Iraqi Journal of Biotechnology, September 2018, Vol. 17, No. 2, 1-12

Integrating Date Palm Biotechnology with community, A Review

Kadhim Mohammad Ibrahim

Professor in Plant Biotechnology, College of Biotechnology, Al-Nahrain University, IRAQ.

Abstract: Date palm (*Phoenix dactylifera* L.) tree is one of the oldest cultivated fruit trees in many regions in Asia and Africa particularly Mesopotamia. The tree has sustained and associated with many human cultures as a source for food, wood, furniture besides the high nutritional value of fruits. The tee has been and still plays a vital role in mitigating harsh environments in the regions where date palm groves are already established. The proposed review focuses on entire utilization of date palm biotechnology in improving the environment, enrichment of flora and fauna diversity, flourishing the tourism, and other aspects of human life. Biotechnology has been integrated almost in all human aspects including the full usefulness of manipulating date palm tree. Widespread of date groves certainly will encourage the biodiversity enriching the ecosystem with a variety of fauna and flora including birds, butterflies, insects, other plant species, and soil microorganisms. Industry based on date palm pruning remains will also flourish the biofuel production. In the last decades, the advances in horticultural and technological practices have been reflected on the date industry shifting into modern agricultural systems. Although, implementation of this progress in some date palm countries is still a slow process. Major challenges are post-harvest and mechanization technologies that are so crucial for building rural societies.

Keywords: date palm, biotechnology, environment, biodiversity, bio products, tissue culture.

Corresponding author: should be addressed (Email: kadhimm2003@yahoo.co.uk).

Introduction:

The magnificent characteristics of date palm tree have initiated a great wish to fully utilize the tree products thus it is a blessed tree (figure 1). Dates grow in various types of soil ranging from light, medium and heavy soils. The tree is the most tolerant to high pH, alkaline and saline conditions (1). It survives at a salt concentration up to 4% and sodicity more than 1%. Date palm trees are so diverse in fruit shape, color, taste, edibility, time of maturity and fruits products.

Figure (1): Date palm is a blessed tree.

Traditionally, date palms are propagated using off shoots (figure 2) emerge from the axillary buds underneath the soil. True to type trees are raised in this method however, a limited number of offshoots is obtained depending upon the tree cultivar. During the whole tree life span, the number of offshoots does not exceed 5 offshoots while, some date palm produce more than 15 offshoots per a tree such as Zahdi which can be exploited for new plantations. Normally, dates are grown at a density of about 120-200 trees per hectare (2). Although some cultivars produce much less than this number and there is a potential risk of diseases transmission (3).

Seeds can be a mean for propagation but they produce heterozygous trees with almost 50% males thus, it is not commonly used (4). Seeds can be stored for years, thus date palm groves are considered as a big seed bank. They germinate easily and are available in large quantities and establish intensive root system (figure 2). Moreover, Shabana *et al*. (5) recorded some new cultivars raised from seeds but in general not for commercial production (4). Although, sexual propagation results in non-true to type trees, but the total advantages obtained are much better than the commercial value of dates yield from the environmental issue. Encapsulated embryos production and forecasting is a new technology requires more intensive studies but still very promising for the future.

Figure (2): Conventional date palm propagation. Left; using seeds giving sexual plants with dense root system. Right; offshoots emerge from axillary buds can be separated and planted for establishment of a true to type plantations.

Possible contribution of date palm in community:

1- Date palm grooves reduce the temperature of the ambient environment and raise the air humidity which lead to save energy and electrical equipment's (6).

2- The tree is an excellent candidate for combating desertification (7). This will lead to change the landscape of deserts converting the derelict land to an oasis.

3- It has the potential of removing soil pollutants.

4- Enhances and enriches the biodiversity (8).

5- Promoting folk industries since tens of such industries depend on date tree bio products (9).

6- The possibility of implementing sustainable agriculture by growing multiple crops utilizing of the semi shade cused by trees, especially for those crops with less requirements of light intensity. It is a customary to grow all citrus species between date palm rows, other farmers grow beans or salad crops. Additional revenue as a result of intercropping can be estimated by $10000 per hectare a year.

7- Modern bioethanol industry can exploit the large quantities of tree pruning remains especially in dense planting (figure 3). The conversion of cellulose, hemicellulose, lignin and other polysaccharides to simple sugar then to bioethanol after fermentation process represents an alternative for fossil fuel with unlimited source of energy (10).

Figure (3): Dense planting of date palm trees produces large biomass can be utilized for bioful production.

8- New date palm grooves can extend honey industry depending on pollen grains produced by the extended plantations. An estimation of one ton of honey can be produced from one hectar taking into consideration the number of male trees grown in such area.

9- Reduction in migrating people from rural to urban areas, thus mitigating the overpopulation in cities. This will improve the working environment in both regions.

10- Labor investment and therfore a reduction in unemploiment which defintly leads to econmic florishment.

11- Activation of folk industries (e.g. house and garden furnuture, composting industry, luxury staff) achieving a supplementary income for people, in addition to conservation of folk industry.

Plant tissue, organ culture as a mean for rapid multiplication:

This technique is applied to all varieties to obtain true-to-type date palm plants. This includes selection of commercial varieties or any variety of interest (Standard tissue culture labs and skilled staff can do the job. Shoot tip explants have been used to obtain organogenic stems of the Moroccan

cultivar Najida. Shoots were proliferated *in vitro* on Beauchese (11) medium (BM). Murashige and Skoog (12) medium (MS) using full, half or third strength supplemented with 2-naphthoxyacetic acid (NOAA) at 0, 0.25, 0.5 $mg.L^{-1}$ + kinetin. Multiplication was achieved on half strength MS + 0.5 $mg.^{L-1}$ NOAA + 0.5 $mg.^{L-1}$ kinetin. The above combination resulted in more than 23 shoots per explant with minimum vitrification. For the purpose of shoot elongation, the same medium components are relevant or using half MS or full MS without need to add plant growth regulators. Rapid shoot elongation was noticed. For rooting, half MS supplemented with 1 $mg.L^{-1}$ NOAA + 1 $mg.L^{-1}$ kinetin, increased mean shoot length up to 15 cm accompanied with approximately 6 roots per explant with an average mean length 3.4 cm. Plantlets were acclimatized in a greenhouse. Noticeably, high survival rate reached 90% was recorded for the plantlets derived from shoots grown on plant growth regulators free medium after two months.

Somatic embryogenesis:

Date palm has routinely been micropropagated in hundreds of tissue culture laboratories. Encapsulated seeds or embryos (somatic or sexual) have been produced and used for different research and crop production objectives (figure 4). Embryos can be encapsulated with the proper gel and used to make seeds or embryos and even plant bombs to reach their targets.

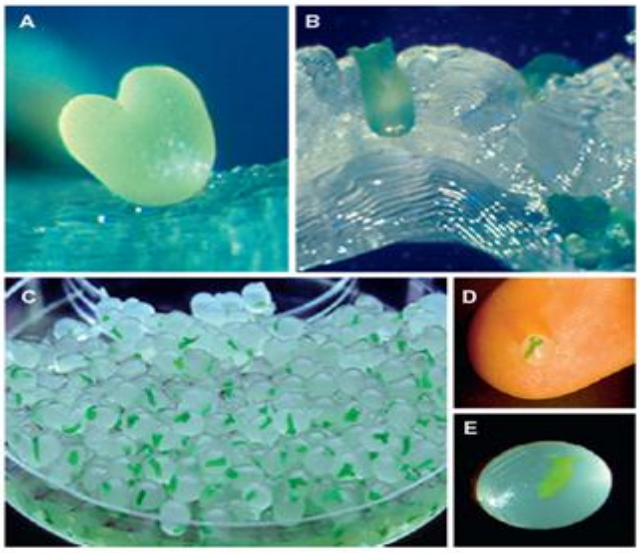

Figure (4): Somatic embryos are a promising mean for mass cultivation of plants. (A) Embryo at the heart like shape). (B) Embedding of embryo in artificial nutrients and gel.

In order to tolerate and sustain the new soil conditions, encapsulation of embryos can be supplemented with nutrients similar to those in the seed endosperm. Other supplements may be added to sustain their growth till they well establish in the soil.

It is some time ago when Thorpe (13) suggested producing whole date palms from somatic embryos, whether directly after proliferation to shoots or indirectly from calli clumps (figure 5).

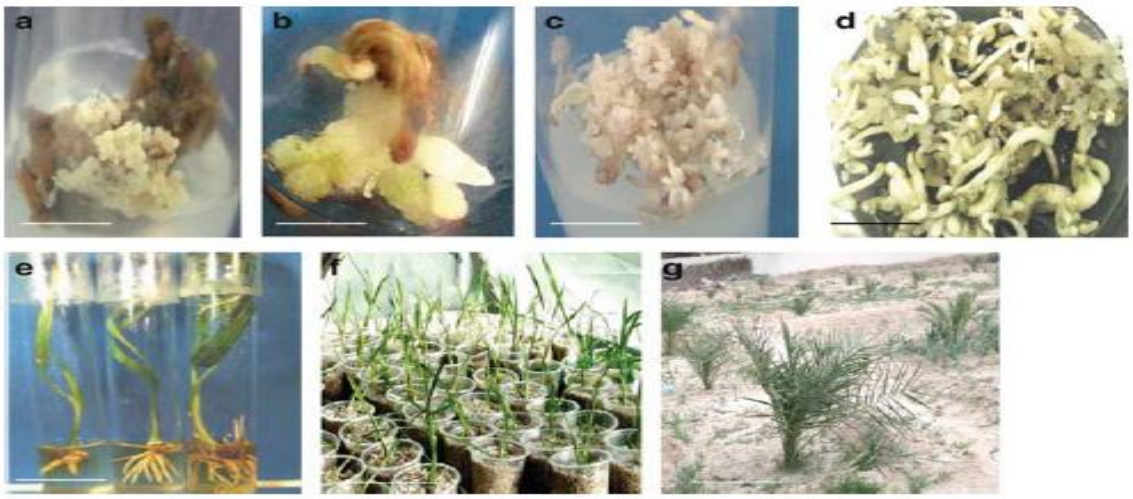

Figure (5): Induction and regeneration of somatic embryos in date palm using young leaflets as explants (a) Embryonic callus at the globular stage. (b) Direct embryogenesis at the lower end of the leaflets. (c) Differentiation of embryonic callus. (d) Mature somatic embryos. (e) Date palm plantlets after weaning (f) Offshoots 3 months old grown inside a glasshouse. (g) Offshoots 2 years old grown in newly established date palm orchard.

Raised date palm trees can be used in combating desertification in arid and semi-arid areas across the world provided a suitable protection from grazing animals and ensuring irrigation sources (figure 6).

Figure (6): Date palm tree raised from somatic embryo grown in the desert and protected from harsh environment and grazing animals paricularly goats.

Micropropagation using shoot tips:

The technique is based on using portions of the terminal apex as explants (14; 15). Shoots appear after some months are used as stock explants after proliferation (figure 7, 8).

Figure (7): Date palm micropropagation using shoot tips. Left; terminal date palm apex. Right; shoot proliferation.

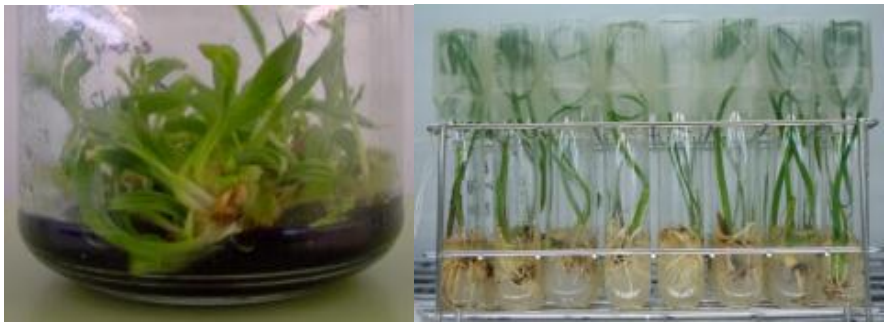

Figure (8): Multiplication stage and proliferation of shoots (left); Rooting of shoots (Right).

Organogenesis technique is based on exploitation of the meristematic shoot tip explants to form new shoots. Plant growth regulators incorporated into the media are used at low concentrations. The plantlets produced are identical to the mother tree since vegetative buds come directly from mother plant tissue. However, the success of this technique is highly dependent on the success of the first multiplication step (initiation) which requires a well-trained staff. Additionally, date palm shoot tip explants are sometimes highly contaminated with internal bacteria. These contaminants are introduced *in vitro* with cultured explants even if they have been well disinfected (16).

Micropropgation using microspores and immature fruits:

Inflorescences are available during spring season and can be used as a source for explants avoiding the destruction of the off shoots, however the technique has been restricted to Barhee and Barban cultivars which are widely grown in Iraq, researchers attempted with other cultivars with the aim of commercial production (14, 17, 18). Zygotic embryos have been induced to from immature fruits 3 – 4 months after pollination (19). Sexual embryos dissected from mature or immature fruits can be easily used as a source for somatic embryos but certainly they will be heterozygous and thus undesirable for commercial production. Inflorescences excised from Barhi and Maktoom cultivars in early spring and cultured on MS medium supplemented with 10.0 µM 2iP plus 5.0 µM NAA (20). Adventitious shoots were proliferated after callus transfer to MS liquid medium containing 10.0 µM 2iP plus 5.0 µM NAA. Direct organogenesis was achieved when spathes and spikes were inoculated onto

MS medium after 2 months. Addition of glutamine to liquid agitated medium increased the number of emerging buds. In fact, intensive experimental work is required for optimization of the technique. Date palm inflorescence culture was also largely investigated by Drira (21). Morphogenetic responses were found dependent on the origin and physiological stage of the explant. Date palm inflorescence culture was studied intensively by (22, 13, 23) who used the technique for the micropropagation of 16 date palm genotypes with good fruit quality. He produced hundreds of well-acclimatized plants belonging to 9 genotypes. Inflorescence-derived plants have shown normal growth and no abnormalities.

Figure (9): Plant material employed for date palm micropropagation protocols using inflorescence tissue: (a) Emerged inflorescence opened under aseptic conditions, (b) Plant material disinfection in sodium hypochlorite solution, (c) Inflorescence explants (1.5-2 cm) ready to be inoculated on culture media. (Cited from Abahmane (16).

Figure (10): Floral explant responses on culture media used: (a) Shoot initiation, (b) Shoot multiplication, (c) Root formation and (d) Carpel development. (Cited from Abahmane (15).

Morphogenesis in tissue cultures:

Various explant sources exhibited different responses to morphogenesis (table 1). Shoot tips excised from plantlets regenerated in vitro showed the highest survival rate reached 95%(24).

Table (1): Morphogenesis obtained from shoot tip cultures derived from various date explant sources

Explant sources (*)	Survival/ treatment (%)	Shoot growth/ culture (%)	Shoot length/ culture (%)	Leaves/ culture	Rooting/ culture (%)
Adult palm	70	85	2.12 ±.71	1.5 ±.5	0
Juvenile offshoot	78	80	2.75 ±.69	2.5 ±.6	0
Seedling	85	100	2.35 ±.65	2.0 ± 0.0	60
Asexual plantlet	95	100	1.67 ±.39	2.2 ±.4	80

(*) 15-20 cultures employed per treatment; results taken 8 weeks after planting.

Plantlet acclimatization:

Acclimatization is a crucial step in date palm micropropagation. Successful date palm production protocols should minimize losses during acclimatization. More than 70% plantlet survival for two cultivars has been reported by (25, 26). Plantations are already established in many countries and reached bearing stage. Currently, tens of laboratories are producing large scale date palms. Date palm plantlets are ready for transplanting they should have gain the following characteristics; two to three healthy and enlarged leaves with no curling phenomenon, a shoot length of at least 10 to 15 cm from stem base to the highest point of the leaves, a shoot base with an onion bulb-like form (also called pear-shaped crown), a well-developed root system with an average of 5 cm in length. Adventitious rooting is obtained by trimming the primary roots to 1 - 1.5cm in length and re-culturing the plant to an agar nutrient medium containing 0.01/0.1 mg/l NAA without charcoal and well acclimatized plant as a final product. Plants are then rinsed in distilled water to remove adhering agar and residual sucrose. A spray with Benlate solution at 0.5 % (or any wide spectrum fungicide) is important since it protects the plant from fungal attack.

Transplanting to the soil medium:

Transplanting should be done as quickly as possible to avoid plant dehydration and root damage. The soil medium must always be sterile and usually consisting of 1 peat: 1 vermiculite (v/v) mixture. Sterile sand with a large grain size is useful to improve drainage. Bark is avoided since it dries out rapidly and causes a water stress situation. Thus, the substrate should be a well-drained with good water retention capacity. The adequate pH to work with should be about 6.5.

Plants are preferably irrigated with 50% Hoagland's solution or 10% MS solution before their incubation into a micro tunnel in an environmentally controlled glasshouse (or a large plastic tunnel) ensuring a high relative humidity (90 - 95 %) and a constant temperature ± 25 - 26°C day time and 21 - 22°C during the night. Bottom heating of the micro tunnel (± 23°C) was found to be very helpful. Tissue culture-derived plants should be adapted to gradually decreasing humidity and gradually increasing light. The light intensity is important during the first 3 to 4 weeks (around 10,000 lux) with a 16 hrs photo period. Benlate can be applied to the foliage once a week, and irrigation using 10% MS solution (or 50% Hoagland) every 3rd or 4th day depending on the hygrometry level of the micro tunnel.

The plastic of the micro tunnel is gradually opened 4 to 6 weeks later, in order to decrease humidity and prepare the plants to adapt to the large glasshouse (or tunnel) conditions which preferably should have a fog system. Plantlets are now ready to be transplanted to larger plastic bags. Water should never be sprayed form the top of the plant. Plants could stay in the glass house (or a tunnel) for a period between 3 to 4 months before their transfer to a less environmentally controlled nursery, which is usually at the farmer's level, for their further hardening-off process.

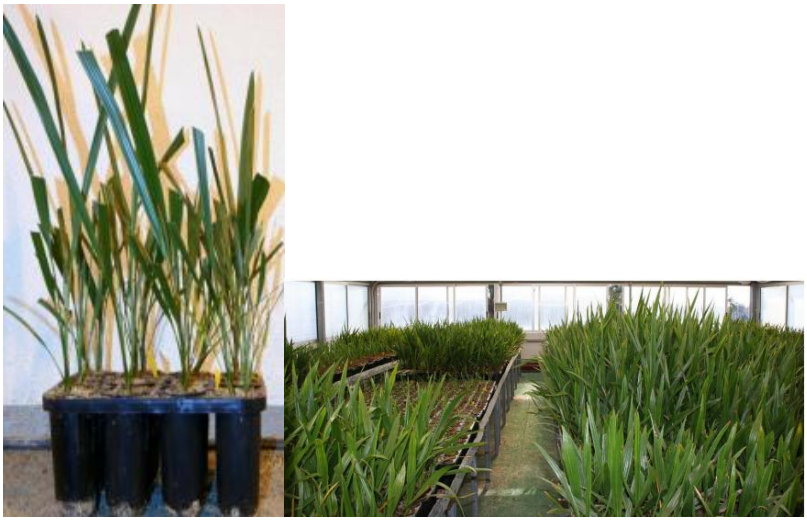

Figure (9): Acclimatized plant (left) and post acclimatization plants (right).

Figure (10): Fruiting of date palm trees raised from tissue culture.

Problems encountered in date palm micropropagation:

Tissue browning:

Browning of cultured explants is a frequent problem in date palm micropropagation. This phenomenon can be dealt during somatic embryogenesis (26) and organogenesis (28). Caffeoylshikimic acids were diagnosed by Loutfi and El Hadrami (29) causing tissues death. Activated charcoal or PVP were added into the culture medium (26; 30). Others incorporated ascorbic acid and/or citric acid with the disinfecting solution (25; 20). Abuhatem et al. (27) studied the

effect of BAP and the number of subcultures on browning occurs in cvs. Boufeggouss and Bouskri. They suggested a frequent transfer of cultures into a fresh medium every 7 days.

Hyperhydricity:

Hyperhydricity is a physiological disorder occurs frequently in date palm somatic embryos and organs as a result of water accumulation in the cultured explants. Type of PGRs, ammonium concentration and the use of cell suspensions lead to increase the intensity of the disorder (30, 28). Thus, the above factors should be manipulated carefully. Othmani *et al*. (26) obtained Somatic embryogenesis and plant regeneration in date palm cv. Boufeggous from embryogenic callus. Al-Khayri (25) improved Somatic embryogenesis when culture medium was supplemented with coconut water. Ibrahim *et al*. (31) recommended the addition of putrescin and salycilic acid to improve somatic embryogenesis. Proper maturation conditions for date palm somatic embryos has to be optimized.

Conclusion:

Date palm trees have many aspects need to be unveiled and exploited thoroughly. Improving the environment, utilization of such tolerant tree in phytoremediation polluted soils, increasing and enriching the species diversity, florishment the economy, all these can be achieved by proper manipulation of date palm trees. The emerging advancement in biotechnology may facilitate the large scale production of trees and ease the cultivation process. Date palm tree is a competitive candidate to replace petrol in flourishing the economy.

Although, numerous works have been published on date palm micropropagation, but still to be optimized particularly for recalcitrant cultivars, shortening the time for plantlets production, and certainly reducing the incidence of physiological disorders. An urgent studies are required on cryopreservation of date palm tissues, somatic embryos and other genetic materials to conserve the presently available cultivars and maintaining the new ones may rise from breeding programs.

References:

1. Chundawat, B. S. (1990). Arid Fruit Culture. Oxford & IBH, New Delhi.
2. Chandra, A. (1993). The Thar Desert in Western Rajasthan. In Towards the rational use of high salinity tolerant plants (Lieth, H. and Al Masoon, A. (eds.) vol. 1. 2:321-223. Performance of date palm in saline alkali soils.
3. Al-Khalifah, N. S. and Askari, E. (2011). Growth Abnormalities Associated with Micropropagation of date palm. In: Date palm biotechnology, Jain, S.M.; Al-Khayri, J.M. and Johnson, D.V. (eds.) Springer, Dordrecht.
4. Zaid, A.; El-Korchi, B. and Visser, H. J. (2011). Commercial date palm tissue culture procedures and facility establishment. In: Date palm biotechnology, Jain SM, Al-Khayri J. M, Johnson DV (eds.) Springer, Dordrecht.
5. Shabana, H. R.; Al-Ani, B. and Mohammed, I. (2014). Investigation of some new cultivars of date palm raised from seed (pit) germination. 5th International Date Palm Conference, Abu Dhabi, UAE, 16-18 March, Abstract No. 38.
6. Ibrahim, K.M. (2010). The role of date palm tree in landscaping. Date Palm Production and Processing Technology Symposium, Sultan Qaboos University. Pp 19-30.

7. Okere, A. U.; Odofin, W. T.; Aladele, S. E. and Fajimi, O. (2010). 4th Inter. Date Palm Conference, UAE, 15-17 March. Abstract No. 38.
8. Ibrahim, K. M. (2006). The role of date palm tree in landscaping. Date Palm Production and Processing Technology Symposium, Sultan Qaboos University. Pp 19-30.
9. Winners Book (2012, 2013, 2014). Khalifa International Date Palm Award. National Media Council.
10. Khierallah, H. S. M.; Bader, S. M.; Ibrahim, K. M. and Al-Jboory, I. (2015). Date Palm Status and Perspectives in Iraq. In: Date Palm Genetic Resources, vol 2. Asia and Europe, Springer Science.
11. Beauchesne, G.; Zaid, A. and Rhiss, A. (1986). Meristematic potentialities of bottom of young leaves to rapidly propagate date palm. Proceedings of the Second Symposium on Date Palm.
12. Murashige, T. and Skoog, F. A. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol..Plant. 15: 473-479.
13. Thorpe, T. A. (1993). In vitro organogenesis and somatic embryogenesis: Physiological and biochemical aspects. In: Morphogenesis in Plants. Roubelakis-Angelakis K.A., Tran Thanh Van K.M. (eds.) Plenum, New York, pp 19-38.
14. Bhaskaran, S. and Smith, R. H. (1992). Somatic embryogenesis from shoot tip and immature inflorescence of Phoenix dactylifera cv. Barhee. Plant Cell Rep. 12: 22-25.
15. Abahmane, L. (2011). Date palm micro propagation via organogenesis. In: Jain S.M., Al-Khayri, J.M., Johnson, D.V. (eds) Date Palm Biotechnology. Springer, Dordrecht, 69-90.
16. Abahmane, L. (2013). Date palm micropropagation by using inflorescence tissues. In: Proceedings of the international symposium on date palm. King Saud University, Kingdom of Saudi Arabia. 911-924.
17. Abul-Soad, A. A. and Mahdi, S. M. (2010). Commercial production of tissue culture date palm (*Phoenix dactylifera* L.) by inflorescence technique. J. Genet. Eng. Biotechnol. 8: 39-44.
18. Abul-Soad, A. A. (2011). Micropropagation of date palm using inflorescence explants. In: Date Palm Biotechnology, Jain, S. M., Al-Khayri, J. M., Johnson, D.V. (eds.), Springer, Dordrecht, 91-117.
19. Reynolds, J. and Murashige, T. (1979). A sexual embryogenesis in callus cultures of palms. In Vitro, 15:383–387.
20. Khierallah, H. S. M.; Al-Hamdany M. H. S.; Abdulkareem, A. A. and Saleh, F. F. (2015). Influence of sucrose and Pacloburtazol on callus growth and somatic embryogenesis in date palm cv. Bream. Int. J. Curr. Res. Aca. Rev. 1: 270-276.
21. Drira, N. (1981).Multiplication végétativeet micropropagation du palmierdattier (*Phoenix dactylifera* L.) à partird'organesprélevéssur la phase adultecultivés in vitro. Specialty doctorate thesis. Fac. Sci. Tunis, Tunisa.
22. Abahmane, L. (1998). Micropropagation of date palm (*Phoenix dactylifera* L.) selected clones by using inflorescence tissues. In: Proceedings of the international conference on date palm. ACSAD, Syria. pp. 256-260.
23. Abahmane, L. (2013). Recent achievements in date palm (*Phoenix dactylifera* L.) micropropagation from inflorescence tissues. Emir. J. Food Agric. 2013. 25 (11): 863-874.
24. Zaid, A. and de Wet, P. F. (2000). Date Palm Propagation. In Date Palm Cultivation. Chapter 5. FAO Corporate Document Repository.
25. Al-Khayri, J. M. (2001). Optimization of biotin and thiamine requirements for somatic embryogenesis of date palm (*Phoenixdactylifera* L.). In Vitro Cell Dev. Biol. Plant. 37: 453-456.
26. Othmani, A.; Bayoudh, C.; Drira, N.; Marrakchi, M. and Trifi, M. (2009). Somatic embryogenesis and plant regeneration in date palm *Phoenix dactylifera* L., cv. Boufeggous is significantly improved by fine chopping and partial desiccation of embryogenic callus. Plant Cell Tiss. Organ Cult. 97: 71-79.
27. Abohatem, M. A; Bakil, Y. and Baazia, M. (2017). Plant regeneration from somatic embryogenic suspension cultures of date palm. Methods Mol. Biol. 1637: 203-214.
28. Mazri, M. A. (2015). Role of cytokinins and physical state of the culture medium to improve in vitro shoot multiplication, rooting and acclimatization of date palm (*Phoenix dactylifera* L.) cv. Boufeggous. J Plant Biochem. Biotechnol. 24: 268-275.

29. Loutfi, K. and El Hadrami, I. (2005). *Phoenix dactilyfera* date palm. In: Biotechnology of fruit and nut crops, Litz, R.E. (ed.) CAB International, Wallingford, pp 144-156.
30. Mazri, M. A. and Meziani, R. (2013). An improved method for micropropagation and regeneration of date palm (*Phoenix dactylifera* L.). J. of Plant Bioch. and Biotech. 22 (2): 176–184.
31. Ibrahim, K. M.; Khierallah, H. S. M. and Hussain, N. H. (2014). Callus growth and somatic embryogenesis as affected by putrescin and salycilic acid in date palm (*Phoenix dactylifera*) var. Bream. Proceedings of the Fifth Inter. Date Palm, Conference. UAE, March16-18, 2014, 223-227.