Mazri and Meziani, Cell Dev Biol 2015, 4:3
DOI: 10.4172/2168-9296.1000160

Cell & Developmental Biology
ISSN: 2168-9296

Review Article Open Access

Micropropagation of Date Palm: A Review

Mouaad Amine Mazri[1]* and Reda Meziani[2]

[1]*Institut National de la Recherche Agronomique, CRRA-Marrakech, UR Agro-Biotechnologie, Laboratoire de Biotechnologie Végétale, Avenue Mohamed 6, BP 533, Marrakech, Morocco*

[2]*Institut National de la Recherche Agronomique, CRRA-Errachidia, UR Systèmes Oasiens, Laboratoire National de Culture des Tissus de Palmier Dattier, Avenue Moulay Ali Chérif, BP 2, Errachidia, Morocco*

Abstract

Somatic embryogenesis and organogenesis are the two pathways of choice for rapid and large-scale propagation of date palm. They have been successfully used for the micropropagation of elite genotypes and have proved their effectiveness for the commercial production of many cultivars. Nevertheless, regeneration through somatic embryogenesis or organogenesis in date palm is still difficult to achieve for recalcitrant genotypes and is often hampered by certain physiological disorders. In the present review, we report the results of a number of studies carried out on date palm micropropagation. It also describes different factors that influence each stage of somatic embryogenesis and organogenesis and the main problems encountered during these two micropropagation processes.

Keywords: *Phoenix dactylifera* L.; Organogenesis; Somatic embryogenesis

Introduction

Date palm (*Phoenix dactylifera* L.) is one of the most important fruit crops cultivated in arid and semi-arid regions. It is distributed throughout the Middle East, North Africa, South Sahel, areas of East and South Africa, Europe and USA [1], with approximately 150 million trees worldwide [2]. Date palm is cultivated for its high productivity and the high nutrient value of its fruit, for preserving ecosystems threatened by desertification and creating appropriate microclimate for agriculture under arid conditions. In addition, date palm cultivation generates considerable opportunities for rural employment, provides a major source of income for farmers and ensures livelihood and food security of the rural areas [3,4].

Date palm can be propagated sexually by seeds or asexually by offshoots. Propagation by seeds cannot be used for the commercial production of elite genotypes due to its heterozygous character [5], and because of the considerable difference between seedlings and vegetatively propagated plants in terms of production potential, fruit maturation and quality, and harvesting time [6]. Propagation by offshoots is a slow technique that is hampered by the restricted number of offshoots produced by a single date palm tree, the low survival rate and the risk of transmitting diseases [7]. Propagation of date palm through in vitro techniques presents an efficient alternative for the conventional methods [8]. Indeed, date palm micropropagation allows rapid and large-scale multiplication of uniform and healthy plants, with neither seasonal effects nor the risk of spreading diseases and pests during plant material exchange [9].

Micropropagation of date palm can be achieved through either somatic embryogenesis or direct organogenesis. Somatic embryogenesis is characterized by the development of a somatic cell into an embryo with a bipolar structure, leading to shoot and root formation. This technique is used in many commercial laboratories for its high potential for rapid and large-scale plant production. On the other hand, organogenesis is characterized by the direct formation of adventitious buds on the explant. This may produce new plants after root regeneration. Organogenesis has lower multiplication efficiency than somatic embryogenesis but it permits the preservation of true-to-typeness of multiplied plants.

The aim of this review is to summarize the literature on date palm micropropagation through somatic embryogenesis and organogenesis, and to highlight the main factors affecting each stage of these two micropropagation techniques. In addition, the main problems encountered during date palm micropropagation are described.

Plant material and disinfection

Shoot tips obtained from offshoots are the most used explants for somatic embryogenesis and organogenesis. The use of shoot tip explants has resulted in successful regeneration in many date palm genotypes such as Iklane and Jihel [10], Khanizi and Mordarsing [11], Khasab and Nabout [12], Barhee, Zardai, Khalasah, Muzati, Shishi, and Zart [13]. Nevertheless, it was reported that, for some date palm genotypes, offshoots may not be available. This makes their micropropagation using shoot tip explants impossible [14]. In such situation, inflorescences might be used. Inflorescence explants are easy to obtain and do not require the sacrifice of offshoots. However, it is important to note that their availability is restricted to early spring [14]. Micropropagation from inflorescence explants was reported in few date palm cultivars such as Barhee [15] and Gulistan [16]. Reynolds and Murashige reported that zygotic embryos derived from green fruits harvested 2-3 months after pollination can be used to induce somatic embryogenesis in date palm [17]. Pinker et al. also used zygotic embryos to induce somatic embryogenesis in cultivars Zahdi, Khistawi, Asabe Elarous and Barban. Nevertheless, such explants are not appropriate for true-to-type propagation [18].

Different methods have been described for explant disinfection, and the main disinfecting solutions used are sodium hypochlorite [19,20] and mercuric chloride [21,22]. In addition, antioxidant additives such as ascorbic acid, citric acid, polyvinylpyrolydon (PVP), anhydrous caffeine and/or sodium diethyldithiocarbonate have been widely used during disinfection in order to reduce explant browning [12,13,19,20].

***Corresponding author:** Mouaad Amine Mazri, Institut National de la Recherche Agronomique, CRRA-Marrakech, UR Agro-Biotechnologie, Laboratoire de Biotechnologie Végétale, Avenue Mohamed 6, BP 533, Marrakech, Morocco, Tel: +212524447882; Fax: +212524446380; E-mail: m.a.mazri@gmail.com

Received May 27, 2015; **Accepted** August 28, 2015; **Published** September 03, 2015

Citation: Mazri MA, Meziani R (2015) Micropropagation of Date Palm: A Review. Cell Dev Biol 4: 160. doi:10.4172/2168-9296.1000160

Micropropagation by somatic embryogenesis

Somatic embryogenesis is the process by which somatic cells develop into somatic embryos after a series of biochemical and morphological changes [8], and the formed embryos are morphologically similar to zygotic embryos. The process of somatic embryogenesis comprises a sequence of stages: embryogenic callus induction, somatic embryo formation (somatic embryogenesis expression), somatic embryo maturation and conversion into plantlet. Somatic embryogenesis is the most commonly used technique for date palm micropropagation, mainly for its high plant production potential [1,9]. However, the success of this technique depends on several factors, including genotype, explant type and plant growth regulators (PGRs) among others. Here we present a review of the existing literature on date palm somatic embryogenesis and factors affecting each step of this regeneration technique.

Embryogenic callus induction and somatic embryo formation

Embryogenic callus induction in date palm is influenced by different parameters such as genotype, explant type, induction period and PGRs. In the case of either shoot tips or inflorescences, high levels of auxins have been used to induce embryogenic calli. 2,4-dichlorophenoxy acetic acid (2,4-D) is reportedly the most effective auxin for embryogenic callus induction in date palm, and it has been used mainly at the concentration of 100 mg/L. Embryogenic callus induction using 100 mg/L 2,4-D was reported in many date palm cultivars such as Khanizi and Mordarsing [11], Khasab and Nabout [12] and Nabout Saif [19]. However, Fki et al. mentioned that high doses of 2,4-D may induce somaclonal variation [9]. Therefore, other researchers used lower 2,4-D concentrations or other auxins in order to induce somatic embryogenesis. For instance, El Hadrami et al. used 5 mg/L 2,4-D to induce somatic embryogenesis in cvs. Iklane and Jihel [10]. Othmani et al. suggested 10 mg/L 2,4-D for cv. Boufeggous [21]. Aslam et al. used 1.5 mg/L 2,4-D for cvs. Barhee, Zardai, Khalasah, Muzati, Shishi, and Zart [13] while Khierallah et al. used 50 mg/L picloram for cv. Bream [20].

The induction of embryogenic calli is a very slow process in date palm. The induction period varies depending on the genotype and may last from few to several months. Othmani et al. reported the formation of somatic embryos after 6-7 months of induction in date palm cv. Boufeggous [21]. In date palm cvs. Amry, Malakaby and Zaghlol, Hassan and Taha observed somatic embryos after 9 months of induction [23] while Eshraghi et al. suggested an induction period of 12 months for cvs. Khanizi and Mordarsing [11].

The formation of somatic embryos (somatic embryogenesis expression) was observed either on PGR-free medium [24] or on medium supplemented with PGRs. In the latter case, low concentrations of PGRs were used [10,23,25].

Other factors such as biotin and thiamine [26], coconut water [12], yeast extract and casein hydrolysate [19], basal formulation of the culture medium [24], silver nitrate [27], carbon source [20,23] and Pacloburtazol [20] were reported to influence somatic embryogenesis in date palm.

Somatic embryo growth, maturation and germination

Many factors have been reported to affect date palm somatic embryo maturation and germination. Somatic embryo maturation in date palm can occur on a semi-solid medium or from suspension-cultured cells in a liquid medium. However, Fki et al. reported that the number of mature embryos increases considerably on liquid medium. In fact, in date palm cv. Deglet Nour, these authors reported the production of 200 embryos from 100 mg fresh weight callus in liquid medium after a 1-month cultivation period, while only 10 embryos were recovered from the same amount of callus on a solidified medium [28]. Therefore, in order to enhance somatic embryo maturation, suspension culture of embryogenic calli has been widely used and was reported in many date palm cultivars such as Jihel and Bousthami Noir [29], Nabout Saif [30], Boufeggouss and Bouskri [31] and Degla Beida [22]. Al-Khayri and Al-Bahrany reported that polyethylene glycol (PEG) stimulates the proliferation of embryos while abscisic acid (ABA) inhibits embryo growth [30]. Al-Khayri found that thiamine and biotin increase the number of embryos and promote their elongation in date palm cv. Khalas [26] while Othmani et al. reported that fine chopping and partial desiccation of embryogenic calli stimulate somatic embryo maturation in cv. Boufeggous [21].

Somatic embryo germination was achieved with different treatments. Zouine and El Hadrami used a combination of 6-benzylamino purine (BAP), indole-3-butyric acid (IBA) and 1-naphthalenacetic acid (NAA) to promote embryo germination in date palm cvs. Jihel and Bousthami Noir [29]. In cv. Boufeggous, Othmani et al. (2009a) indicated that 1 mg/L NAA resulted in the germination and conversion into plantlets of 81% of somatic embryos [21]. Al-Khayri reported that the concentration of NAA, IBA as well as the medium strength influence somatic embryo germination [32]. In date palm cvs. Malkaby and Barhee, germination frequencies of 83.3 and 60.0 % were observed, respectively, after 3 hours of desiccation [33]. Ibrahim et al. reported that the germination frequency of somatic embryos varies with the genotype used [33]. In date palm cv. Degla Beida, the germination frequency ranged from 8.66 to 35.2% depending on the texture of the culture medium [22]. Al-Khayri and Al-Bahrany reported that the size of somatic embryos (small, medium, large and very large) also influences their germination frequency [30].

Plantlet acclimatization

Acclimatization of date palm plantlets derived from somatic embryos was successfully reported in many cultivars. Al-Khayri reported a survival rate of 72-84 % after ex vitro transfer of cvs. Khasab and Nabout Saif plantlets [12]. In date palm cvs. Boufeggous and Deglet Nour, Othmani et al. observed the survival rates of 60 and 80%, respectively [21,34]. Other authors demonstrated that additives such as 5-aminolevulinic acid-based fertilizer [35] and ammonium nitrate and gibberellic acid [36], as well as inoculation with rhizosphere bacteria [37] promote date palm plantlet acclimatization.

Micropropagation by adventitious organogenesis

Organogenesis is the process by which explants undergo changes which lead to the formation of a unipolar structure (shoot or root primordium) with a vascular connection to the parent tissues [38]. In the case of date palm, the purpose of this technique is the formation of adventitious buds directly on the explant without the callus formation step. Date palm organogenesis comprises several steps: initiation of adventitious buds, shoot bud multiplication, shoot elongation and rooting then plantlet acclimatization [39-42]. The success of this regeneration pathway depends on several factors, which are presented in this section.

Adventitious bud initiation

The formation of adventitious buds on date palm explants depends on many factors such as media components, genotype, and time period

of plant material collection [6]. Various culture media were suggested for adventitious bud formation, depending on the cultivar. From offshoot-derived explants, Beauchesne et al. suggested half-strength Murashige and Skoog (MS) medium [43] supplemented with 1-5 mg/L 2-naphthoxyacetic acid (NOA), 1 mg/L NAA, 1 mg/L indole-3-acetic acid (IAA) and 0.1-3 mg/L 6-(dimethylallylamino) purine (2iP) [44]. Khierallah and Bader recommended MS medium supplemented with 2 mg/L 2ip, 1 mg/L BAP, 1 mg/L NAA and 1 mg/L NOA for cv. Maktoom [45]. Al-Mayahi suggested MS medium supplemented with 1 mg/L BAP and 0.5 mg/L thidiazuron (TDZ) for cv. Hillawi [46]. For cv. Zaghlool, Bekheet used MS medium supplemented with 2 mg/L 2ip and 1 mg/L NAA [41] while Hussain et al. used MS medium supplemented with 4 mg/L IBA and 1 mg/L BAP for cvs. Asil, Hussaini and Zaidi [47]. According to Al-Khateeb, low PGRs concentrations promote the formation of buds while high concentrations induce abnormal growth without bud formation [48].

Studies on adventitious bud formation from inflorescence explants are very scarce. Loutfi and chlyah indicated that shoot primordia is formed mostly on Greshoff and Doy medium supplemented with 0.5 mg/L NAA, 2 mg/L BAP and 1 mg/L 2iP [49]. In a recent review of the literature, Abahmane reported that the combination of one auxin and two cytokinins is effective for bud formation on inflorescence explants [50].

As regard to the period of offshoot removal, Beauchesne et al. suggested a period starting from the end of dates harvesting and lasting until the beginning of flowering [44]. Aissam reported that the explants taken between October and February show the highest buds formation rate [51], whereas Zaid et al. reported that the best period for the in vitro culturing of offshoot-derived explants is from the onset of flowering [6].

Shoot bud multiplication

Many factors influence shoot bud multiplication in date palm, especially the basal formulation of the culture medium, the genotype and PGRs. Abahmane mentioned that the main basal formulation used is MS at full or half-strength, supplemented with PGRs at low concentrations as compared with the bud initiation stage [39]. Zaid et al. reported that for shoot bud multiplication, NAA, NOA, IAA, BAP and kinetin might be used at 0.5-5 mg/L [6]. Beauchesne et al. suggested half-strength MS medium supplemented with 2 mg/L NOA, 1 mg/L NAA, 1 mg/L IAA, 0.5 mg/L BAP, 1 mg/L 2iP and 1-5 mg/L kinetin [44]. For cultivar Khalas, Aslam and Khan used 7.84 µM BAP for high shoot bud multiplication [52]. Khierallah and Bader recommended MS medium with a combination of 1 mg/L NAA, 1 mg/L NOA, 4 mg/L 2iP and 2 mg/L BAP for date palm cv. Maktoom [45] while Khan and Bi Bi found that MS medium containing 0.5 mg/L BAP and 0.5 mg/L kinetin yields the highest number of shoots per explant in cv. Dhakki. In a previous work on cv. Najda, we found that the best medium for shoot bud multiplication was half-strength MS medium supplemented with 0.5 mg/L NOA and 0.5 mg/L kinetin, which yielded an average of 23.5 shoot bud per explant after 3 months of multiplication [53]. Mazri recommended MS medium containing 2.5 µM IBA and 2.5 µM BAP for cv. 16-bis (22.3 shoot buds per explant) while he recommended half-strength MS medium supplemented with 3 µM IBA and 3 µM BAP for cv. Boufeggous, which showed 22.9 shoot buds per explant [54]. Al-Mayahi suggested MS medium containing 1 mg/L BAP and 0.5 mg/L TDZ for cv. Hillawi, which resulted in an average of 18.2 buds per culture [46]. Other factors such as the medium texture [54,55], cultivation in bioreactors [56], explant size and density [57] and carbon source [56,58,59] were also reported to affect shoot bud multiplication of date palm.

Shoot elongation, rooting and plantlet acclimatization

Shoot elongation and rooting may be achieved either on a medium containing PGRs or on a PGR-free medium. Beauchesne et al. suggested the use of half-strength MS medium supplemented with 1 mg/L NAA, 0.5 mg/L BAP, 0.5 mg/L kinetin and 1-3 mg/L gibberellin for shoot elongation [44]. El Sharabasy et al. reported that the use of 0.1 mg/L NAA has a better effect on shoot elongation as compared to IBA and IAA [60]. The use of liquid medium was also reported to promote shoot elongation [61]. As regard to shoot rooting, Bekheet recommended 1 mg/L NAA, which showed better results than IAA or IBA at the same concentration [41]. In a previous work on cv. Najda, we compared media with and without PGRs. Our results showed that shoot elongation is fast in media supplemented with PGRs, with high root formation rates. However, shoots cultured on PGR-free media had wider and greener leaves, and exhibited higher survival rates after acclimatization [53]. This shows that plantlet acclimatization might be influenced by previous culture conditions. Along this line, it has been shown that the texture of the elongation-rooting medium influences the survival rate of plantlets after ex vitro transfer. Indeed, the use of a liquid medium just before plantlet acclimatization showed lower survival rates as compared to a semi-solid medium [54,61]. On the other hand, increasing the level of sucrose in the elongation-rooting medium increases the survival rate of plantlets during acclimatization [59]. Other factors such as the nature of the substrate [60] and the application of gamma aminobutyric acid [62] were reported to influence plantlet acclimatization.

Problems Encountered in Date Palm Micropropagation

Tissue browning

Browning of in vitro cultured explants is a frequent problem in date palm micropropagation. This phenomenon was encountered during somatic embryogenesis [31] and organogenesis [54]. It is due to the high levels of caffeoylshikimic acids contained in date palm tissues [63] and leads to explant death. In order to control tissue browning, activated charcoal or PVP have been incorporated into the culture medium by many researchers [e.g., 21-23,53]. In addition, many other authors have used ascorbic acid and/or citric acid in the disinfecting solution in order to prevent browning [e.g., 19,20,23,27]. Abohatem et al. studied the effect of BAP and the rate of subcultures on tissue browning of date palm cvs. Boufeggouss and Bouskri and concluded that the transfer of cultures on a fresh medium every 7 days allows a considerable reduction of tissue browning [21]. Mazri reported that TDZ increases tissue browning during shoot bud multiplication of date palm cv. Boufeggous and leads to explant death [54].

Hyperhydricity

Hyperhydricity is a common physiological disorder in date palm somatic embryogenesis [64] and organogenesis [53,54]. It is the result of the accumulation of water in the cultured explants. Many factors such as PGRs, ammonium concentration and liquid media may increase the intensity of hyperhydricity [53,54,59,65]. In order to reduce the incidence of this phenomenon, Al-Khateeb suggested the use of solid media, increasing agar concentrations and decreasing PGRs and ammonium concentrations [65].

Precocious rooting

Root formation may take place during the shoot bud multiplication stage. This phenomenon decreases the multiplication efficiency of shoot buds since nutrients are absorbed by roots [65]. According

to Al-Khateeb (2008b), high auxin concentrations, low mineral concentrations and incubation of cultures in darkness increase the frequency of precocious rooting [65]. Mazri reported that the cytokinin type and medium texture also influence precocious rooting [54].

Genetic variability in regenerants

The genetic conformity of date palm plants micropropagated through somatic embryogenesis has been controversially discussed. Kumar et al., Othmani et al. and Othmani et al. studied the genetic fidelity of somatic embryogenesis derived plantlets using AFLP, ISSR and RAPD primers and confirmed the genetic stability of regenerants [34,66,67]. In contrast to these authors, Saker et al. observed genetic variability in 4% of the micropropagated plants of date palm cv. Zaghloul using RAPD markers [68]. In cultivars Sakkoty, Gandila and Bertamoda, Saker et al. reported 2.6, 0.8 and 1% level of polymorphism using AFLP markers, respectively [69]. Moghaieb et al. reported higher level of polymorphism, with 37.8% variability in the micropropagated plants of cv. Ferhi using RAPD primers [70]. Low concentrations of auxins, mainly 2,4-D, low numbers of subcultures and the use of juvenile explants were suggested to decrease the risk of somaclonal variation [9,71,72].

Conclusions and Future Prospects

Micropropagation of date palm either through somatic embryogenesis or through organogenesis was reported for many cultivars, and several factors have been revealed to influence these regeneration systems.

Date palm micropropagation presents an efficient way for the large-scale propagation of genotypes resistant to bayoud, a very dangerous disease caused by the fungus *Fusarium oxysporum* f. sp. *albedinis*, which had decimated more than 12 million trees during the last century. Plantlets of bayoud-resistant genotypes are used to rehabilitate palm groves ravaged by this fungus. Micropropagation also allows the large-scale propagation of cultivars of high fruit quality, in order to satisfy the high demand of farmers and consumers.

Despite the numerous works published on date palm micropropagation, research is still needed to optimize culture conditions for the newly selected genotypes and recalcitrant cultivars, to shorten the time needed to produce plantlets, and to reduce the incidence of physiological disorders. It is also important to carry out studies related to the application of somatic embryogenesis to genetic transformation, synthetic seeds production and cryopreservation of embryogenic cultures.

References

1. Jain SM (2012) Date palm biotechnology: Current status and prospective-an overview. Emir J Food Agric 24: 386-399.
2. Al-Khayri JM, Jain SM, Johnson DV (2015) Date palm genetic resources and utilization. In: Africa and the Americas, Al-Khayri JM, Jain SM, Johnson DV (eds) Springer, Dordrecht.
3. Jain SM, Al-Khayri JM, Johnson DV (2011) Date palm tissue culture: a pathway to rural development. In: Date palm biotechnology. Jain SM, Al-Khayri JM, Johnson DV (eds) Springer, Dordrecht.
4. Rajmohan K (2011) Date palm tissue culture: A pathway to rural development. In: Date palm biotechnology. Jain SM, Al-Khayri JM, Johnson DV (eds) Springer, Dordrecht.
5. Tisserat B (1982) Factors involved in the production of plantlets from date palm callus cultures. Euphytica 31: 201-214.
6. Zaid A, El-Korchi B, Visser HJ (2011) Commercial date palm tissue culture procedures and facility establishment. In: Date palm biotechnology, Jain SM, Al-Khayri JM, Johnson DV (eds.) Springer, Dordrecht.
7. Al-Khalifah NS, Askari E (2011) Growth Abnormalities Associated with Micropropagation of date palm. In: Date palm biotechnology, Jain SM, Al-Khayri JM, Johnson DV (eds.) Springer, Dordrecht.
8. Quiroz-Figueroa FR, Rojas-Herrera R, Galaz-Avalos RM, Loyola-Vargas VM (2006) Embryo production through somatic embryogenesis can be used to study cell differentiation in plants. Plant Cell Tissue Organ Cult 86: 285-301.
9. Fki L, Masmoudi R, Kriaâ W, Mahjoub A, Sghaier B, et al. (2011a) Date palm micropropagation via somatic embryogenesis. In: Date palm biotechnology, Jain SM, Al-Khayri JM, Johnson DV (eds) Springer, Dordrecht.
10. El Hadrami I, Cheikh R, Baaziz M (1995) Somatic embryogenesis and plant regeneration from shoot-tip explants in *Phoenix dactylifera* L. Biol Plant 37: 205-211.
11. Eshraghi P, Zaghami R, Mirabdulbaghi M (2005) Somatic embryogenesis in two Iranian date palm cultivars. Afr J Biotechnol 4: 1309-1312.
12. Al-Khayri JM (2010) Somatic embryogenesis of date palm (*Phoenix dactylifera* L.) improved by coconut water. Biotechnol 9: 477-484.
13. Aslam J, Khan SA, Cheruth AJ, Mujib A, Sharma MP, et al., (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.
14. Abul-Soad AA (2011) Micropropagation of date palm using inflorescence explants. In: Date palm biotechnology, Jain SM, Al-Khayri JM, Johnson DV (eds.), Springer, Dordrecht, pp 91-117.
15. Bhaskaran S, Smith RH (1992) Somatic embryogenesis from shoot tip and immature inflorescence of *Phoenix dactylifera* cv. Barhee. Plant Cell Rep 12: 22-25.
16. Abul-Soad AA, Mahdi SM (2010) Commercial production of tissue culture date palm (*Phoenix dactylifera* L.) by inflorescence technique. J Genet Eng Biotechnol 8: 39-44.
17. Reynolds J, Murashige T (1979) Asexual embryogenesis in callus cultures of palms. In Vitro 15:383–387.
18. Pinker I, Ibraheem YM, Böhme MH (2009) Propagation of some date palm cultivars by using tissue culture methods. Acta Hortic 839: 71-78.
19. Al-Khayri JM (2011a) Influence of yeast extract and casein hydrolysate on callus multiplication and somatic embryogenesis of date palm (*Phoenix dactylifera* L.). Sci Hortic 130: 531-535.
20. Khierallah HSM, Al-Hamdany MHS, Abdulkareem AA, Saleh FF (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. Othmani A, Bayoudh C, Drira N, Marrakchi M, Trifi M (2009a) 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.
22. Boufis N, Khelifi-Slaoui M, Djillali Z, Zaoui D, Morsli A, et al., (2014) Effects of growth regulators and types of culture media on somatic embryogenesis in date palm (*Phoenix dactylifera* L. cv. Degla Beida). Sci Hortic 172: 135-142.
23. Hassan MH, Taha RA (2012) Callogenesis, somatic embryogenesis and regeneration of date palm *Phoenix dactylifera* L. cultivars affected by carbohydrate sources. Int J Agric Res 7: 231-242.
24. Al-Khayri JM (2011b) Basal salt requirements differ according to culture stage and cultivar in date palm somatic embryogenesis. Am J Biochem Biotechnol 7: 32-42.
25. Eke CR, Akomeah P, Asemota O (2005) Somatic embryogenesis of Date palm (*Phoenix dactylifera* L.) from apical meristem tissues from “Zebia” and “Loko” landraces. Afr J Biotechnol 4: 244-246.
26. Al-Khayri JM (2001) Optimization of biotin and thiamine requirements for somatic embryogenesis of date palm (*Phoenix dactylifera* L.). In Vitro Cell Dev Biol Plant 37: 453-456.
27. Al-Khayri JM, Al-Bahrany AM (2001) Silver nitrate and 2-isopentyladenine promote somatic embryogenesis in date palm (*Phoenix dactylifera* L.). Sci Hortic 89: 291-298.
28. Fki L, Masmoudi R, Drira N, Rival A (2003) An optimised protocol for plant

regeneration from embryogenic suspension cultures of date palm, *Phoenix dactylifera* L., cv. Deglet Nour. Plant Cell Rep 21: 517-524.

29. Zouine J, El Hadrami I (2007) Effect of 2,4-D, glutamine and BAP on embryogenic suspension culture of date palm (*Phoenix dactylifera* L.). Sci Hortic 112: 221-226.

30. Al-Khayri JM, Al-Bahrany AM (2012) Effect of abscisic acid and polyethylene glycol on the synchronization of somatic embryo development in date palm (*Phoenix dactylifera* L.). Biotechnol 11: 318-325.

31. Abohatem M, Zouine J, El Hadrami I (2011) Low concentrations of BAP and high rate of subcultures improve the establishment and multiplication of somatic embryos in date palm suspension cultures by limiting oxidative browning associated with high levels of total phenols and peroxidase activities. Sci Hortic 130: 344-348.

32. Al-Khayri JM (2003) In vitro germination of somatic embryos in date palm: effect of auxin concentration and strength of MS salts. Curr Sci 84: 101-104.

33. Ibrahim IA, Hassan MM, Taha RA (2012) Partial desiccation improves plant regeneration of date palm in vitro cultures. Wudpecker J Agric Res 1: 208-214.

34. Othmani A, Bayoudh C, Drira N, Marrakchi M, Trifi M (2009b) Regeneration and molecular analysis of date palm (*Phoenix dactylifera* L.) plantlets using RAPD markers. Afr J Biotechnol 8: 813-820.

35. Awad MA (2008) Promotive effects of 5-aminolevulinic acid based fertilizer on growth of tissue culture derived date palm plants (*Phoenix dactylifera* L.) during acclimization. Sci Hortic 118: 48-52.

36. Darwesh RSS, Zaid ZE, Sidky RA (2011) Effect of ammonium nitrate and GA3 on growth and development of date palm plantlets in vitro and acclimatization stage. Res J Agric Biol Sci 7: 17-22.

37. Farrag HMA, Abeer HE, Darwesh (2011) Growth Promotion of Date Palm Plantlets Ex vitro by Inoculation of Rizosphere Bacteria. J Hortic Sci Ornamen Plants 3: 130-136.

38. Thorpe TA (1993) In vitro organogenesis and somatic embryogenesis: Physiological and biochemical aspects. In: Morphogenesis in Plants. Roubelakis-Angelakis KA, Tran Thanh Van KM (eds.) Plenum, New York, pp 19-38.

39. Abahmane L (2011) Date palm micropropagation via organogenesis. In: Jain SM, Al-Khayri JM, Johnson DV (eds) Date palm biotechnology. Springer, Dordrecht, pp 69-90.

40. Al-Khalifah NS, Askari E, Shanavaskhan AE (2013) Date palm tissue culture and genetical identification of cultivars grown in Saudi Arabia. KACST Press, Riyadh, Saudi Arabia.

41. Bekheet SA (2013) Direct organogenesis of date palm (*Phoenix dactylifera* L.) for propagation of true-to-type plants. Sci Agr 4: 85-92.

42. Al Maari KW, Al Ghamdi AS (1998) Effect of seasonal variation on the multiplication of date palm through tissue culture. Proceedings of the International Conference on Date Palm.

43. Murashige T, Skoog FA (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Phys Planta 15: 473-479.

44. Beauchesne G, Zaid A, Rhiss A (1986) Meristematic potentialities of bottom of young leaves to rapidly propagate date palm. Proceedings of the Second Symposium on Date Palm.

45. Khierallah HSM, Bader SM (2007) Micropropagation of date palm (*Phoenix dactylifera* L.) var. Maktoom through direct organogenesis. Acta Hortic 736: 213–224.

46. Al-Mayahi AMW (2014) Thidiazuron-induced in vitro bud organogenesis of the date palm (*Phoenix dactylifera* L.) CV. Hillawi. Afr J Biotechnol 13: 3581-3590.

47. Hussain I, Rashid H, Muhammad A, Quraishi A (2001) In vitro multiplication of date palm. Proceedings of the Second International Conference on Date Palm.

48. Al-Khateeb AA (2006) Role of cytokinin and auxin on the multiplication stage of date palm (*Phoenix dactylifera* L.) cv. Sukry. Biotechnol 5: 349-352.

49. Loutfi K, Chlyah H (1998) Vegetative multiplication of date palm from the in vitro cultured inflorescence: effect of some growth regulator combinations and organogenetic potential of various cultivars. Agron 18: 573-580.

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

51. Aissam S (1990) Observations histologiques sur l'organogenèse et le développement des bourgeons du palmier dattier (*Phoenix dactylifera* L.) en culture in vitro. Thèse Doctorat Troisième Cycle.

52. Aslam J, Khan SA (2009) In vitro micropropagation of khalas date palm (*Phoenix dactylifera* L.), an important fruit plant. J Fruit Orn Plant Res 17: 15-27.

53. Mazri MA, Meziani R (2013) An improved method for micropropagation and regeneration of date palm (*Phoenix dactylifera* L.). J Plant Biochem Biotechnol 22: 176-184.

54. Mazri MA (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.

55. Al-Khateeb AA, Alturki SM (2014) A Comparison of Liquid and Semi-Solid Cultures on Shoot Multiplication and Rooting of Three Date Palm Cultivars (*Phoenix dactylifera* L.) in Vitro. Adv Env Biol 8: 263-269.

56. Fki L1, Bouaziz N, Kriaa W, Benjemaa-Masmoudi R, Gargouri-Bouzid R, et al. (2011) Multiple bud cultures of 'Barhee' date palm (*Phoenix dactylifera*) and physiological status of regenerated plants. J Plant Physiol 168: 1694-1700.

57. Mazri MA (2013) Effect of basal medium, explant size and density on the in vitro proliferation and growth of date palm (*Phoenix dactylifera* L.) cultivar '16-bis'. Not Sci Biol 5: 332-337.

58. Al-Khateeb AA1 (2008) Regulation of in vitro bud formation of date palm (*Phoenix dactylifera* L.) cv. Khanezi by different carbon sources. Bioresour Technol 99: 6550-6555.

59. Mazri MA (2014) Effects of plant growth regulators and carbon source on shoot proliferation and regeneration in date palm (*Phoenix dactylifera* L.) '16-bis'. J Hortic Sci Biotechnol 89: 415-422.

60. El Sharabasy SF, Bosila HA, Ibrahim IA (2001) Micropropagation studies on Zaghlool and Sewi cvs. of date palm (*Phoenix dactylifera* L.): III. Plantlet acclimatization. Proceedings of the Second International Conference on Date Palm.

61. Mazri MA (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 Ornament Hortic Plants 2: 225-231

62. Awad MA (2007) Effect of anti-stress substances and elemental sulphur application on growth and stress tolerance of tissue culture derived date palm (*Phoenix dactylifera* L.) plantlets cv. Khadrawy during acclimatization. Proceedings of the Fourth International Symposium on Date Palm.

63. Loutfi K, El Hadrami I (2005) *Phoenix dactilyfera* date palm. In: Biotechnology of fruit and nut crops, Litz RE (ed) CAB International, Wallingford, pp 144-156.

64. McCubbin MJ, Zaid A (2007) Would a combination of organogenesis and embryogenesis techniques in date palm micropropagation be the Answer? Acta Hortic 736: 255-259.

65. Al-Khateeb AA (2008b) The problems facing the use of tissue culture technique in date palm (*Phoenix dactylifera* L.). Sci J King Faisal Univ 9: 85-104.

66. Kumar N, Modi AR, Singh AS, Gajera BB, Patel AR, et al. (2010) Assessment of genetic fidelity of micropropagated date palm (*Phoenix dactylifera* L.) plants by RAPD and ISSR markers assay. Physiol Mol Biol Plants 16: 207-213.

67. Othmani A, Rhouma S, Bayoudh C, Mzid R, Drira N, et al (2010) Regeneration and analysis of genetic stability of plantlets as revealed by RAPD and AFLP markers in date palm (*Phoenix dactylifera* L.) cv. Deglet Nour. Int Res J Plant Sci 1: 48-55.

68. Saker MM, Bekheet SA, Taha HS, Fahmy AS, Moursy HA (2000) Detection of somaclonal variations in tissue-culture derived date palm plants using isoenzyme analysis and RAPD fingerprints. Biol Plant 43: 347-351.

69. Saker MM, Adawy SS, Mohamed AA, El-Itriby HA (2006) Monitoring of cultivar identity in tissue culture-derived date palms using RAPD and AFLP analysis. Biol Plant 50: 198-204.

70. Moghaieb REA, Abdel-Hadi AA, Ahmed MRA (2011) Genetic stability among date palm plantlets regenerated from petiole explants. Afr J Biotechnol 10:14311-14318.

71. El Hadrami A, Daayf F, Elshibli S, Jain SM, El Hadrami I (2011) Somaclonal Variation in Date Palm. In: Date palm biotechnology, Jain SM, Al-Khayri JM, Johnson DV (eds.) Springer, Dordrecht.

72. Khan S, Bi Bi T (2012) Direct shoot regeneration system for date palm (*Phoenix dactylifera* L.) cv. Dhakki as a means of micropropagation. Pak J Bot 44: 1965-1971.