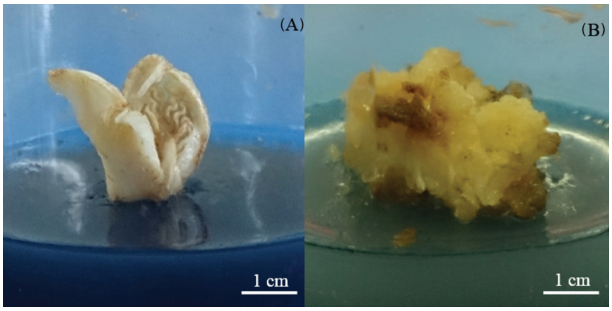


**Fig. 1** Initiation stage (A) and callus formation stage (B) 7 mth after culture on modified Murashige and Skoog medium under dark conditions, where initial callus was soft tissue (like absorbent cotton)

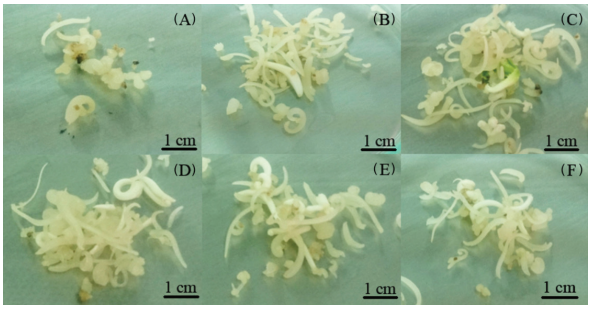


**Fig. 2** Embryogenesis of date palm cultured in media supplemented with2,4-dichlorophenoxyacetic acid at: (A) 0 mg/L; (B) 3 mg/L; (C) 6 mg/L; (D) 9 mg/L; (E) 12 mg/L; (F) 15 mg/L

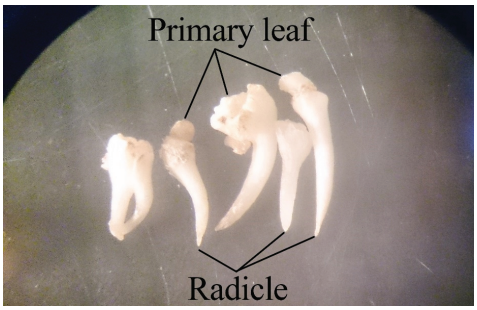


**Fig. 3** Characteristics of embryo cultured in liquid medium consisting of a long, fang-like radicle and a primary leaf not yet functioning nor proliferating


**Fig. 4** Micropropagation of date palm at important stages: (A) callus and organogenesis phase; (B) elongation stage (C) ready-to-transplant stage

**Table 1** Effects of 2, 4-dichlorophenoxyacetic acid (2,4-D) on embryogenesis of date palm in Murashige and Skoog liquid medium after 4 wk in culture.

| Medium | 2,4-D (mg/L) | Embryo weight (g) |
|---|---|---|
| 1 | 0 (control) | 0.27±0.05$^{d}$ |
| 2 | 3 | 2.02±0.32$^{bc}$ |
| 3 | 6 | 2.59±0.29$^{a}$ |
| 4 | 9 | 2.13±0.26$^{b}$ |
| 5 | 12 | 1.97±0.22$^{bc}$ |
| 6 | 15 | 1.65±0.27$^{c}$ |

Different lowercase superscripts within a column indicate significant (95% level) differences determined using Duncan's new multiple range test.

**Table 2** Mean±SD of number of roots and root length (cm) of date palm cultured in rooting medium supplemented with different concentrations of NAA

| | Root induction | | |
|---|---|---|---|
| Medium | NAA (mg/L) | Number of roots | Root length (cm) |
| 1 | - | 0.25±0.44$^{e}$ | 0.27±0.50$^{d}$ |
| 2 | 0.3 | 1.85±1.27$^{d}$ | 3.17±1.33$^{c}$ |
| 3 | 0.6 | 2.70±1.38$^{c}$ | 4.55±0.61$^{ab}$ |
| 4 | 0.9 | 5.20±1.06$^{a}$ | 4.92±0.52$^{ab}$ |
| 5 | 1.2 | 4.50±1.10$^{b}$ | 5.04±0.75$^{a}$ |

NAA = 1-naphthaleneacetic acid; MS = Murashige and Skoog
Different lowercase superscripts within a column indicate significant (95% level) differences determined using Duncan's new multiple range test.

*Sterilization and callus induction*

The preferred antioxidant solutions are ascorbic acid and citric acid that are used to reduce the formation of phenolic compounds, such as 150 mg/L each of ascorbic acid and citric acid (Al-Khalifah et al., 2013) after sterilization or 150 mg/L ascorbic acid and 100 mg/L citric acid (Gabr and Abd-Alla, 2010) before sterilization. However, the current experiment used double-immersion in antioxidant both before and after sterilization or every time a cut was made, because after cutting, the tissue wounding released phenolic compounds so the techniques adopted were suitable to avoid browning.

Surface sterilization is an important step in the disinfection of the offshoots used to generate the explants. Sterilization of date seed has used sulfuric acid (Sané et al., 2006), ethanol (Al-Khayri, 2010), mercuric chloride (Al-Khalifah et al., 2013) and Clorox® (sodium hypochlorite) (Gabr and Abd-Alla, 2010; Al-Khayri, 2010; Al-Khalifah et al., 2013) onoffshoots or inflorescence (Abahmane, 2013). The choice of chemical depends on the explant properties selected but based on the same principle of sterilization. Multiple sterilizations should be used to reduce the contamination rate as well. However, a low concentration should be used of a chemical suitable for the tissue as much of the internal tissue is susceptible to strong chemicals.

*Callus induction*

The most extensively used medium for date palm tissue culture is MS medium. However, a plant growth regulator (PGR) is often added to the MS medium, such as 100 mg/L 2,4-D and 3 mg/L 2iP (Asemota et al., 2010; Al-Khateeb, 2008; El-Din et al., 2007; Eshraghi et al., 2005 and Tisserat, 1983). Adding 1.5 g/L activated charcoal to the medium avoided browning during initiation and callus induction (Al-Khalifah et al., 2013). Culturing involvingincubation at 25 ± 1°C under completely dark conditions has been shown to be essential for callus formation, and this is a normal technique used for callus induction. The 2,4-D plays an important role in this stage in promoting cell division in the plant cell (Al-Khalifah et al., 2013) and the current study used the same conditions for callus induction.

The explants were induced in MS medium supplemented with 100 mg/L 2,4-D, 3 mg/L kinetin and 1 mg/L BAP to achieve production of callus. The current results were very similar to those of Asemota et al. (2010); Aslam and Khan (2009); Al-Khateeb (2008); El-Din et al. (2007); Eshraghi et al. (2005); Eke et al. (2005); Zaid (2003); Daguin and Letoutze (1988) and Tisserat (1983) who used 100 mg/L 2,4-D and 3 mg/L 2iP added to the culture medium. The ratios of auxin to cytokinin were similar, with the difference being kinetin and BAP were used in the current study.

*Somatic embryogenesis*

Sané et al. (2006) induced embryogenesis from cell suspensions in liquid medium supplemented with 2 mg/L 2,4-D; the suspensions were then cultivated for 1 mth in a liquid medium. The hormones added can vary to induce embryogenesis, though the synthetic growth regulator 2,4-D is regularly used, with 6 mg/L 2,4-D in the current study being the most effective concentration for somatic embryogenesis just a low concentration but it produced better results than higher concentrations. An excessive concentration can cause genetic disorders (El Hadrami et al., 2011), so this influenced the level used in the current study. After embryogenesis, the next stage is organogenesis, involving cell division, so 2,4-D is usually avoided (Hoffmann et al., 2013).

*Elongation and root induction*

The leaves emerging early in the elongation stage were light green at the shoot tip. At this stage it was not necessary to use the PGR, as the plantlets needed only nutrients important for growth and development. The environment (temperature, humidity, photoperiod) also was important. The leaves usually appeared before many roots, though in some cases they appeared simultaneously. Root induction usually requires the addition of auxin in the culture medium. For example, Khierallah and Bader (2007) used 1.0 mg/L NAA for root induction consistent with the current study, which was supplemented with 0.9 mg/L NAA to promote the maximum number of roots (5.20 roots), while 1.2 mg/L NAA produced the longest roots (5.04 cm). The current results were consistent with the study by Khierallah and Bader (2007). Good *in vitro* plantlets should have complete roots and leaves.

The current study considered the micropropagation of date palm through callogenesis and somatic embryogenesis until the produced date palm plantlets were ready for transplanting. Different PGR chemicals were used to induce callus and organs, for example 2, 4-D, kinetin, BAP and NAA depending on the stage. From initial culture

up to ready-for-transplanting takes about 2 yr and this study reported all stages of growth, whereas previous works have reported only some stages, such as somatic embryogenesis (Aslam et al., 2011), callus induction (Zayed and Abd Elbar, 2015; Zayed et al., 2016) and shoot elongation and acclimatization (Mazri, 2012). The current study highlighted that all parts of the process must be meticulously carried out, using advanced skills in callus induction. Acclimatization is necessary before transplanting to ensure survival in the greenhouse.

## Conflict of Interest

The authors declare that there are no conflicts of interest.

## Acknowledgements

The authors thank the Center of Excellence in Postharvest Technology, Naresuan University, Thailand. The Thailand Research Fund-Research and Researchers for Industry (RRi) Ph.D Scholarship (PHD57I0011) and Sam So Import & Export International Co., Ltd., provided financial support.

## References

Abahmane, L. 2013. Recent achievements in date palm (*Phoenix dactylifera* L.) micropropagation from inflorescence tissues. Emir. J. Food Agric. 25: 863–874.

Al-Khalifah, N.S., Askari, E., Shanavaskhan, A.E. 2013. Date Palm Tissue Culture and Genetical Identification of Cultivars Grown in Saudi Arabia. KACST Press.Riyadh, Saudi Arabia.

Al-Khalifah, N.S., Shanavaskhan, A.E. 2012. Micropropagation of Date Palms. Asia-Pacific Consortium on Agricultural Biotechnology (APCoAB) and Association of Agricultural Research Institutions in the Near East and North Africa (AARINENA). Riyadh, Saudi Arabia.

Al-Khateeb, A. 2008. Comparison effects of sucrose and date palm syrup on somatic embryogenesis of date palm (*Phoenix dactylifera* L.). Am. J. Biotech. Biochem. 4: 19–23.

Al-Khayri, J.M. 2010. Somatic embryogenesis of date palm (*Phoenix dactylifera* L.) improved by coconut water. Biotechnol. 9: 477–484.

Al-Khayri, J.M. 2013. Factors affecting somatic embryogenesis in date palm (*Phoenix dactylifera* L.) In: Aslam, J., Srivastava P.S., Sharma M.P., (Eds.)., Somatic Embryogenesis and Genetic Transformation in Plants. Narosa, New Delhi, pp. 15–38.

Asemota, O., Eke, C.R., Emoghene, B.O., Aisueni, N.O., Adetunji, O.A., Gidado, R.M., Solomon, B.O. 2010. Investigation of somatic embryogenesis for in vitro cultures of date palm (*Phoenix dactylifera* L.). Acta Hort. 882: 225–231.

Aslam, J., Khan, S.A. 2009. In vitro micropropagation of Khalas date palm (*Phoenix dactylifera L.*) as important fruit plant. J. Fruit Ornam. Plant Res. 17: 15–27.

Aslam, J., Khan, S.A., Cheruth, A.J., Mujib, A., Sharma, M.P., Srivastava P.S. 2011. Somatic embryogenesis, scanning electron microscopy, histology and biochemical analysis at different developing stages of embryogenesis in six date palm (*Phoenix dactylifera* L.) cultivars. Saudi J. Biol Sci. 18: 369–380.

Badawy, E.M., Afaf, M.A.H., El-Bana, A., Gehan, M.Y. 2005. Propagation of date palm (*Phoenix dactylifera*) plants by using tissue culture technique. Arab J. Biotech. 8: 343–354.

Beauchesne, G. 1983. Vegetative propagation of date palm (*Phoenix dactylifera* L.) by *in vitro* culture. In: Proceedings of 1st Symposium on Date Palm. Al-Ahssa, Saudi Arabia, pp. 698–699.

Daguin, F., Letouze, R. 1988. Regeneration of date palm (*Phoenix dactylifera* L.) by somatic embryogenesis, improved effectiveness by dipping in a stirred liquid medium. Fruits 43: 191–194.

Eke, C.R., Akomeah, P., Asemota, O. 2005. Somatic embryogenesis in date palm (*Phoenix dactylifera* L.) from apical meristem tissues from 'zebia' and 'loko' landraces. Afr. J. Biotechnol. 4: 244–246.

El-Din, Z., Amal, F.M., Abd. El-Rasoul, M., Ibrahim, I.S., Aly, A.S., Sharaf Eldeen, H.A.M. 2007. Micropropagation of some date palm cultivars: Changes of some chemical constituents related to embryogenesis. Acta Hort. 736: 233–241.

El Hadrami, A., Daayf, F., Elshibli, S., Jain, S.M., El Hadrami I. 2011. Somaclonalvariation in date palm. In: Jain, S.M., Al-Khayri, J.M., Johnson, D.V. (Eds.)., Date Palm Biotechnology. Springer Netherlands. The Netherlands, pp. 183–203.

Eshraghi, P., Zarghami, R., Mirabdulbghi, M. 2005. Somatic embryogenesis in two Iranian palm cultivars. Afr. J. Biotechnol. 4: 1309–1312.

Ferry, M. 2011. Potential of date palm micropropagation for improving small farming systems. In: Jain, S.M., Al-Khayri, J.M., Johnson, D.V. (Eds.)., Date Palm Biotechnology. Springer. The Netherlands, pp.15–28.

Fki, L., Masmoudi, R., Kriaa, W., Mahjoub, A., Sghaier, A. B., Mzid R., Mliki, A., Rival, A., Drira, N. 2011. Date palm micropropagation via somatic embryogenesis. In: Jain, S.M., Al-Khayri, J.M., Johnson, D.V. (Eds.). Date Palm Biotechnology. Springer. The Netherlands, pp. 47–68.

Gabr, M.F., Abd-Alla, M.M. 2010. Micro propagation of *Phoenix dactylifera* L. var karama. New York Sci J. 3: 64–69.

Hoffmann, F., Fortier, T., Hoffmann-Tsay, S.S. 2013. Micropropagation of Date Palm (*Phoenix dactylifera*). Phoenix Institute of Desert Agriculture, San Diego, California.

Johnson, D.V. 2011. Date palm biotechnology from theory to practice. In: Jain, S.M., Al-Khayri, J.M., Johnson, D.V. (Eds.)., Date Palm Biotechnology. Springer Netherlands. The Netherlands, pp. 1–11.

Khierallah, H.S.M., Bader, S.M. 2007. Micropropagation of Date Palm (*Phoenix dactylifera* L.) var. Maktoom through Direct Organogenesis. Acta Hort. 736: 213–224.

Masmoudi-Allouche, F., Gargouri-Bouzid, R., Kriaâ1, W., Saïdi, M.N., Jamoussi, H., Abdelkefi, Y., Drira. 2011. Investigation of genetic variability related to the in vitro floral hermaphrodism induction in Date palm (Phoenix dactylifera L.). Afr. J. Biotechnol. 10: 10567–10574.

Mazri, M.A. 2012. Effect of liquid media and in vitro pre-acclimatization stage on shoot elongation and acclimatization of date palm (*Phoenix dactylifera* L.) cv. Najda. J. Orna Hort Plan. 2: 225–231.

McCubbin, M.J., Van Staden, J., Zaid, A. 2000. A southern African survey conducted for off-types on date palm production using somatic embryogenesis. In: Proceedings of the date palm international symposium. Windhoek, Namibia, pp. 68–72.

Mujib, A., Cho, M.J., Predieri, S., Banerjee, S. 2004. In Vitro Application in crop improvement. Exford IBH Public, New Delhi, India.

Rhiss, A., Poulain, C., Beauchesne, G. 1979. La culture *in vitro* appliqué a la multiplication vegetative du palmier dattier (*Phoenix dactylifera* L.). Fruits. 34: 551–554. [in French]

Sané, D., Aberlenc-bertossi, F., Gassama-dia, Y.K., Sagna, M., Trouslot, M.F., Duval, Y., Borgel, A. 2006. Histocytological analysis of callogenesis and somatic embryogenesis from cell suspensions of date palm (*Phoenix dactylifera)*. Ann Bot. 98: 301–308.

Sharma, D.R., Dawra, S., Chowdhyry, J.B. 1984. Somatic embryogenesis and plant regeneration in date palm (*Phoenix dactylifera* L.) cv. 'Khadrai' through tissue culture. Indian J. Exp. Biol. 22: 496–598.

Tisserat, B. 1979. Propagation of date palm (*Phoenix dactylifera* L.) *in vitro*. J. Exp Bot. 30: 1275–1283.

Tisserat, B. 1983. Development of new tissue culture technology to aid in the cultivation and crop improvement of date palms. In: Proceedings of 1st Symposium on the Date Palm in Saudi Arabia. Al-Ahssa, Saudi Arabia, pp. 126–139.

Zaid, A., de Wet, P.F. 2002. Date palm propagation. In: Zaid, A. (Eds.)., Date Palm Cultivation. Available source: http://www.fao.org/docrep/006/Y4360E/y4360e00.htm #Contents. 18 Febuary 2020

Zaid, Z. 2003. Comparative studies on the production of date palm cultivars via tissue culture technique. Ph.D. Dissertation, Department of Pomology of Agriculture, Cairo University. Cairo, Egypt.

Zayed, E.M.M., Abd Elbar, O.A. 2015. Morphogenesis of immature female inflorescences of date palm *in vitro*. Ann. Agric. Sci. 60: 113–120.

Zayed, E.M.M., El Din, A.F.M.Z., Manaf, H.H., Abdelbar, O.H. 2016. Floral reversion of mature inflorescence of date palm *in vitro*. Ann. Agric. Sci. 61: 125–133.