INDIAN JOURNAL OF
ARID HORTICULTURE
2016, Vol. 11 (1-2):1-15

Date Palm propagation through tissue culture

P. N. Sivalingam*, Dhurendra Singh, Arjit Chaturvedi, Eshu Arora, Shiva Parihar and R. Bhargava
ICAR-Central Institute for Arid Horticulture, Beechwal, Bikaner, India
*Corresponding author: Email- pnsivalingam@gmail.com
(Received: 10.02.2015; Accepted: 4.012.2015)

Abstract

Date palm is one of the important arid fruit crops, naturally propagated through offshoots and seeds. These methods of propagation are not sufficient to meet the demand of genetically uniform large scale plantation of female date palm plants. This demand has been realized through tissue culture methods; embryogenesis and organogenesis. Initially somatic embryogenesis (SE) protocols have been developed for several cultivars of date palm in different countries for its large scale multiplication and planted in the field. After noticing abnormalities in some of the date palm plants developed through SE due to somaclonal variations, organogenesis method has been developed though it has low multiplication rate. Now-a-days date palm is commercially produced by organogenesis method in Morocco, UAE and Saudi Arabia. Research focus has also been given on identification of somaclonal variations in date palm plants produced by SE method by molecular markers, increasing multiplication rate in organogenesis method by modifying cultural conditions, automation of protocols, identification of exact cells involving in embryo formation and their mechanisms, etc. In India, recently SE protocol developed by public sector for commercial use and several public and private sectors involved in production of date palm plants by tissue culture approach. However many challenges ahead still needs to be resolved are; precise identification of somaclonal variants in *in vitro*, increasing multiplication rate in organogenesis, reducing duration in juvenile phase for callus induction in SE and shoot bud formation in organogenesis, developing genotype independent tissue culture protocols.

Key words: Date palm, embryogenesis, organogenesis, propagation

Introduction

Date palm (*Phoenix dactylifera* L., family-*Arecaceae*) is a dioecious, tall ever green, cross-pollinated, perennial monocotyledonous and most important fruit crop of the arid regions of the world (Taha *et al.,* 2001). This can successfully tolerate adverse environmental conditions such as drought, salinity and high temperature which are the peculiar conditions of desert (Bekheet *et al.*, 2008). It has domesticated and thought to have originated in the Southern Near East (Zohar and Spiegel Roy, 1975). It is distributed in Middle East and Arab countries, North Africa and South Sahel, areas of East and South Africa and Asia even in parts of Europe and USA. Total world production of dates was 7.55 million tonnes (MT) from 1.1 million hectare (Mha) land (~100 million date palm trees) (FAO, 2013) and Egypt alone produced 1.47 MT during 2012. In India date palm cultivated in approximately 0.0168 Mha with the production of 0.125 MT.

Date palm is naturally propagated through sexually by seeds and asexually by suckers. Being a dioecious crop, the male and female flowers are in separate tree allowing for cross pollination which creates heterogeneity among the progenies. Moreover, the seed propagated plants are approximately 50 per cent are male plants which produce no fruits. More over few male trees are enough to pollinate female trees in the field. Another disadvantage is the growth and multiplication of seed propagated seedlings is slow and takes 8-10 years or more to yield fruit. Traditionally, the propagation of date palm is achieved by using offshoots that are produced from axillary buds. These offshoots are true to type as they are asexually propagated and it comes to the fruiting 2-3 years earlier than seed propagated date palm trees. The disadvantage of using offshoot propagations are, (i) offshoots are produced only early stage of life cycle of tree (10-15 years) depending upon the genotypes (ii) limited in number per tree (maximum 15-20). The use of tissue culture is the most suitable approach for large scale plant multiplication in vegetatively propagated crops as it offers several advantages like, pest and disease free seedlings, large scale multiplication, genetically uniform plants of same sex, easy and fast exchange of planting material and germplasm exchange and economically reliable if produced in large scale (Zaid and Arias-Jimenez, 2002).Therefore, large scale production of female planting material of elite genotypes of date palm depends on tissue culture.

Micropropagation through organogenesis and embryogenesis methods has been successfully applied in date palm. Until now, the micropropagation of date palm has been limited success, World market need about 2 million date palms per year and approximately 25 research groups

are working world wide. Commercially, date palm plants produced through tissue culture by somatic embryogenesis in Israel, UAE, USA, UK and France and by organogenesis in Morocco, UAE and Saudi Arabia are produced through organogenesis. This review focuses on propagation of date palm plants through tissue culture in the World and in India by two methods (i) Organogenesis and (ii) Somatic embryogenesis.

Organogenesis

The regeneration of date palm plantlets through organogenesis was achieved in several countries outside India. This process involves initiation of meristematic shoot buds, shoot multiplication, shoot elongation and rooting, and acclimatization (Loutfi and El Hadrami, 2005; Zaid and Arias-Jimenez, 2002). The important and successful examples are also given in Table 1.

***Initiation of meristematic shoot buds*:** Most of the meristamatic shoot bud initiation from date palm cultures have been established from the internal face of young bases taken from offshoots (Beauchesne *et al.,* 1986; Beauchesne, 1988; Asemota and Eke, 2006; Khirellah and Badar, 2007; Abdul-Soad, 2006; Zaid and Tissert, 1983; Balal and El Deep, 1997). Other explants like petiole of female date palm (Costa and Aloufa, 2006) and male inflorescence (Bennasseur, 2007) are also used for shoot bud initiation. The meristamatic bud induction usually takes 8-10 months under dark conditions in order to reduce tissue browning and phenolic compounds accumulation and to stimulate cell division (Loutfi and El Hadrami, 2005; Bennasseur, 2007; Abd El Bar and El Dawayati, 2014). The meristem induction from an explant depends on several factors and also by their interactions, which includes composition of culture medium, genotype of explants, stage and age of offshoots collected from mother plants etc. Procambial cells which are going to develop as vascular strand are has the ability to form proembryos not other cells or other differentiated tissues (Abd El Bar and El Dawayati, 2014).

Most of the researchers used MS media with different types and combinations of plant growth regulators and additives. Induction and formation of proembryo needs higher amount of cytokinin (Abd El Bar and El Dawayati, 2014). The growth harmones; auxins like NAA, NOA, IAA and IBA and cytokinins like BA, kinetin, and 2iP, occasionally TDZ were used in various combinations and concentrations. The explants responded better in combination of auxins and cytokinins, which are; (i) 0.01-1 mg/L of NAA+1 mg/L of NOA+ 1-4 mg/L of 2iP+1-5mg/L of BA (Asemota and Eke, 2006; Khirelladha and Badar, 2007) (ii) 4mg/L of IBA or IAA+0.5mg/L of NAA+ 1-5 mg/L BA or 2iP (Balal and El Deep, 1997; Mater, 1990), (iii) 5.4 µM of NAA+4.9 µM of IBA+5-27 µM of NOA + 0.5 µM 2iP (Poulain *et al.,* 1979) and (iv) 5.4 µM of NAA + 5.7 µM of IAA+5-27 µM of NOA + 0.5-14.8 µM 2iP (Beauchesne *et al.,* 1986) (v) 1 mg/L NAA + 3mg/L 2ip + 3 mg/L BA (Khan and Bi, 2012) (vi) 2mg/L 2ip + 1mg/L NAA (Bekheet, 2013) and (vii) 3.0 mg l/L 2-iP + 0.1 mg l/L NAA (Chander Bhan *et al.*, 2013). Direct embryo formation from shoot tip explant of date palm requires 2mg/L BA only (Abd El Bar and El Dawayati, 2014). The significant variations in the frequency of induction were found in the cultures of different genotypes and cultivars in the same medium (Beauchesne, 1982) indicate their necessity of specific culture medium for each genotype. Induction requires the culture requires low light intensity (1000-1400 lux) with temperature at 27±1^{o}C and sub- culturing once in a month. With these efforts the number of buds obtained was 2 to 6.2 (Asemota and Eke, 2006; Khirelladha and Badar, 2007; Mater, 1990). It was also observed that explants from offshoot and inflorescence develop roots much earlier than bud initiation and the reason for this is not known (Anjarne and Zaid, 1993; Loufti, 1999).

***Shoot multiplication*:** The shoot multiplication from the meristematic buds of date palm mainly depends on the culture conditions, growth hormones and cultivars. In most of the researchers used the same concentration of auxins and cytokinins at both bud induction and multiplication stages where the success rate was very low. The best combination and concentration of growth hormones at the stage of multiplication are; (i)10.5 µM of NOA+5.4 µM of NAA+5.7 µM of IAA: 2.2 µM of BA+ 5.0 µM of 2iP + 4.6-23 µM of kinetin in one case, (ii) 1.0 mg/L of NAA +1.0 mg/L of NOA : 4.0 mg/L of 2iP+2.0 mg/L of BA (Beauchesne *et al.,* 1986; Asemota and Eke, 2006; Khirellah and Badar, 2007), (iii) 0.5 mg/L of TDZ + 1.0 mg/L of 2iP (Hussain *et al.,* 2001), (iv) 5 mg/L 2ip or/ and 2mg/L Kinetin (Bekheet, 2013) and (v) 0.5 mg/L BA + 0.5 mg/L Kinetin + 1 mg/L NAA (Khan and Bi, 2012). However, auxin and cytokinin ratios maintained less than one for the cultures initiated from young inflorescences (Loutfi and Chlyah, 1998). In this case, 2.5 µM of NAA+ 4.4 – 8.8 µM of BA + 5 µM of 2iP were used in the media and cultures were incubated in the light intensity of 180 µmol/m^2/s with 16 h photoperiod. More shoots (12.6 buds) from single date palm bud were obtained where the cultures induced from shoot tip and maintained in auxin and cytokinin ratio of more than one in liquid media with shaking (40 rpm) for 8 weeks compared to solid media (Khirellah and Badar, 2007). The multiplied date palm shoots at this stage resembled rosette.

***Shoot elongation and rooting*:** This stage also depends on hormones used in the media and other cultural conditions. This process involves the transfer of multiplied shoot buds into a medium with high auxin: cytokinin ratio (Beauchesne *et al.,* 1986; Loutfi and Chlyah, 1998). Combination of 5.4 µM of NAA + 2.2 µM of BA + 2.3 µM of kinetin or 10.8

µM of NAA + 4.4 µM of BA (or 5.0 µM of 2iP) was found optimum for elongation. GA_3 of 3-9 µM also used for 15 – 20 days during first and second subculture in order to improve shoot elongation (Loutfi, 1989). However other studies revealed that GA_3 (0.1-0.5 mg/L) alone or in combination with NAA (0.1 mg/L) were used to elongate the shoot (Khirellah and Badar, 2007; Abul-Soad *et al.*, 2006). Generally, shoot elongation takes place within 2 months. No special medium is required for rooting as date palm forms root easily during elongation time. If not, it has been suggested that the roots can be induced by adding 0.1 - 1.5 mg/L of NAA into the elongation medium (Abul-Soad *et al.*, 2006; Shakya and Saker, 1998; Khan and Bi, 2012; Bekheet, 2013).

***Acclimatization*:** Acclimatization of date palm plantlets derived from tissue culture is the critical steps for their survival. The conditions of plants in MS medium are in low light intensity, high humidity, aseptic and mostly heterotropic in nature. But in the field, the seedlings are exposed to high light intensity, low humidity, autotrophic in nature and in addition to that the seedling has to survive in the microbial and other organisms in a particular ecosystem. So, the gradual impose of these factors on the date palm seedlings will improve their survival rate in the field. It has also suggested that the plantlets with root and shoot transferred to high sucrose (100 g/l) medium (Beauchesne *et al.,* 1986) before taken to the soil get more vigor and seemed to adopt well in green house during hardening stage. The gradual lifting of plastic cover in the culture room and greenhouse assists in the formation of cuticle layer and regulation of stomatal activities. Among the methods used for acclimatization of date palm seedlings the mixture containing 2 part peat moss: 1 part perlite or equal volume of peat moss and vermiculite under high humidity were found best and 80 per cent of plants were survived in the field after three months (Khirellah and Badar, 2007; Bekheet,, 2013).

Embryogenesis

It is the process or phenomenon of induction of embryogenic culture and the development of somatic embryo from these date palm cultures, and utilized various explants (Reynolds and Murashige, 1979). The breakthrough came in 1979 when Tisserat (1979) used growth media supplemented with high concentration of auxins (2, 4-D) and activated charcoal and produce date palm plantlets successfully. Since then, this technique has been used effectively by various commercial laboratories producing date palm micropropagation by somatic embryogenesis mainly located in Israel, UAE and other Arab countries, USA, UK, French, Egypt and Morocco (Loutfi and El Hodami, 2005; Jain, 2007). There are several research groups have demonstrated the micropropagation of date palm plant through somatic embryogenesis (Taha *et al.,* 2001; Sane *et al.,* 2006; Esraghi *et al.,* 2005; Eke *et al.,* 2005; Fki *et al.,* 2003; Bhargava *et al.,* 2003; Alkhateep, 2008; Alkhayri, 2003; Kackar *et al.,* 1989; Yadav *et al.,* 2001; Bekheet *et al.,* 2008; Asemota *et al.,* 2007, El-Bellaj, 2000; Loutfi, 1999; Sharon and Chandramati, 1998; Sharma *et al.,* 1986, Mater, 1986; Zaid and Tisserat, 1983; Othamani *et al.*, 2009a; Sidky and Eldawyati, 2012). Somatic embryogenesis method is ideal one for clonal propagation of woody and fruit plant (Jain and Gupta, 2005) and it has huge potential for large scale propagation of date palm because of its automation, long term storage and amenable for genetic manipulation (Jain, 2007). Somatic embryogenesis involves several steps *viz*., induction of callus from explants, maintenance and pro-embryogenic calli, development and maturation of somatic embryo, germination of somatic embryo and acclimatization. The summary of some important examples are given in Table 1.

***Induction of callus from explants*:** This phase involves the conversion of differentiated tissue into dedifferentiated tissue, which is group of cells called callus. Several factors such as type and age of explants, genotypes, plant growth medium, additives, growth regulators and other cultural conditions including agents like surface sterilizing agents, fungicides and bactericides etc. which are very critical for successful induction of date palm embryogenic culture. Importantly callogenesis phase is heavily depends on genotypes used (Sane *et al.*, 2012).

Explants: Various explants have been used to initiate callus from date palm. The explants used were shoot tip of young seedling and offshoots of 1-3 year old (Taha *et al.,* 2001; Mater, 1990; Sane *et al.,* 2006; Eshraghi *et al.,* 2005; Eke *et al.,* 2005; Bhargava *et al.,* 2003; Tisserat, 1979; Alkhateep, 2008; Kackar *et al.,* 1989; Yadav *et al.,* 2001; Bekheet *et al.,* 2007; 2008; El-Bellaj, 2000; Loutfi, 1999; Sharma *et al.,* 1986; Zaid and Tisserat, 1983; Veramandi and Navarro, 1996; Letouze *et al.,*1998; Khan *et al.,* 1983; Al-Khayri, 2011), inflorescence (Fki *et al.,* 2003; Tisserat, 1979; Tissert and DeMason, 1980; Drira, 1981; Sidky and Eldawyati, 2012; Abdul-Soad and Mahdi, 2010), mature and immature zygotic embryos (Bekheet *et al.,* 2008; Mater, 1986), leaf segments excised from seedling and offshoots (Fki *et al.,* 2003; Poulain *et al.,* 1979; Yadav *et al.,* 2001; Khan *et al.,* 1983; Othmani *et al.*, 2009a; Kurup *et al.*, 2014), leaf and meristamatic tissues excised from *in-vitro* grown date palm plants (Jain, 2007), auxiliary bud (Khan *et al.,* 1983; Quarishi *et al.,* 1997), stem, young merisematic roots (Sharma *et al.,* 1988; Zaid and Tisserat, 1983; Khan *et al.,* 1983; Yadav *et al.,* 2001). The most frequently used explants are apical shoot tip and lateral buds as they are more response in callus induction and other downstream process (Jain, 2007).

Culture medium: It is essential to supply adequate nutrients to the date palm tissue during *in-vitro* conditions. The most common media used for micropropagation of date palm through somatic embryogenesis is MS medium. Rarely, B_5 medium (Bhargava *et al.,* 2003; Abdul-Soad and Mahdi, 2010), Eauwan's medium (Asemota *et al.,* 2007), RM 1965 medium (Bekheet *et al.,* 2007), W and WPM medium for date palm cv. Khusab, SH and NN medium for cv. Berny and SH, W and MS medium for cv. Barhee (Al-khayri, 2011) are used in date palm tissue culture. Several research groups modified its composition upto some extent by adding additives for various purposes like vitamins, adenine sulphate, thiamine, glycine, glutamine, myo-inosital, silver nitrate (Al-khayri and Albrany, 2007) and other substances like asparagine, di-hydrogeno ammonium nitrate and casein hydrolysate (Abdul, Rahim *et al.,* 2001; Letouze, 1998), date syrup (Alkhateep, 2008) and biotin (Sane *et al.*, 2006) are used for callus growth and development. Activated charcoal and poly venyl pyrolidine are being used to reduce tissue browning due to phenolics. Citric acid and ascorbic acid are used as antioxidant. Sucrose 20-30 g/l used as carbon and energy source in tissue culture media in addition to that date palm syrup of 5-6 per cent also replace or along with sucrose found enhance the callus growth and multiplication. In most cases, agar (0.7-0.8 %) used as gelling agent of the medium for date palm tissue culture, phytogel is also used, but in limited level. KNO_3 is essential source of nitrogen form for callogenesis for optimum callus formation (50 mM including ammoniacal source) (Asemota, 2007).

Hormones: Generally high concentration of auxin used in most of the cases to induce callus, where auxin and cytokinin ratio also maintains high. Among plant growth regulators, 2, 4-D is commonly used either alone or in combination with cytokinin in the date palm tissue culture medium. Other auxins like picloram, NAA and NOA also used. Earlier researchers used relatively high concentration of 2, 4-D (450.5-901.0 µM) in the medium with activated charcoal for callus induction (Loutfi and El-Hadrami, 2005). Recently, several studies also have showed that less concentration of 2, 4-D is also enough to induce callus and further enhanced by addition of BA or adenine sulfate (Sane *et al.*, 2012). Based on the reports, 2, 4-D concentration up to 100 mg /l with 3-5 mg/L of BA/2iP alone or with 0.5 mg/L kinetin was found effective (or) 0.1mg/L of IAA/10 mg/L of NAA/ 4.0 mg/L of IBA with 1-3 mg/L of either BA/ 2 iP/ Kinetin or 53.7 µM NAA + 7.4 µM 2ip was also found to induce date palm callus (Al-Khayri and Al-Bahrany, 2003; Hussain *et al.,* 2001; Khan *et al.,* 1983; Asemota *et al.,* 2007; Al-Khayri, 2003; Al-khayri, 2011; Kurup *et al.,* 2014) or equal concentration of IAA and kinetin with 2,4-D also produce callus (Mater 1986; Abdul-Soad and Mahdi, 2010). Recent reports suggest that the usage of lower concentration of 2,4-D (10 mg/L) would be beneficial for callus in induction, and other down stream process (Othmani *et al.,* 2009 a,b&c).

***Surface sterilization*:** To avoid microbial contamination, outer surface and other endophytic organism frequently contaminate the tissue and not amenable for *in-vitro* culturing and other process. The surface sterilizing agents like $HgCl_2$ 0.01-0.1 per cent for 5 minutes to 1.0 hrs (Kackar *et al.,* 1989; Yadav *et al.*, 2001; Fki *et al.,* 2003; Al-Khayri and Al-Baharany, 2003; Bhargava *et al.,* 2003; Othmani *et al.*, 2009a) or 2-5 per cent NaOCl for 10-20 minutes (Eshraghi, *et al.,* 2005; Alkhateeb, 2008; Asemota *et al.,* 2007; Bekheet *et al.,* 2007) were used in most cases. Some researchers used 70 per cent ethanol for surface sterilization for 1-2 minutes with $HgCl_2$ and with NaOCl treatments (Bekheet *et al.,* 2007). After surface sterilization tissue were washed three times with sterilized distilled water.

Cultivars: Several cultivars of date palm used for somatic embryogenesis are; Zaghlool (Taha *et al.,* 2001), Deglet Nour (Fki *et al.,* 2003) Sukari, Barhee, Kalas (Rao *et al.,* 2001), Amsekshi (Sane *et al.,* 2006), Khuneizi (Al-Khateep, 2008; Kurup *et al.,* 2014), Mardarsing (Eshraghi *et al.,* 2005), land race like Zebia and loko (Eke *et al.,* 2005), Suckary (Al Khateep, 2008), Khadrawy (Yadav *et al.,* 2001; Kacker *et al.,* 1989), Halawy, Medjool (Yadav *et al.,* 2001), Sayar (Bhargava *et al.,* 2003), Barhy (Al-Khalifah *et al.,* 2006; Smith and Aynsley, 1995) , Deglet Bey (Othmani *et al.,* 2009a), Khusab, Berny and Barhee (Al-Khayri, 2011) Gulistan (Abdul-Soad and Mahdi, 2010). More than 50 cultivars imported from various countries since 1985 to standardize somatic embryogenesis protocol for multiplication (Letouze *et al.,* 1998). Those are Barhi, Khalas, Zahidy, Medjool (from USA), Barhi, Khalas, Nabtha Saif, Kowaiz, Owaid, Halawy, Umm Dahan, Saggai, AbuMan, Rothna (from UAE), Medjool (from Morocco).

After surface sterilization, explants of date palm were cut into appropriate size generally 0.4 to 10 mm as callus induction requires cut ends of tissue and placed on appropriate culture medium. These cultures are incubated in dark for 4-6 months at 25 -30^{o}C for callus induction. Callus induction period varied from minimum of three months (Rao *et al.,* 2001) to eight months (Veramandi and Navaro, 1996) depending upon many factors and cultural conditions. Most of the cultures induced callus in dark but dim light also favours callus induction (Reuveni and Kipnis, 1974). During this period the cultures were subcultured once in a month. However, the duration of subculture varies according to the conditions and other factors including genotype, medium composition, growth regulators, etc. Minimum period of subculturing was reported from one week (Rao *et al.,* 2001) and maximum of two months (Fki *et al.,* 2003). Embryogenic potential of developed calli again depends on

the genotypes and explants derived from offshoot or inflorescence (El hadrami *et al.,* 1995; Loutfi, 1999), etc. The exact place of callus induction in the explant has been reported and probably from the vascular tissues particularly vessels (Sane *et al.,* 2006). These induced calli are so-called primary calli. Chooping of these primary calli grow fast and produce friable globular calli which more amenable for embryo induction. But what is/are factor(s) involving in the reactivation of somatic cells at the beginning in inducing callus is not known.

***Maintenance*:** It involves induced calli from date palm explants need to be further multiplied to increase the size and developing calli should be pro-embryogenic in nature. During maintenance, the induced calli can be multiplied in the same medium and compositions used for induction. Several factors contributing to multiplication phase have been identified by many research groups. They are: (1) proliferation of calli and further formation of embryo depends on hormone concentrations. At this phase the calli should be maintained in low auxin and cytokinin like (i) 0.45 µM of 2, 4-D and 2.2 µM of BA (El Hadrami *et al.,* 1995); (ii) 2.5 mg/L of 2,4-D with 0.25 mg/L kinetin and 0.1mg/L NAA (Bhargava *et al.,* 2003); (iii) only auxin -1 mg/L of 2,4-D (Fki *et al.,* 2003); (iv) 2.7 µM of NAA and 0.44 µM of BA (Loutfi, 1999); (v) 1.5 mg/L 2ip + 10 mg/L NAA (Al-Khayri 2011) and (vi) hormone free medium (Letouze *et al.,* 1998; Yadav *et al.,* 2001, Tisserat, 1979; Sharma *et al.,* 1986; Bhaskaran and Smith, 1992; Kurup *et al.*, 2014), (2) subculturing once in a week (Rao *et al.,* 2001) to two months (Fki *et al.,* 2003) interval, most cases subculturing was done once in a month, (3) chopping of primary calli into small pieces during first or second subculturing also increased their fresh weight and growth level (Sane *et al.,* 2006; Fki *et al.,* 2003), (4) culturing of calli in liquid medium with either low auxin (0.5 mg of 2, 4-D) (Fki *et al.,* 2003) or hormone free (Letouze and Daguin, 1989; Bhaskaran and Smith, 1992). After a short period in liquid medium (up to 1 month) it can be transferred to solid medium with low auxin could increase the calli fresh weight up to 4 fold when compared to continuous subculturing in solid medium. These cell suspensions are initiated by inoculating 5g of fresh weight of friable calli in to 50-100 ml of liquid medium in 250 ml flasks with shaking (100 rpm) at 27±1°C with 15 h photoperiod. The culture can be subcultured once in a month and each subculturing the culture was filtered through 500 µm mesh fabric. Culture retaining this filter will be taken to next stage and the suspension culture passing this filter will be in same liquid medium for further multiplication (Letouze and Daguin, 1989). Date syrup (up to 6 %) (AlKhateep, 2008), 37.5 to 50 µM $AgNO_3$ (Al-Khayri and Al-Bahrany, 2004), KNO_3 as nitrogen source in combination with ammoniacal form of nitrogen source (Asemota *et al.,* 2007) and di-hydrogeno ammonium citrate (Letouze *et al.,* 1998) also increase the date palm callus fresh weight and development.

***Somatic embryo development and maturation*:** Development of somatic embryo from pro-embryogenic calli is the critical steps in somatic embryogenesis. The large scale multiplication of somatic embryo decides its applicability in commercialization/ industrialization of tissue culture raised date palm plants. Many factors and their interactions involving in the development and maturation process of somatic embryo. This includes composition of medium, growth hormones, cultural conditions and genotype/ cultivars. Most of the research groups used MS medium as base nutrient with additives. In most cases, somatic embryo formation is induced by transforming the embryogenic calli in to either hormone free medium (Al-Khayri, 2003; Rao *et al.,* 2001; Tisserat, 1979; Eke *et al.,* 2005; Letouze *et al.,* 1998; Yadav *et al.,* 2001; Kackar *et al.,* 1989; Sane *et al.,* 2006; Othmani *et al.*, 2009a&c) or low concentration of auxin, 0.1 mg/L of 2,4-D (Othmani *et al.*, 2009b), 1.0-2.5 mg/L of 2, 4-D containing media (Fki *et al.,* 2003; Bhargava *et al.,* 2003) or media containing 0.05 mg/L of NAA and 1 mg/L of 2iP (Al-Baiz *et al.,* 2000) or 0.5 mg/L NAA + 0.25 mg/L BA (Kurup *et al.*, 2014) and kept either in dark (Tisserat, 1979; Al-Khayri, 2003; Rao *et al.,* 2001; Al Khateep, 2008; Yadav *et al.,* 2001) or in low light intensity (28-50 µmol/m^2/s or up to 300 lux) for 16 h photoperiod (Eke *et al.,* 2005; Letouze *et al.,* 1998; Fki *et al.,* 2003; Kackar *et al.,* 1989; Bhargava *et al.,* 2003). Somatic embryo also induced by culturing callus in liquid medium followed by transform them in to solid media (Fki *et al.,* 2003). During this stage, individual embryogenic cells rapidly divide and develop in to globular pro-embryo. These globular pro-embryos forms break zones with thick pecto cellulosic walls which delimited the pluricellular pro-embryos (Sane *et al.,* 2006). The anatomy of somatic and zygotic embryos is similar but the difference is the low accumulation of intracellular proteins in somatic embryo, the reason is not known. Maturation of somatic embryos is also governed by many factors and it seems that they need some stress for enough maturation. Increasing sucrose concentration (60g/l) in the medium produces large number of somatic embryos and they are large in size and stout. The similar effects also induced by using date syrup of 2.6 % (Al Khateep, 2008), intermittent culturing of somatic embryo in liquid media devoid of hormones with ½-strength MS medium for one month period than culturing continuously on solid medium also improves their maturation and development. It has also suggested that at this stage somatic embryo may be required less concentration of IAA (0.1 mg/L) or BA (0.1 mg/L) (Bhargava *et al.,* 2003) or silver nitrate (up to 37.5 µM) (Al Khayri and Al-Bahrany, 2004) or partial desiccation (Fki *et al.,* 2003) or addition of 0.1 µM of abscisic acid (ABA) (El-Bellaj, 2000) under low light

intensity for their maturation and development. During this phase, suspension culture of embryogenic calli facilitates large scale production of date palm somatic embryos (Fki *et al.,* 2003; Letouze *et al.,* 1998). Even somatic embryo rquire 0.5- 2.0 mg/L of 2,4-D for their further proliferation (Othmani *et al.*, 2009a).

Suspension culture method: To develop large scale, high throughput and automation production of somatic embryo is possible using suspension culture of embryogenic calli in 'bio reactor system' (Jain, 2007). The somatic embryo production through liquid culturing increased up to 20 fold (10-200 somatic embryo/ month/ 100 g of calli) (Fki *et al.,* 2003), repeated culturing of suspension culture up to three years produced 200±20 embryo/ gram of calli (Yadav *et al.,* 2001). Using temporary immersion system (TIS) shoots emerged from intact and fragmented juvenile leaf explants of date palm in RITA for 15 min per day enhanced multiplication of 8.4 shoots per shoot (Fki *et al.*, 2011). This is double the multiplication compared to solid media. Inoculation of callus of date palm cv. Barhee in MS medium with 10mg/L 2,4-D and 1.5 mg/L 2ip and subculturing once in 4 weeks enhanced somatic embryos to the tune of 3.5 fold (Al-Khayri, 2012). Liquid MS medium supplemented with 0.1 mg/L NAA and 1.5 mg/L activated charcoal enhanced 6-16 fold somatic embryos production within 16 weeks (Ibraheem *et al.*, 2013). MS media with 0.1 mg/L NAA + 0.05 mg/L BA was found optimum for suspension culture for date palm cv. Halawy and Khalas (Anonymous, 2014).

***Somatic embryo germination*:** Matured date palm somatic embryos can germinate on the medium used for its development and maturation. The germination also occurred in hormone free medium (Rao *et al.,* 2001) with little success. However, it has been well documented that addition of NAA @ 0.1-2.0 mg/L (0.54 µM) to the growth medium increased the germination and other development of plantlets (Kacker *et al.,* 1989; Yadav *et al.,* 2001; Fki *et al.,* 2003; Eke *et al.,* 2005; Sane *et al.,* 2006; Khan *et al.*, 1983; Mater, 1986). Along with NAA, addition of cytokinin like BA @ 0.1 to 2.0 mg/L also increases the germination of date palm somatic embryo (Khan *et al.,* 1983; Kackar *et al.,* 1989). Addition of only BA @0.1mg/L (Bhargava *et al.,* 2003) or IBA @ 0.2-0.4 mg/L (Al- Khayri, 2003) or 0.1 mg/L 2,4-D (Othmani *et al.*, 2009a) or 0.1 mg/L NAA + 0.1 mg/L kinetin (Abdul-Soad and Mahdi, 2010) also induced the germination. Germination of somatic embryo was also obtained in MS medium supplemented with 1 mg/L NAA + 0.1 mg/L 2,4-D (Othmani *et al.*, 2009b) and 0.1 mg/L NAA alone (Al-Khayri, 2011) or with 0.25 mg/L BA (Kurup *et al.*, 2014). Reducing moisture content of the somatic embryos from 90 to 75 per cent by desiccation and cutting back of tip of the somatic embryo increases the rate germination from 25 to 80 per cent (Fki *et al.,* 2003). During culturing, the cotyledon sheath elongate to several centimeter and split open, by which the plumule pierced out from and radicle also come out (Tisserat *et al.,* 1979).

***Acclimatization*:** Date palm seedlings have 10-15 cm long with two to three leaves with distinct tap root with two to three tap root stage is suitable to transfer in to soil. Before transfer in to greenhouse, the plantlets kept in pre-acclimatization chamber with 3.87 cm^2 air chamber area increases the survival rate to 92 per cent in the field (Bhargava *et al.,*, 2003). Some of the critical factors i.e., environmental conditions, composition of soil, and pesticide treatment against insect pest and disease causing organisms (Loutfi and El Hardrami, 2003) are important for survival of date palm plantlets. The acclimatization procedures are similar to that of plantlets derived through organogenesis. Other methods also suggested for initial planting is mixing of peat and gravette or peat mass and vermiculite in equal proportions. First 2 to 3 weeks the seedlings must be kept in high levels of relative humidity and gradually brought to glasshouse conditions. During this period, 25-30^oC temperature can be maintained. The hardening takes for several months in the field. The two months old date palm plantlets regenerated from somatic embryos were transferred to pot mixer of peat moss and sand (2:1 ratio) or soil: peat moss: vermiculite (1:1:1) of equal ratio (Al-Khayri 2011) or peat: sand: dehydrated cow manure (1:1:1 ratio) (Kurup *et al.*, 2014)and cultivated in green house under natural sunlight with 25±2^oC and 80-90% RH. Under this process, 60.2% of plantlets were survived in the field (Othmani *et al.,* 2009a&b; Kurup *et al.*, 2014).

Problems and possible solutions in micropropagation: There are many problems during micropropagation of date palm through tissue culture (Table 1). The important problems are described under.

During in-vitro culturing

Tissue browning: This is one of the major problems in date palm tissue culture not only in date palm and in all the species belongs to the family *Arecaceae*. It includes browning of date palm explants and the adjacent media, which is assumed to be due to the oxidation of polyphenols like caffeoyl shikimic acids (190 to 430 µg/g of fresh weight) in to quinones. Quinones are more toxic to the tissue than phenol (Maier and Metzlium, 1965). The high content of methoxy-substituted phenols i.e., sinapic and ferulic derivatives have been associated with cell wall rigidity and loss of cell division together tissue browning (El-hardrami *et al.,* 1995). To minimize tissue browning, several additives and practices are being followed. These include incubation of culture in dark, frequent subculturing, pre-soaking of explants in antioxidant like solution containing citric acid (150 mg/L) and ascorbic acid (100 mg/L) solution before

surface sterilization (Zaid and Tisserat, 1983; Othmani *et al.,* 2009a), adding these antioxidants and addition of combination of absorbents like citrate, adenine, and glutamine (Rhiss *et al.,* 1979), or addition of activated charcoal or poly venyl pyrolidine (PVP) to the culture medium. Among all these additives, use of activated charcoal is more preferable to reduce tissue browning as others are toxic to plant tissue at higher concentrations. The charcoal has been used by most of the researchers to control tissue browning. It was also suggested that the medium should be added with high concentration of growth regulators when activated charcoal added to the medium (Fridborg and Erikson, 1975).

Microbial contamination: In addition to the normal tissue culture contaminants like bacteria and fungi, the endophytic microorganisms living inside date palm tissue including the meristem are also growing along with explants and contaminate the cultures. Such endophytes, *Bacillus circulans* was identified in date palm tissue culture initiated from offshoot tissues (Bouguedoura, 1993). Antibiotics such as gentamycin are often used to control such bacteria. However, the success was not appreciable (Chekaoui, 1997). In some cases, tissues are also not able to differentiate into callus when the antibiotics are added to the media. Most of the fungal contaminants identified in date palm tissue culture are *Aspergillus*, *Alternaria* and *Penicillium* and bacteria are *Bacillus, Staphylococcus* and *Proteus* (Abass, 2013 a & b).

During ex-vitro culturing

The plants derived through tissue culture are exhibit genetic variation or somaclonal variations and showing abnormalities in the field.

Somaclonal/ genetic variations: This is one of the major limitations in micropropagation of plants through somatic embryogenesis. Somaclonal variation has the characters of induced mutagenesis that may be strongly influenced by oxidative stress at excision of the tissue and also geneotype dependant. It is influenced by technique used for propagation, nature of mother plant (chimera), types of growth regulators used, type of explant used (ploidy gradients: apex to root), age of cultures (more than one year), medium composition and incubation conditions (Zaid and Arias-Jimener, 2002). The *in-vitro* production of date palm plants by somatic embryogenesis requires the application of high concentrations of auxins like 2, 4-D or NAA for callus induction, which are known to be associated with genetic instability in date palm and other plants. DNA methylation or activation of retro-transposable elements expression or both during stress may be associated with this genetic change (Jain, 2001). To reduce somaclonal variation in tissue culture raised date palm plants, the ratio between explants and the number of plants raised should be maximum of 1 : 2000 (Sivalingam *et al.*, 2014).

Abnormalities in the field: Tissue culture raised date palm plants showing various kinds of abnormalities like hapaxanthic axillary shoot (shoot which produce terminal inflorescence after 1-2 year growth and then die) (Sudhersan *et al.,* 2001; Sudhersan and El-Nil, 2004), twisted inflorescence, broader leaves, excessive vegetative growth, leaf variegation, dwarfing, leaf whitening, bastard offshoots, delayed flowering time, pollination failure, abnormal fruiting and seedlessness (Zaid and Al-Kaabi, 2003; Cohen *et al.,* 2004).

***Possible Solutions*:** The variation and abnormalities found more in date palm plants produced via somatic embryogenesis than organogenesis. These somaclonal variations and abnormalities can be minimized by scanning the nature of the genome by molecular tools and enzyme analysis techniques before planting to the field. The enzyme analysis techniques like isozyme analysis of peroxidase, shikimat dehydrogenase, acid phosphatase, esterase, polyphenol oxidase (Zirdar *et al.*, 2008), and DNA fingerprinting techniques through markers like Random Amplified Polymorphic DNA (RAPD) (Letouze *et al.,* 1998; Saker *et al.,* 2000; Saker *et al.,* 2006; Javoukey *et al.,* 2000; Ali *et al.,* 2007) and Amplified Fragment Length Polymorphism (AFLP) are techniques to identify the variations of tissue culture raised date palm plants by comparing with the mother date palm plant from where the explants were taken. Fki *et al.,* (2003) analyzed the ploidy level of date palm seedlings produced by somatic embryogenesis method using floricytometry technique and found no change in ploidy level.

Micropropagation of date palm in India

In India, production of date palm plants by tissue culture started from 1984 by somatic embryogenesis at various research Institutions and Universities (Sharma *et al.*, 1984; 1986; 1988; Yadav *et al.,*2001; Bhargava *et al.,* 2003; Kackar *et al.,* 1989). These plants were planted in the field of respective Institutions such as CCS Haryana Agricultural University, Hisar (cv.Khadrawy, Halawy, and Medjool), SK Rajasthan Agricultural University, Bikaner (cv. Sayar and Jaglool) and ICAR-Central Arid Zone Research Institute (ICAR-CAZRI), Jodhpur (cv. Khadrawy). None of these developed protocols were commercialized. Recently Anand Agricultural University (AAU), Anand successfully developed date palm propagation protocol through tissue culture for local elite cultivar. These plants are being evaluated under field conditions at different centres such as ICAR-Central Institute for Arid Horticulture (ICAR-CIAH), Bikaner, Date Palm Research Station, Mundra and ICAR-CAZRI, Jodhpur under ICAR sponsored net work project 'Production and demonstration of tissue culture raised plants

under three locations and collection and maintenance of elite germplasm of date palm'. Under these project AAU, Anand also developing tissue culture protocol for cv. Barhee and ICAR-CAZRI for cv. Muscut-2. In addition many private companies such as ACE Biotech, Hyderabad; Atul Ltd, Jodhpur; Kachchh Crop Services Ltd, Gajod; Sarjan Biotech, Bhuj; ECO Cell, Bhuj and Saliah Dates Nursery/Dates India, Krishnapuram, are being actively involved in the production of date palm plants of different cultivars through tissue culture in India (Shah, 2014; Sivalingam *et al.*, 2014). ICAR-CIAH is also engaged in developing tissue culture protocol for date palm cv. Halawy and cv. Khalas. Suspension culture method was standardized to enhance somatic embryo formation for these date palm cultivars (Anonymous 2014).

Future perspectives

Commercial date palm micropropagation has been well established and private companies are located in Israel, UAE, USA, UK, France and Morocco. In addition to meet their local demand, it has been started exporting tissue culture grown date palm plants to other countries. The tissue culture grown plants are mostly by somatic embryogenesis, these date palm trees are in the field and yielding fruits. Some of the places have shown some variation and abnormalities in the field. This is the major challenge for companies producing date palm seedlings through tissue culture. Some method to identify the variations at the early stage has also in process such as DNA finger printing techniques and an alternate route like production through organogenesis method to avoid variation which is already started by private companies in Morocco, UAE and Saudi Arabia. Here, the challenge is low multiplication rate. These methods of micropropagation will be highly useful in production of disease-free date palm seedlings as bayoud disease causing organism spreads through vascular tissues and causing huge economic losses to the growers. Date palm requires prolonged hot dry summer, moderate winter, rain free period at the time of fruit ripening and requires more water. In India, the north-western region includes the Thar Desert of Rajasthan, part of Gujarat and Haryana is very suitable for date palm cultivation (More *et al.*, 2011). The major bottle necks in this region are, i) non-availability of suitable cultivars for various uses, ii) non-availability of large number of planting materials, and iii) irrigation facility. Irrigation facilities in desert areas are made available through canal (IGNP) water. The main concerns which needs to be addressed are, (i) normalization of date palm tissue culture procedures for cultivars independent or develop suitable procedure for most of the necessary cultivars (ii) identification of somaclonal variation techniques precisely at early stage (before going to field) for date palm plants produced through somatic embryogenesis method (iii) reduction of time requirement of shoot bud initiation and increasing multiplication rate in organogenesis method and callus induction time in embryogenesis method (iv) methods to remove endophytic organisms which pose contamination during culturing *in vitro*
.

Table 1: Problems associated with the techniques used for commercial micropropagation of date palm

Types	Somatic embryogenesis	Organogenesis
Initiation	-Destruction of offshoots with the use of shoot tip meristem. -Browning of explants leading to death of tissues (± 30%). -Length of time taken to produce nodular callus and somatic embryo approx. 6-12 months. -Use of 2, 4-D in high concentrations increasing risk of somaclonal variation - Contamination losses - Varietal response differences	-Destruction of offshoots with the use of shoot tip meristem. -Browning of explants leading to death of tissues. -Length of time for shoot proliferation is long (up to one year). -No somaclonal variation - Contamination losses
Multiplication	- Callus has limited life span and more sensitive to environmental changes - Risk of variation - Good multiplication rates (± 7) - Vitrification of callus -Unsynchronized embryo development (small and large) - Loss of totipotency in some varieties	- Yield of the technique per offshoot is low -Selection of plantlets is critical - Multiplication rates are low - Light sensitive - Loss of totiponcy in some varieties - Precocious rooting sometimes occurs-decreasing regeneration capacity
Elongation	-Not necessary -Low efficient rooting (two stage rooting)	-Necessary step -Not all plants are sufficiently rooted

Rooting Acclimatization	-Hardening-off is generally 80 % successful (location dependent)	 -Hardening-off is generally high (90-98%) location dependent

(adapted from McCubbin and Zaid, 2007 and modified)

Details of explant, media and hormones used in date palm tissue culture with their response

S. No.	Explant	Media and Hormones	Response	Reference
I. ORGANOGENESIS				
1.	Off Shoot	Bud initiation stage (MS media) (i) 0.01-1 mg/L NAA + 1 mg/L NOA + 1-4 mg/L 2ip + 1-5 mg/L BA (ii) 4 mg/L IBA or IAA + 0.5 mg/L NAA + 1-5 mg/L BA or 2ip (iii) 5.4 µM NAA + 4.9 µM IBA + 5-27 µM NOA + 0.5 µM2ip (iv) 5 mg/L 2ip (v) 1 mg/L NAA + 3 mg/L BA + 3 mg/L 2ip (vi) 3 mg/L NAA + 0.1 mg/L NAA Multiplication stage (i) 1.0 mg/L NAA + 1.0 mg/L NOA + 4 mg/L 2ip + 2 mg/L BA (ii) 0.5 mg/L TDZ + 1.0 mg/L 2ip (iii) 5 mg/L 2ip+ 2 mg/L kinetin+5mg/L silver nitrate (iv) 0.5 mg/L BA + 0.5 mg/L Kinetin + 1 mg/L NAA Shoot elongation 0.1-0.5 mg/L GA_3 alone / along 0.1-1.0 mg/L NAA Rooting 0.1-1.5 mg/L NAA	6.2 -8.1buds/ explant 6.8-12.6 buds/ explant	Asemota and Eke, 2006; Khierallah and Bader, 2007 Costa and Alofa, 2006; Balal and El deep, 1997 Poulain *et al.,* 1979; Bekheet, 2013; El Bar and El Dawayati, 2014; Khan and Bi, 2012; Chander Bhan *et al.*, 2013) Asemota and Eke, 2006; Khierallah and Bader, 2007 Hussain *et al.,* 200; Bekheet, 2013; Khan and Bi, 2012 Khierallah and Bader, 2007; Abul-Soad, 2006; Chander Bhan *et al.*, 2013 Abul-Soad, 2006; Shawky and Saker, 1998; Bekheet, 2013; Khan and Bi, 2012
II. SOMATIC EMBRYOGENESIS Callus Induction				
1.	Seedling shoot tip	MS media with 2 mg/L 2,4-D	-	Shane *et al.,* 2006
2.	Shoot tip	MS media with 50 mg/L 2,4-D + 3 mg/L 2ip	5 months	Esraghi *et al.,* 2005
3.	Shoot tip	MS media with 0.1 mg/L 2,4-D + 3 mg/L 2ip	-	Mc Cubbin and Zaid, 2007
4.	Shoot tip	MS media with 100 mg/L 2,4-D + 3 mg/L 2ip	-	Eke *et al.,* 2005
5.	Shoot tip	MS media with 2.5 - 50 mg/L 2,4-D + 0.1 mg/L IAA	2-3 months	Bhargava *et al.,* 2003
6.	i) Immature leaf ii) Inflorescence	i) MS media with 10 mg/L 2,4-D ii) MS media with 0.5 mg/L 2,4-D	-	Fki *et al.,* 2003
7.	Shoot tip / bud	0.1-100 mg/L NAA or 2,4-D + 3 mg/L 2ip	3-4 months	Tisserat, 1979
8.	Shoot tip	MS media with 100 mg/L 2,4-D + 3 mg/L 2ip	-	Al Khateep, 2008
9.	Shoot tip	MS media with 10 mg/L NAA + 1.5 mg/L 2ip	4 months	Al Khayri and Al-Bahrany, 2003
10.	Shoot tip	MS media with 100 mg/L 2,4-D + 5 mg/L 2ip	Initiation 3-4 weeks	Yadav *et al.,* 2001

11.	Leaf	MS media with 10 mg/L 2,4-D	6-8 months	Othmani *et al.,* 2009
12.	Shoot tip	B5 media with 2.5-15 mg/L 2,4-D + 0.1 mg/L IAA	2-3 months	Bhargava *et al.,* 2003
13.	Leaf	Eauwan's media with 15 mg/L NAA	NR	Asemota *et al.,* 2007
14.	Tender leaf	MS media with 100 mg/L 2,4-D	2-3 months	Kurup *et al.*, 2014
15.	Inflorescence	MS media with 0.1 mg/L 2,4-D+0.1 mg/L IAA+5.0 mg/L NAA	2-3 months	Abdul-Soad & Mahdi, 2010
Somatic Embryo (SE) Formation				
1.		MS media without hormone	SE formed	Tisserat, 1979; Yadav *et al.,* 2001; Al Khayri, 2003; Fki *et al.,* 2003 ; Othmani *et al.,* 2009a&c
2.		MS media with 0.1 mg/L 2,4-D	SE formed	Othmani *et al.,* 2009b
3.		MS media wth 1.0-2.5 mg/L 2,4-D	SE formed	Bhargava *et al.,* 2003; Fki *et al.,* 2003
4.		MS media with 0.5mg/L NAA + 0.25 mg/L BA	SE formed	Kurup *et al.*, 2014
5.		MS media with 5 mg/L 2,4-D + 1.0 mg/L 2iP	SE formed	Abdul-Soad & Mahdi, 2010
Somatic Embryo Germination				
1.		MS media with 0.1-2.0mg/L NAA	SE germinated	Khan *et al.,* 2003; Yadav *et al.,* 2001; Fki et al ., 2003; Eke *et al.,* 2005; Sane *et al.,* 2006.
2.		MS media with 0.1 mg/L BA	SE germinated	Bhargava *et al.,* 2003
3.		MS media with 0.2 – 0.4 mg/L IBA	SE germinated	Al Khayri, 2003
4.		MS media with 0.1 mg/L 2,4-D	SE germinated	Othmani *et al.,* 2009a
5.		MS media with 1 mg/L NAA + 0.1 mg/L 2,4-D	SE germinated	Othmani *et al.,* 2009b
6.		MS media with 0.5mg/L NAA + 0.25 mg/L BA	SE germinated	Kurup *et al.*, 2014
7.		MS media with 0.1mg/L NAA + 0.05 mg/L BA	SE germinated	Abdul-Soad & Mahdi, 2010

References

Abass, M. H. 2013a. A PCR ITS-RFLP method for identifying fungal contamination of date palm (*Phoenix dactylifera* L.) tissue cultures. *African Journal of Biotechnology*. 12 (32): 5054-5029.

Abass, M. H. 2013b. Microbial contamination of date palm (*Phoenix dactylifera* L.) in Iraqi tissue culture laboratories. *Emirates Journal of Food and Agriculture*. 25 (11): 875-882.

Abd El Bar, O. H. and El Dawayati, M. M. 2014. Histological changes on regeneration *in vitro* culture of date palm (*Phoenix dactylifera* L.) leaf explants. *Australian Journal of Crop Science*. 8 (6): 848-855.

Abdel-Rahim, E.A., Abdel-fatah, O.M., El-Shemy, H.A. and El-Samei, A. 2001. Growth of date palm callus as affected by amino acids as organic nitrogen source. In: Proc. of the First International Conference on Date Palm, Al-Ain, UAE. pp. 234-240.

Abul-Soad, A. A. and Mahdi, S. M. 2010. Commercial production of tissue culture date palm (*Phoenix dactylifera* L.) by inflorescence technique. *Journal of Genetic engineering and Biotechnology*. 8 (2):39-44.

Abul-Soad, A.A., Zaid, Z.E. and Sidky, 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 : 789-802

Al-Baiz, A.A., Mouli, C.K. and Al-Oraini, F. 2000. Suspension cultures from embryogenesis. In; Proc. of the Date Palm International Symposium, Windhoek, Namibia, 22-25 Feb.,2000 pp.36-40.

Ali, T.A., Jubrail, J.M. and Jasim, A.M. 2007. The Use of RAPDs for the Detection of genetic Stability of

Regenerated Plantlets of Barhee Palm in Iraq. In: Proc. of Third International Date Palm Conference. *Acta Horticulturae*, ISHS 736 : 127-131.

Al-Khalifah, N.S., Khan, F.A., Askari, E. and Hadi, S. 2006. *In vitro* culture and genetic analysis of male and female date palm (*Phoenix dactylifera* L.). *Acta Horticulturae,* 725 : 653-661.

Alkhateeb, A. 2008. Comparison effects of sucrose and date palm syrup on somatic embryogenesis of date palm (*Phoenix dactylifera* L). *American Journal of Biotechnology and Biochemistry*, 4 : 19-23.

Al-Khayri, J. M. 2011. Basal salt requirements differ according to culture stage and cultivars in date palm somatic embryogenesis. *American Journal of Biochemistry and Biotechnology*. 7 (1): 32-42.

Al-Khayri, J. M. 2012. Determination of the date palm cell suspension growth curve, optimum plating efficiency, and influence of liquid medium on somatic embryogenesis. Emirates Journal of Food and Agriculture. 24 (5): 444-455.

Al-Khayri, J.M. 2003. *In vitro* germination of somaticembryos in date palm: Effect of auxinconcentration and strength of MS salts. *Current Science*, 84 : 680-683.

Al-Khayri, J.M. and Al-Bahrany, A.M. 2004. Genotype-dependent *in vitro* response of date palm (*Phoenix dactylifera* L.) cultivars to silver nitrate. *Scientia Horticulturae*, 99 : 153-162

Al-Khayri, J.M. and Al-Bahrany, A.M. 2007. Silver nitrate and 2 isopentyladenine promote somatic embryogenesis in date palm (*Phoenix dactylifera* L.). *Scientia Horticulturae*, 89 : 291-298.

Anjarne, M. and Zaid, A. 1993. Effects de certains equilibres hormonaux sur l'enracinement precoce des tissus de palmier dattier (*Phoenix dactylifera* L.). *Al Awamia*, 82 : 197-210.

Anonymous (2014) Annual Report 2013-14, Central Institute for Arid Horticulture, Bikaner, Rajasthan 132pp.

Asemota, O. and Eke, C.R. 2006. Direct shoot development from date palm culture. RUN article 3094. (Ref. http;//www.runetwork.de/html/en/index.html?article_id=3094)

Asemota, O., Chukwuemeka, R.E. and Joshua. O. O. 2007. Date palm *in vitro* morphogenesis in response to growth regulators, sucrose and nitrogen. *African Journal of Biotechnology*, 6 (20) : 2353-2357.

Bakeet, S. 2013. Date palm biotechnology in Egypt (Review article). *Applied Science Reports*, 3 (3): 144-152.

Balal, N.A. and EI Deep, M.D. 1997. Direct organogenesis of date palm (*Pheonix dactylifera* L.) *in vitro* . *Assuit Journal of Agricultural Sciences*,28 (2) : 67-77.

Beauchesne, G. 1982. Vegetative propagation of date palm (*Phoenix dactylifera* L.) by *in vitro* cultures. In: Date Palm Symposium, Al Hassa, Saudi Arabia. pp. 698-699.

Beauchesne, G. Zaid, A. and Rhiss, A. 1986. Rapid propagation of date palm (*Phoenix dactylifera* L.) through tissue culture. In: Proc. of the Second Symposium on Date Palm, Saudi Arabia, pp. 87-93.

Bekheet S.A., Taha, H.S., Saker, M.M. and Solliman, M.E. 2007. Application of cryopreservation technique for *in vitro* grown date palm (*Phoenix dactylifera* L.) cultures. *Journal of Applied Sciences Research,* 3 : 859-866.

Bekheet, S. 2013. Direct organogenesis of date palm (*Phoenix dactylifera* L.) for propagation of true – to-type plants. *Scientia Agriculturae*, 4 (3): 85-92.

Bekheet, S.A., Taha, H.S.,Hanafy, M.S. and Solliman 2008. Morphogenesis of sexual embryos of date palm cultured *in vitro* identification of sex type. *Journal of Applied Science Research*, 4: 345-352.

Bennasseur, E.K. 2007. Large Scale In Vitro Propagation of a rare and unique male date palm (*Phoenix dactylifera* L.) using inflorescences technique. *Acta Horticulturae* ISHS, 736 : 243-247.

Bhargava, S.C., Saxena, S.N. and Sharma, R. 2003. *In vitro* multiplication of *Phoenix dactylifera*. *Journal of Plant Biochemistry and Biotechnology*, 12 : 43-47.

Bhaskaran, N. and Smith, S. 1992. Somatic embryogenesis from shoot tip and immature inflorescence of *Phoenix dactylifera* L. cv. Barhee. *Plant Cell Reports*, 12 : 22-25.

Bouguedoura, N. 1993. Theme 2: Contamination des cultures. In: *Compte Rendu des travaus de l'atelier sur la culture in vitro du palmier dattier, Sfax* 12-16 Juillet. FAO/PNUD.

Chander Bhan, Sivalingam, P.N., Singh, D., and Sharma M.K. 2013. Studies on *in-vitro* organogenesis in date palm. *Indian Journal of Horticulture,* 70: 475-479

Cherkaoui, B. 1997. Isolement, identification et lute les contaminations bacteriennes en culture *in vitro* chez *Phoenix dactylifera* L. These de Doctorat de 3eme cycle, niversite Cadi Ayyad, Marrakesh, Morocco.

Cohen, Y., Korchinsky, R. and Tripler, E. 2004. Flower abnormalities cause abnormal fruit setting in tissue culture propagated date palm (*Phoenix dactylifera* L.). *Journal of Horticultural Science and Biotechnology*, 79 : 1007-1013

Costa, N.M. and Aloufa, M.A.I. 2006. Direct organogenesis in *Phoenix dactylifera* L. through cotyledonar petiole. *Pesquisa Agropecuaria Tropical*, 36 : 195-198.

Drira, N. 1981. Multiplication végétative et micropropagation du palmier dattier (*Phoenix dactylifera* L.) à partir d'organes prelevés sur la phase adulte cultivés "*in vitro*". Thése pour le titre de Docteur de Specialité. Faculté des Sciences de Tunis. 138 p.

Eke, C.R., Akomeah, P. ans Asemota, O. 2005. Somatic embryogenesis in date palm (*Phoenix dactylifera* L.) from apical meristem tissues from 'zebia' and 'loko' landraces. *African Journal of Biotechnology*, 4 : 244-246.

El Hadrami, I., Cheikh, R. and Baaziz, M. 1995. Somatic embryogenesis and plant regeneration from shoot-tip explants in *Phoenix dactylifera* L. *Biologia Plantarum*, 37 : 197-203.

El-Bellaj, M., El-Jaafari, S. and El-Hadrami, I. 2000. IAA oxidase: regulator and potential marker of somatic embryogenesis in date palm (*Phoenix dactylifera* L.). *Cahiers Agricultures*, 9 : 193-195.

Eshraghi, P., Zarghami, R. and Mirabdulbaghi, M. 2005. Somatic embryogenesis in two Iranian date palm cultivars. *African Journal of Biotechnology,* 4 : 1309-1312.

FAO (2013) http://faostat.fao.org

Fki, L., Bouaziz, N., Kriaa, W., Benjemaa-Masmoudi, R., Gargouri-Bouzid, R., Rival, A. and Drira, N. (2011) Multiple bud cultures of 'Barhee' date palm (*Phoenix dactylifera*) and physiological status of regenerated plants. *Journal of Plant Physiology*, 168: 1694-1700

Fki, L., Masmoudi, R.,Drira, N. and Rival, A. 2003. An optimised protocol for plant regeneration from embryogenic suspension cultures of date palm, *Phoenix dactylifera* L., cv. Deglet Nour. *Plant Cell Reporter*, 21: 517–524.

Fridborg, G. and Eriksson, T. 1975. Effects of activated charcoal on growth and morphogenesis in cell cultures. *Plant Physiology*, 34: 306-308.

Hussain, I., Rashid, H. and Quraishi, A. 2001. *In vitro* multiplication of date palm. In: Second International Conference on Date Palm. 24-27 March, Al-Alin, U.A.E.

Ibraheem, Y., Panikar, I. and Bohme, M. 2013. A comparative study between solid and liquid cultures relative to callus growth and somatic embryo formation in date palm (*Phoenix dactylifera* L.) cv. Zaghlool. *Emirates Journal of Food and Agriculture,* 25 (11) 883-898.

Jain, S. M. 2007. Recent advances in date plam tissue culture and mutagenesis. *Acta Horticulturae*, 736 : 205-211.

Jain, S.M. 2001. Tissue culture-derived variation in crop improvement. *Euphytica*, 118 : 153-166.

Jain, S.M. and Gupta, P.K. (eds.) 2005. Protocols for Somatic Embryogenesis in Woody Trees. Springer

Javoukey, M., Daguin, F. and Letouze, R. 2000. Somatic embryogenesis, an efficient tool for date palm (*Phoenix dactylifera* L.) industrial micropropagation. Characterization and genetic stability of original offshoots and regenerated plantlets by RAPD markers. Intern. Symp. on Methods and Markers for Quality Assurance in Micropropagation, Cork, Irish Republic, 24-27 August, 1999. Acta Horticulturae 530 : 237-241.

Kackar, N.C.;. Solanki, K.R and. Joshi, S.P. 1989. Micropropagation of Date palm (*Phoenix dactylifera* L.) cv. Khadrawy using tissue culture technique. *Annals of Arid Zone*, 28 : 137-141.

Khan, M.A.,Khalil, M.S. and Al-Kahtani, M.S. 1983. *In vitro* culture of different tissues of date palm (*Phoenix dactylifera* L.) offshoot. Proc. of the First Symposium on the date palm in Saudi Arabia.

Khan, S. and BI BI, T. 2012. Direct shoot regeneration system for date palm (*Phoenix dactylifera* L.) cv. dhakki as a means of micropropagation. *Pakistan Journal of Botany*, 44 (6): 1965-1971.

Khierallah, H.S.M and Bader, S.M. 2007. Micropropagation of date palm (*Phoenix dactylifera* L.) var. Maktoom through direct organogenesis. *Acta Horticulturae*, 736 : 213-217.

Kurup, S. S., Aly, M. A. M., Lekshmi, G. and Tawfil, N. H. 2014. Rapid *in vitro* regeneration of date palm (*Phoenix dactylifera* L.) cv. Kheneizi using tender leaf explants. *Emirates Journal of Food and Agriculture*, 26 (6): 539-544.

Letouze, R. and Daguin, F. 1989. L'embryogenese somatique: des possibilities nouvelles pour la micro-propagation du palmier dattier (*Phoenix dactylifera* L.). In: *Compte Rendu 2eme seminaire maghrebin sur la multiplication rapide du palmier datier,* 9-12 Octobre, Marrakech, Maroc, pp. 51-58.

Letouze, R. Daguin, F., Satour, P., Hamama, L. and Marionnet, F.1998. Somatic embryogenesis and mass micropropagation of date palm (*Phoenix dactylifera* L.) characterization and genetic stability of regenerated plantlets by RAPD markers. In: Proc. of the First International Conference on Date Palm. Al-Ain, UAE. pp. 158-167.

Loutfi, K. 1989. Multiplication vegetative du palmier datier (*Phoenix dactylifera* L.) a partir de la culture *in vitro* d'explants inflorescentiels. These de Doctrorat de 3eme cycle, Universite Cadi Ayyad, Marrakesh, Morocco.

Loutfi, K. 1999. Organogenese et embryogenese somatique a patir des tissus floraux du palmier dattier (*Phoenix dactylifera* L.) cultives *in vitro*. Aspects histologiques et caryologie des vitroplants. These

de Doctorat d'Etat, Universite Cadi Ayyad, Marrakesh, Morocco.

Loutfi, K. and Chlyah, H. 1998. Vegetative multiplication of date palms from *in vitro* cultured inflorescences: effect of some growth regulator combinations and organogenetic potential of various cultivars. *Agronomie*, 18 : 573-580.

Loutfi, K. and El-Hadrami 2005. *Phoenix dactylifera* Date Palm. *In:* Biotechnology of fruit and nut crops-book edited by Litz, R.E. pp.144-156

Maier, V.P. and Metzlium, D.M. 1965. Quantitative changes in date polyphenols and their relation to browning. *Journal of Food Science*, 30 : 80-84.

Mater, A.A. 1986. *In vitro* propagation of date palm (*Phoenix dactylifera*. L.). *Date Palm Journal* , 4 : 137-152.

Mater, A.A. 1990. Effect of auxin-cytokinin on micropropagation of date palm. *J. King Saud Univ., Agricultural Sciences*, 2 : 211-223.

McCubbin, M. and Zaid, A. 2007. Would a Combination of Organogenesis and Embryogenesis Techniques in Date Palm Micropropagation be the Answer? *Acta Horticulture,* 736 : 255-258.

More, T.A., Sivalingam, P.N. and Singh, D. 2011. Datepalm. In: Advances in Horticulture Biotechnology— Regeneration Systems — Fruit Crops, Plantation Crops and Spices (Volume I) (eds: H.P. Singh, V. Parthasarathy and Nirmal Babu). Westville Publishing House, New Delhi 279-298pp

Othmani, A., Bayoudh, C., Drira, N. and Trifi, M. 2009a. *In vitro* cloning of date palm *Phoenix dactylifera* L., cv. Deglet Bey by using embryogenic suspension and temporary immersion bioreactor (TIB). Biotechnol. & Biotechnol. EQ. 23/2009/2: 1181-1188

Othmani, A., Bayoudh, C., Drira, N., Marrakchi, M. and Trifi, M. 2009b. Somatic embryogenesis and plant regeneration in date palm Phoenix dactylifera L., cv. Boufeggous is significantly improved by fine chopping and partial desiccation of somatic embryogeneic callus. *Plant Cell Tissue Organ Culture*, 97: 71-79.

Othmani, A., Bayoudh, C., Drira, N., Marrakchi, M. and Trifi, M. 2009c. Regeneration and molecular analysis of date palm (*Phoenix dactylifera* L.) Plantlets using RAPD markers. *African Journal of Biotechnology,* 8(5): 813-820.

Poulain, C., Rhiss, A. and Beauchesne, G. 1979. Multiplication vegetative en culture in vitro du palmier dattier (*Phoenix dactylifera* L.). *C R Acad Agric France,* 11 : 1151-1154.

Quarishi, A., Hussain, I., Ahmed, M., Rashid and Mohammed, L. 1997. Sustained multiplication of long term embryogenesis culture of date palm and their field performance. *Pakistan Journal of Botany,* 29(1) : 135-141.

Rao, A.M., Reddy, S.K., Rao, V.R.M. and Bhalla, J.K. 2001. Clonal propagation of date palm through somatic embryogenesis. *Indian Forester,* 127 : 463-465.

Reuveni, O. 1979. Embryogenesis and plantlets growth of date palm (*P. dactylifera* L.) derived from callus tissues. *Plant Physiology*, 63 : 138 (Abstr.).

Reuveni, O. and Kipniss, H.L. 1974. Studies of the *in vitro* culture of date palm (*P. dactylifera* L.) tissues and organs. The Volcani. Inst. Agric. Research Pamphlet No: 145.

Reynolds, J.F. and Murashige. T. 1979 Asexual embryogenesis in callus cultures of palms. *In vitro*. 15 : 383-387.

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

Saker, M.M., Adawy, S.S., Mohamed, A.A. and El Itriby, H.A. 2006. Monitoring of cultivar identity in tissue culture derived date palms using RAPD and AFLP analysis. *Biologia Plantarum*, 50 : 198-204.

Saker, M.M., Bekheet, S.A., Taha, H.S., Fahmy, A.S. and Moursy, H.A. 2000. Detection of somaclonal variations in tissue culture derived date palm plants using isoenzyme analysis and RAPD fingerprints. *Biologia Plantarum,* 43 : 347-351.

Sane, D., Aberlenc Bertossi, F., Gassama Dia, Y.K., Sanga, M., Trouslot, M.F., Duval, Y. and Borgel, A. 2006. Hostological analysis of callogenesis and somatic embryogenesis from cell Suspensions of date palm (*Phoenix dactylifera*). *Annals of Botany*, 98 : 301-308

Sane, D., Aberlenc-Bertossi, F., Diatta, L. I. D., Gueye, B., Daher, A., Sagna, M., Duvel, Y. and Borgel, A. 2012. Influence of growth regulators on callogenesis and somatic embryo development in date palm (*Phoenix dactylifera* L.) sahelian cultivars. *The Scientica World Journal*, 1-8.

Shah, J.J. 2014 Date palm cultivation in India: An overview of activities. *Emir. J. Food Agric*., 26: 987-999

Shakya, A.B. and Saker, M.M. 1998. *In vitro* propagation of Egyption Date palm: II. Direct and indirect shoot proliferation from shoot tip explant of (*Phoenix dactylifera L).* cv. Zaghlool. Proceedings of FICDP 150- 157.

Sharma, D.R., Dawra, S. and Chowdhry, J.B. 1984. Somatic embryogenesis and plant regeneration in date palm (*Phoenix dactylifera* L.) cv. 'Khadrai' through tissue culture. *Indian Journal of Experimental Biology,* 22 : 496-498.

Sharma, D.R., Deepak, S. and Chowdhury, J.B. 1986. Regeneration of plantlets from somatic tissues of date palm (*Phoenix dactylifera* L.) *Indian Journal of Experimental Biology* 24 : 763-766.

Sharma, D.R., Yadav, N.R. and Chowdhury, J.B. 1988. Somatic embryogenesis and plantlet regeneration from shoot tip calli of wild date palm (*Phoenix sylvestris* Roxb.). *Indian Journal of Experimental Biology* 26 : 854-857.

Sharon M.S. and Chandramati, P. 1998. Somatic embryogenesis and plant regeneration from leaf primordial of *Phoenix dactylifera* cv. Yakubi. *Indian Journal of Experimental Biology* 36 : 525-529.

Sidky, R. A. and Eldawyati, M. M. 2012. Proliferation of female inflorescences explants of date palm. *Annals of Agricultural Science*. 57 (2): 161-165.

Sivalingam, P.N., Bhargava, R., Sharma, S.K. and Murlidharan, C.M. 2014. Date Palm. *Current Science*. 107: 1373-74

Smith, R.J. and Aynsley, J.S. 1995. Field performance of tissue cultured date palm (*Phoenix dactylifera*) clonally produced by somatic embryogenesis. *Principes* 39 : 47-52.

Sudhersan, C. and El-Nil, M.A. 2004. Axillary shoot production in micropropagated date palm (*Phoenix dactylifera*). *Current Science* 86 : 771-773.

Sudhersan, C., El-Nil, M.A. and Hussain, J. 2001. Hapaxanthic axillary shoots in date palm plants grown *in vivo* and in vitro. *Palms* 45 : 84-89.

Taha, H.S., Bekheet, S.A., Hanafy, M.S. and Solliman, M.E. 2001 Factors affecting *in vitro* ultiplication of date palm. *Journal of Applied Science Research* 4 (4) : 345-352.

Tisserat, B. 1979. Propagation of date palm *in vitro*. *Journal of Experimental Botany*. 30(6) : 1275-1283.

Tisserat, B. 1981. Date palm tissue culture. Advances in Agricultural Technology, Western Series, United States Department of Agriculture 17 : 50.

Tisserat, B.H. and De Mason, D. 1980. A histological study of the development of adventive embryos in organ cultures of *Phoenix dactylifera* L. *Annals of Botany* 45 : 465-472.

Vermendi, J. and Navarro, L. 1996. Influence of physical conditions of nutrient medium and sucrose on somatic embryogenesis of date palm. *Plant Cell Tissue and Organ Culture* 45(2) : 159-164.

Yadav, N., Yadav, R.C., Chowdhury, V.K. and Chowdhury, J.B. 2001. Explant and cultivar response to *in vitro* clonal propagation of female date palm. In: IInd International Conference on Date Palm, Al- Ain, UAE, 25-27 March, 2001 pp. 491-499.

Zaid, A. and Al-Kaabi, H. 2003 Plant off types in tissue culture derived date palm (*Phoenix dactylifera* L.). *Emirates Journal of Agricultural Sciences* 15 : 17-35.

Zaid, A. and Arias-Jimenez, E.J. 2002. Date Palm Cultivation. Food and Agricultural Organization of the United Nations, Rome, 2002. (http://www.fao.org/DOCREP/006/Y4360E/y4360e09.htm)

Zaid, A. and Tisserat, B.H. 1983. *In vitro* shoot tip differentiation in *Phoenix dactylifera* L. *Date Palm Journal* 2(2) : 163-182.

Zirdar, S., Mousawi, M. and Ansari, N.A. 2008. Genetic stability in Date Palm Micropropagation. *Asian Journal of Plant Sciences* 7 : 775-778.

Zohar, D. and Spiegel-Roy, P. 1975. Beginning of fruit growing in the Old World. *Science* 187 : 319-327.