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REVIEW

Date palm micropropagation: Advances and applications

Micropropagação de tamareira: Avanços e aplicações

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ABSTRACT

Date palm (*Phoenix dactylifera* L.) is a fruit tree resilient to adverse climatic conditions predominating in hot arid regions of the Middle East and North Africa. The date fruit contains numerous chemical components that possess high nutritional and medicinal values. Traditional propagation by offshoots is inefficient to satisfy current demands for date palm trees. Alternatively, micropropagation provides an efficient means for large-scale propagation of date palm cultivars. Both somatic embryogenesis and organogenesis, either directly or indirectly though the callus phase, have been demonstrated in date palm in vitro regeneration. Culture initiation commonly utilizes shoot-tip explants isolated from young offshoots. Recently, the immature inflorescences of adult trees were utilized as an alternative nondestructive source of explants. In addition to the nature of the explant used, successful plant regeneration depends on the cultivar, composition of the culture medium and physical status. Challenges of date palm micropropagation include long in vitro cycle, latent contamination, browning, somaclonal variation as well as ex vitro acclimatization and transplanting. A remarkable amount of research investigating these factors has led to optimized protocols for the micropropagation of numerous commercially important cultivars. This has encouraged the development of several international commercial tissue culture laboratories. Molecular characterization provides an assurance of genetic conformity of regenerated plantlets, a key feature for commercial production. This article describes date palm micropropagation protocols and also discusses recent achievements with respect to somaclonal variation, molecular markers, cryopreservation and future prospects.

Index terms: Cryopreservation; somatic embryogenesis; somaclonal variation; organogenesis; molecular marker.

RESUMO

A tamareira (*Phoenix dactylifera* L.) é uma arvore frutífera adaptada à condições climáticas adversas predominantemente em regiões áridas do Oriente Médio e Norte Africano. As tâmaras possuem vários componentes químicos com alto valor medicinal e nutricional. A propagação tradicional por estacas não é suficiente para satisfazer a demanda por mudas e assim, a micropropagação apresenta-se como uma alternativa eficiente para a produção de mudas em larga escala. Embriogênese somática e organogênese, tanto direta quanto indireta via calos, tem sido usada para obter a regeneração *in vitro* de tamareira. O inicio do cultivo *in vitro* normalmente utiliza meristemas excisados de brotações jovens. Recentemente, inflorescências imaturas de árvores adultas são usadas como fonte alternativa de explantes não destrutiva. Além da origem do explante, o sucesso da regenerção depende do cultivar, da composição do meio de cultura e de condições físicas. Desafios na micropropagação de tamareira incluem um longo ciclo *in vitro*, contaminação, escurecimento do tecido, variação somaclonal além do enraizamento e aclimatização *ex vitro*. Diversos estudos investigando esses fatores tem conduzido à otimização de protocolos de micropropagação de inúmeros cultivares comerciais proporcionando o estabelecimento de vários laboratórios de cultura de tecidos de plantas. A caracterização molecular permite uma segura conformidade genética do material regenerado, considerado uma característica chave na produção comercial. Essa revisão descreve protocolos de micropropagação de tamareira e aborda as mais recentes conquistas relacionadas à variação somaclonal, marcadores moleculatres, criopreservação e perspectivas futuras.

Termos para indexação: Criopreservação; embriogênese somática; variação somaclonal; organogênese; marcador molecular.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a 'tree of life' which belongs to the family Arecaceae; it is distributed mainly in the Middle East and North Africa. Over the last century, date palm agriculture has spread to Australia, Southern Africa and the Americas. The distribution of date palm is very peculiar because of the inherent requirement for hot climate which is necessary for successful pollination and fruit setting (Chao; Krueger, 2007). Date palm cultivation has been recorded for thousands of years and over that time selection procedures have developed more than 3,000 known varieties of date palm around the world (Johnson, 2011). Date fruits possess important nutritional and medicinal values and as a part of religious practice, dates

are consumed by people all over the world (Vayalil, 2012). Dates are a good source of essential minerals, which enhance their nutritional value (Al-Shahib; Marshall, 2002, 2003; Elleuch et al., 2008). The aqueous extracts of dates possess antioxidant activity (Al-Farsi et al., 2007; Biglari; AlKarkhi; Easa, 2008; Saafi et al., 2009). Phenolic compounds present in dates, have beneficial effects on human health and act against cancer and cardiovascular diseases (Vayalil, 2012). Dates are a high energy source consisting of carbohydrates 44-88% in addition to proteins 2.3-5.6% and fats 0.2-9.3% (El Hadrami; El Hadrami, 2009). Palm syrup, palm sugar, vinegar, wine and honey are made from the date fruits (Chao; Krueger, 2007). The annual international market value (including import and export) of the date crops reached nearly 1.9 billion USD (FAOSTAT, 2013), from which a country can build a strong economic platform. The vegetative part of the date tree is used as a raw material for roof coverings for houses, wooden boats, timber for wood industry and making numerous handicrafts (Al-Khayri; Jain; Johnson, 2015a, 2015b).

In recent years, because of overexploitation, the diversity of the date palm groves has declined. The production and utilization of the date fruits also varies from country to country due to the influence of current environmental conditions. There are a number of elements which hinder the production of date palm, such as major pests and diseases, salinity and drought, poor harvest and postharvest practices (Al-Khayri; Jain; Johnson, 2015a, 2015b). Date palm is conventionally propagated vegetatively by offshoots. Due to their heterozygous nature, seeds cannot be used for the propagation of commercially elite cultivars because they produce off-type propagules (Abahmane, 2011). The number of offshoots produced by a date palm tree during their life span is only about 20-30 (Zaid; El-Korchi; Visser, 2011). The survival rate of offshoots in the field is low and chances of disease transmission are high (Abahmane, 2011). These factors hinder meeting the increasing agro-industrial demand for propagules. The use of micropropagation techniques eliminates these restrictions and allows for large-scale production of healthy, disease-free, true-to-type plants.

Since the early reports of date palm in vitro regeneration (Tisserat, 1979, 1982) numerous researchers have described different approaches to achieve date palm micropropagation: somatic embryogenesis (Al-Khayri, 2003, 2005; Fki et al., 2003, 2011a; Mazri et al., 2017; Naik; Al-Khayri, 2016; Othmani et al., 2009a; Roshanfekrrad et al., 2017) and organogenesis (Bekheet, 2013; Jazinizadeh et al., 2015; Khan; Bi, 2012; Khierallah; Bader, 2007; Meziani et al., 2015, 2016). In addition to providing a means for rapid clonal propagation of date palm (Khierallah; Bader, 2007), tissue culture techniques can be utilized for the production of synthetic seeds (Bekheet et al., 2002), cell suspension culture (Othmani et al., 2009b), cryopreservation (Fki et al., 2013), somaclonal variation introduces stress tolerant, disease resistance and with high quality fruits (El Hadrami; El Hadrami, 2009; Jain, 2001), the production of secondary metabolites (El-Sharabasy, 2004) and commercial production of date palm gives considerable profit to both public-sector and private agencies (Zaid; El-Korchi; Visser, 2011).

The present article gives an update of the current approaches of date palm micropropagation with emphasis on the plant regeneration through somatic embryogenesis and organogenesis and use of a bioreactor. It highlights key factors that influence in vitro differentiation and culture growth including tissue browning, hyperhydricity, explant type, light and composition of culture medium. Also addressed are concerns related to potential somaclonal variation, molecular detection techniques, utilization of tissue culture in germplasm conservation and commercial production.

MICROPROPAGATION

The decades of efforts on date palm micropropagation by scientists achieved the goal of using different parameters which affect the in vitro development of date palm such as explant source (Figure 1A), age of explant, size of explant, intensity and quality of light, temperature, pH of the medium, plant hormones, culture medium and age of culture (Al-Khayri, 2013; Mazri, 2015; Mazri; Meziani, 2015; Mazri et al., 2016). This is an efficient alternative to the conventional means of propagation. Somatic embryogenesis and organogenesis are the two main modes of micropropagation of date palm, which are widely accepted throughout the tissue culture arena in the world (Bhansali, 2010). The utilization of bioreactors is also making a significant contribution toward micropropagation of different plant species (Frómeta et al., 2017; Gomes et al., 2016; Othmani et al., 2011).

Somatic embryogenesis

Somatic embryogenesis can be defined as the development of somatic embryos from somatic cells, which undergo a series of morphological and biochemical changes (Quiroz-Figueroa et al., 2006). Somatic embryogenesis includes a series of stages viz. embryogenic callus initiation, formation of somatic embryo, development of somatic embryo, maturation and plantlet formation. In date palm micropropagation somatic embryogenesis is considered as the most potent tool to achieve large-scale production.

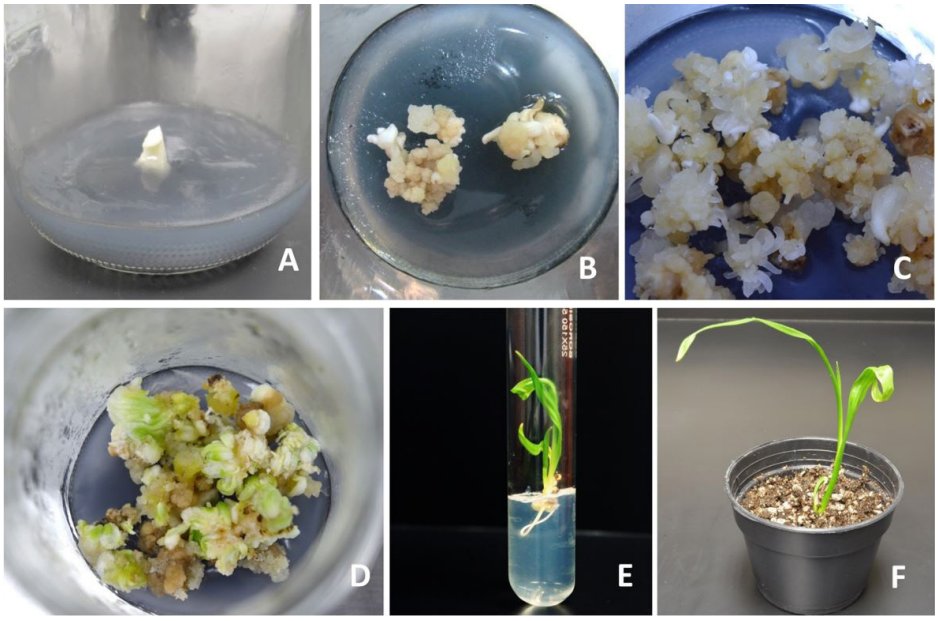

Figure 1: Micropropagation of date palm. (A) Shoot tip culture, (B) Embryogenic callus induction, (C) Embryogenic callus proliferation, (D) Embryo formation, (E) Rooting and (F) Transplanted plant.

Embryogenic callus initiation and embryo formation

The embryogenic calli initiation process in the date palm is very slow; this is mainly due to the tree nature of the plant. The embryogenic callus initiation and proliferation (Figure 1B and 1C) process depends on various parameters like the type of genotype, explant source, plant hormones, and culture condition. To induce embryogenic callus in most of the cases for either shoot tip or inflorescence, a high concentration of auxins is used. Most researchers suggest 2,4-dichlorophenoxyacetic acid (2,4-D) as the most efficient auxin to induce embryogenic callus used at 100 mg L^{-1} concentration (Al-Khayri, 2005; Al-Khayri, 2010; Eshraghi; Zaghami; Mirabdulbaghi, 2005; Naik; Al-Khayri, 2016). Fki et al. (2011a) reported that high concentrations of 2,4-D causes somaclonal variations. Many researchers applied low concentration of 2,4-D such as 1.5, 5 and 10 mg L^{-1} to induce somatic embryo (Aslam et al., 2011; El Hadrami; Cheikh; Baaziz, 1995; Othmani et al., 2009a). Depending on the genotype the somatic embryo formation (Figure 1D) period also varies from a few to several months (Eshraghi; Zaghami; Mirabdulbaghi, 2005; Hassan; Taha, 2012; Othmani et al., 2009a). Recently Mazri et al. (2017) reported somatic embryogenesis from the adventitious bud of date cv. Najda, where the MS medium was supplemented with 2,4-D, kinetin (KN) or 6-dimethylallylamino purine (2iP). Proximal leaf segment showed embryogenesis only in the medium supplemented with 2,4-D or picloram.

Development of somatic embryo and maturation

Various factors are responsible for the development of somatic embryo and maturation. Date palm has the capacity to develop as a mature embryo in both semisolid and liquid medium. The authors reported in the date palm cv. Deglet Nour, 1-month old suspension culture produces 200 embryos from 100 mg fresh weight callus inoculum, on the other hand it produces 10 embryos on semisolid medium (Fki et al., 2003). In the date cv. Khalas, application of thiamine and biotin showed an increased number of embryos and also helps in the elongation process (Al-Khayri, 2001). The embryogenic calli with fine chopping and partial desiccation significantly improves the embryo maturation in cv. Boufeggous as reported by Othmani et al. (2009a).

Plantlet formation

Somatic embryo germination or plantlet formation depends on different factors. The size of the somatic embryos also varies the germination percentage in date palm (Al-Khayri; Al-Bahrany, 2012). Al-Khayri (2003) also reported that the somatic embryo germination is influenced by the strength of the medium and concentration

of the 1-naphthalenacetic acid (NAA) and indole-3-butyric acid (IBA) used. Somatic embryo germination with 81% plantlet conversion was achieved with 1 mg L^{-1} NAA concentration (Othmani et al., 2009b). The hormone combination of 6-benzylamino purine (BAP), IBA and NAA also influences the plantlet formation or rooting (Figure 1E) in dates (Zouine; El Hadrami, 2007). However, Mazri et al. (2017) achieved somatic embryo germination in hormone-free MS medium.

Acclimatization of plantlet

The success of acclimatization is hidden in the nature of the substrate which should be high in organic matter, possess optimal water-holding capacity and proper aeration attributes (Hegazy, 2008). Plantlets derived from date palm somatic embryos have been successfully acclimatized in many cultivars. Kurup et al. (2014) found nearly 60% survival rate of cv. Kheneizi when transferred to pots with a peat/vermiculite mixture of 2:1. Othmani et al. (2009a, 2009b) reported 60 and 80% survival rates in date palm cvs. Boufeggous and Deglet Nour, respectively. Al-Khayri (2010) observed 72-84% survival rate in cvs. Khasab and Nabout Saif plantlets after ex vitro transfer (Figure 1F).

Organogenesis

Organogenesis refers to the development of organ tissue with a vascular connection and finally which influences the plant formation from an explant with or without the intermediate callus stage. Date palm micropropagation favors the direct organogenesis of an explant without callus formation stage. To produce elite cultivars from rapid clonal propagation, direct organogenesis techniques have been widely adopted (Khierallah; Bader, 2007). There are various steps involved in date palm organogenesis: adventitious bud formations, multiplication of shoot bud, shoot elongation, and rooting (Abahmane, 2011; Bekheet, 2013; Mazri; Meziani, 2013, 2015).

Adventitious bud formations

The formation of adventitious buds from date palm explants depends on several factors. Based on the findings of Al-Khateeb (2006) a high concentration of plant hormones induces abnormal growth of the tissue without bud formation, and low concentrations influence the formation of adventitious buds. Many researchers have found that different auxin and cytokinin combinations promote bud formation in different date palm cultivars. Cultivar Maktoom induced buds in the MS medium (Murashige; Skoog, 1962) supplemented with 1 mg L^{-1} BAP, 2 mg L^{-1} 2iP, 1 mg L^{-1} NAA and 1 mg L^{-1} 2-naphthoxyacetic acid (NOA) (Khierallah; Bader, 2007). Bekheet (2013) reported that MS medium fortified with 2 mg L^{-1} 2iP and 1 mg L^{-1} NAA promotes buds in cv. Zaghlool, while Al-Mayahi (2014) used cv. Hillawi which induced buds in the MS medium supplemented with 1 mg L^{-1} BAP and 0.5 mg L^{-1} thidiazuron (TDZ).

Multiplication of shoot buds

Shoot bud multiplication is influenced by various factors such as medium composition, genotype and plant hormones. Khierallah and Bader (2007) mentioned that the date palm cv. Maktoom showed higher shoot-bud multiplication in MS medium with a hormone combination of 1 mg L^{-1} NAA, 1 mg L^{-1} NOA, 4 mg L^{-1} 2iP and 2 mg L^{-1} BAP. Mazri and Meziani (2013) found that half-strength MS medium augmented with 0.5 mg L^{-1} NOA and 0.5 mg L^{-1} KN produced 23.5 shoot buds per explant after 3 months of multiplication in cv. Najda. Al-Mayahi (2014) reported production of an average of 18.2 buds per culture in cv. Hillawi, in the MS medium containing 1 mg L^{-1} BAP and 0.5 mg L^{-1} TDZ.

Shoot Elongation and rooting

The medium with or without hormone promotes shoot elongation and rooting in date palm. Mazri and Meziani (2013) found that in the cv. Najda shoot elongation was faster in the medium supplemented with hormones when compared to hormone free medium, which also adds high frequency of root formation, the hormone free medium showed wider and green leaves with optimum survival rates. Bekheet (2013) suggested 1 mg L^{-1} NAA induces better and optimum rooting at the same concentration IAA or IBA. Meziani et al. (2015) reported cv. Mejhoul shoots grew an average of 13.4 cm with an average 4.6 roots number per shoot with wide and green leaves from 3 months old hormone-free half MS medium.

Acclimatization of plantlet

Acclimatization of plantlets is the final step of micropropagation. Mazri and Meziani (2013) achieved good survival frequency in cv. Najda when a peat-gravel mixture was used as the substrate. Meziani et al. (2015) reported after 3 months of observation in the greenhouse the cv. Mejhoul plantlets showed 88% survival rate. The cv. Boufeggous shoots from semisolid medium, found the highest survival rate up to 92.5% whereas the shoots from stationary liquid media culture showed a survival rate of 50% after 3 months in the greenhouse (Mazri, 2015).

Bioreactor

In recent years micropropagation protocols have used liquid media instead of semisolid media. The production of somatic embryos in liquid culture systems was 17-fold higher than on semisolid medium (Othmani et al., 2011). The Temporary Immersion Bioreactor (TIB) showed that shoot clusters were regenerated 5.5-fold faster as compared to semisolid medium (Othmani et al., 2011). The bioreactor system may be used in the micropropagation optimization process, which needs less manipulation of medium, reduces the cost of gelling agents, experienced labor, laboratory space and induces the multiplication frequency of the particular plant species (Gomes et al., 2016). Recently, Frómeta et al. (2017) successfully achieved the regeneration of the Barberton daisy (*Gerbera jamesonii*) using TIB system. Ramírez-Mosqueda and Iglesias-Andreu (2016) reported the micropropagation of the vanilla orchid (*Vanilla planifolia*) in different bioreactor systems such as TIB, Gravity Immersion Bioreactors (GIB) and Recipient for Automated Temporary Immersion (RITA). The high number of shoots observed in the TIB followed by RITA and GIB and optimum rooting was found in TIB compared to GIB and RITA. Gomes et al. (2016) evaluated the regeneration of somatic embryos of oil palm (*Elaeis guineensis*) by using semisolid, RITA and Temporary Immersion System (TIS) and found TIS was most suitable for regeneration. Whereas, the use of TIB system in date palm (cv. Deglet Bey) in vitro regeneration was reported by Othmani et al. (2009b, 2011). Improved embryogenic callus and proliferation of regenerated shoots was observed in TIB system compared to semi-solid medium.

MAJOR PROBLEMS AND FACTORS AFFECTING THE DATE PALM MICROPROPAGATION

In date palm micropropagation various problems are encountered like tissue browning, hyperhydricity and genetic variation. The major factors are as follows: nature of the explant, light and medium composition used. The problems and factors affecting micropropagation are briefly discussed in this section.

Tissue browning

The browning of the explant is the most frequent problem in date palm tissue culture which finally leads to the death of the tissue. During micropropagation both somatic embryogenesis (Abohatem; Zouine; El Hadrami, 2011) and organogenesis (Mazri, 2015) encounter this problem. The reason is that date tissue contain high levels of phenolic compounds which are toxic to the tissue and finally causes its death (Loutfi; El Hadrami, 2005). During the surface sterilization procedure, ascorbic acid and citric acid are used to control browning in the explant tissue (Al-Khayri, 2010; Khierallah et al., 2015; Naik; Al-Khayri, 2016). In order to prevent this browning many researchers add activated charcoal and polyvinylpyrrolidone (PVP) in the culture medium (Al-Khayri, 2010; Mazri; Meziani, 2013; Naik; Al-Khayri, 2016).

Hyperhydricity

Hyperhydricity refers to the accumulation of water content in the cultured tissue. This physiological disorder is common in somatic embryogenesis and organogenesis of date palm (Mazri, 2015; Mazri; Meziani, 2013; McCubbin; Zaid, 2007). Kriaa et al. (2012) reported when hydrated calli were cultured on the low concentration of 2,4-D for a long period, calli will induce somatic embryos or shoots. The factors such as plant hormones, liquid media and concentration of ammonia used are responsible for hyperhydricity (Al-Khateeb, 2008; Mazri; Meziani, 2015). The use of high agar concentration in the semisolid media and low concentration of plant hormones and ammonium concentration will help in the reduction of formation of hyperhydricity in the cultured tissue (Al-Khateeb, 2008).

Nature of explant

In the beginning era of date palm tissue culture, researchers used different kind of explants: embryos, immature fruits, roots, leaf petioles, lateral buds, shoot tips, pieces of stem and rachilla tissue (Sharma; Deepak; Chowdhury, 1986; Tisserat, 1979). After decades of research it has been proved that date palm micropropagation is most responsive with explants having meristematic origin and these includes apical shoot tips, lateral buds and leaf primordial isolated from shoot tip (Aslam et al., 2011; Khan; Bi, 2012). The meristematic cells or tissue respond quickly to the components of the culture medium. However, Khan and Bi (2012) reported the multiplication of date palm cv. Dhakki through direct shoot regeneration where different explants such as shoot tips, leaf primordia and apical meristem were used, among these shoot tips emerged as a most promising explant with highest capacity for direct shoot regeneration. In recent years use of the inflorescence as an explant has increased, where harvesting of the offshoots is not required. Micropropagation from inflorescence was achieved in date

palm cvs. such as Gulistan, Aseel, Dedhi, Gajar, Kashoowari, Khar, Kharbline, Khormo, Deglet Noor, Blombek, Menakher and Barhee (Abahmane, 2013; Abul-Soad, 2012; Abul-Soad; Mahdi, 2010; Kriaa et al., 2012).

Effect of light

Light serves as an external factor to regulate the growth and development of in vitro plants. The broad spectrum of fluorescent lamps with a wavelength range from 380-750 nm are used as a light source for tissue culture (Kim et al., 2004). Light intensity and type of light affect date palm micropropagation (Al-Mayahi, 2016; Meziani et al., 2015). Meziani et al. (2015) evaluated the organogenesis of date palm cv. Mejhoul using different levels of light intensities. The results explained that the 2000-3000 lux light intensity enhances shoot elongation and greening but reduction in the shoot bud proliferation was observed. Darkness and low light intensity (500 lux) significantly induced advanced rooting. The 1000 lux light intensity during the multiplication stage showed optimal growth of culture with respect to shoot buds per explant, greening an advanced rooting. Recently Al-Mayahi (2016) conducted an experiment to test the effect of combinations of red + blue light emitting diode (18:2) (CRB-LED) and white fluorescent light on direct organogenesis by induction of adventitious buds from shoot tip and multiplication shoots of date palm cv. Alshakr. The results showed CRB-LED performed better in the production of shoot numbers than white fluorescent light. CRB-LED also significantly increased the total soluble carbohydrate, starch, free amino acids, peroxidase activity, potassium, magnesium and sodium contents of the in vitro shoots.

Medium composition

Tissue culture media is composed of a wide range of micro and macro elements, these compositions of basal media regulates the growth and development of tissue in the in vitro culture. The date palm tissue culture also showed different growth patterns with respect to the various media composition (Al-Khayri, 2011). Al-Khayri (2011) tested five different types of media such as MS medium, W medium (White, 1963), NN medium (Nitsch; Nitsch, 1969), SH medium (Schenk; Hildebrandt, 1972), Woody Plant Medium, WPM (Lloyd; McCown, 1981) to evaluate the callus growth and somatic embryogenesis. Optimal callus growth was attained in cv. Barhee using SH, W and MS media, cv. Berny using SH and NN medium, in cv. Khusab using W and WPM media. The best embryo production was seen in cv. Barhee on W and SH media, cv. Berny using WPM and MS media and cv. Khusab using W and SH media. The optimum regeneration percentage obtained in the cv. Barhee occurred on W and WPM media, cv. Berny on WPM medium and cv. Khusab using W medium. Mazri et al. (2016) evaluated the effect of various concentrations of major mineral salts including ammonium nitrate, potassium nitrate, calcium chloride dehydrate, potassium dihydrogen phosphate and magnesium sulfate heptahydrate on shoot bud proliferation of date palm cv. Mejhoul, they also tested the different concentration of L-glutamine and myo-inositol. The results showed that maximum number of shoot buds per explant obtained on the medium fortified with 825 mg L^{-1} NH_4NO_3, 1900 mg L^{-1} KNO_3, 220 mg L^{-1} $CaCl_2.2H_2O$, 170 mg L^{-1} KH_2PO_4, 370 mg L^{-1} $MgSO_4.7H_2O$ as well as 1 g L^{-1} L-glutamine, 2 g L^{-1} myo-inositol and 30 g L^{-1} sucrose. The media supplemented with 1650 mg L^{-1} NH_4NO_3 significantly influenced the frequency of hyperhydricity. The results also showed that the number of shoot buds per explant was significantly affected by the concentration of L-glutamine, and myo-inositol present in the medium.

In addition to the mineral salts in tissue culture medium, the carbon source also plays an important role in the tissue growth. In the tissue culture of different plant species and also in the date palm the major carbon source used is sucrose at 30 g L^{-1} (Al-Khayri, 2013). In addition to the sucrose, other carbon source such as sorbitol, maltose, mannitol or commercial granulated sugar are also being used to improve the callus growth and shoot bud proliferation in date palm (Al-Khayri, 2013; Mazri et al., 2016). Mazri et al. (2016) evaluated the various concentrations of carbon sources to improve the shoot bud multiplication in date palm cv. Mejhoul. The experiments revealed the medium supplemented with 30 g L^{-1} sucrose obtained the optimal number of shoot buds per explant when compared to sorbitol, mannitol and commercial granulated sugar. Low and high concentrations of sucrose did not induce optimum number of shoot buds. The superior morphology of the shoot buds was generated from sucrose supplemented media when compared to buds obtained from the media augmented with sorbitol, mannitol and commercial granulated sugar.

VARIATION IN MICROPROPAGATED PLANTS

Somaclonal variation

The morphological and genotypical differences observed in micropropagated plants are called somaclonal variations. Sometimes micropropagated plants carry advantageous characters with respect to commercial and

agronomical interest which finally leads to some new varieties (El Hadrami et al., 2011). Somaclonal variation act as a raw material for the mass propagation, cryopreservation and synthetic seed production. Date palm micropropagation and somatic variation introduces new genotypes with stress tolerant, disease resistant and with high quality fruits (El Hadrami; El Hadrami, 2009; Jain, 2001). Research has showed that somaclonal variation frequency is depend on the age of the tissue cultured date palm (Saker et al., 2000). The high concentration of 2,4-D induced 25% somaclonal variation and observed change in the leaf morphology and also cause poor flower pollination leads to low quality date fruit (Fki et al., 2011b). To reduce the risk of somaclonal variation researchers suggest using juvenile explants, low auxin concentration, especially 2,4-D and minimum numbers of subcultures (El Hadrami et al., 2011; Fki et al., 2011a, 2011b; Khan; Bi, 2012). The use of high concentration of plant growth regulators induces somaclonal variation in date palm (Al-Mazroui et al., 2007).

Molecular characterization

The date palm is heterozygous and its out-breeding nature creates progeny with 50% male and 50% female trees that are not true-to-type (Othmani et al., 2010). In commercial micropropagation it is very important to test the genetic conformity/true-to-type of the regenerated plants. Several researchers have used molecular markers (AFLP, ISSR and RAPD) to confirm the genetic conformity of the tissue-cultured plants (Kumar et al., 2010; Othmani et al., 2009c, 2010). The use of RAPD primers showed micropropagated date cv. Ferhi with high level of polymorphism and 37.8% of variability (Moghaieb; Abdel-Hadi; Ahmed, 2011). The variation in tissue-cultured plants is also observed in cvs. Barhee and Khalas which display dwarfism and abnormal flower development (Al-Kaabi; Zaid; Ainsworth, 2007; Zaid; Al-Kaabi, 2003). Kumar et al. (2010) evaluated 27 micropropagated date plants with 160 RAPD and 21 ISSR primers, in which 30 RAPD and 12 ISSR primers produced a total of 347 reproducible monomorphic band; the results suggested that the micropropagated plants are true-to-type. Aslam, Khan and Naqvi (2015) established the somatic embryo derived regeneration protocol for the six cvs. Barhee, Zardai, Khalasah, Muzati, Shishi and Zart, in which the RAPD profile showed a similar banding pattern for micropropagated plants and mother plants and confirmed the genetic stability. Abass, Al-Utbi and Al-Samir (2017) screened the genetic toxicity of hormones on date palm callus using RAPD and protein profile. Low concentration of 2,4-D and Dicamba showed no polymorphism in RAPD primers and protein profile when compared to the control profile. The high concentration of hormones induced more polymorphism in both the tested markers compared to control.

GERMPLASM CONSERVATION

Micropropagation methods help in the germplasm conservation of many plant species. The disappearance of date palm farms due to urbanization, and which also leads in the declining of the genetic diversity of the date palm. Shortage of arable land, adverse climatic conditions, diseases and natural disasters are some major factors affecting the diversity of date palm (Bekheet; Taha, 2013; Zaid et al., 1999). There is a crucial need for the conservation of date palm germplasm and better utilization of available genetic resources. Although farmers are playing an important role in the conservation of the date palm germplasm, through cultivating different varieties in their traditional groves (Bekheet; Taha, 2013), they tend to propagate a limited number of commercially desired cultivars. The use of modern techniques are necessary to conserve date palm germplasm. The establishment of a germplasm bank based on available in vitro technologies for this important species is yet to be realized.

Cryopreservation

One of the modern conservation method is cryopreservation, where genetic materials like shoot tips, callus, cell suspension, microspore and somatic embryos are stored long-term under super-low temperatures (-196 ºC in liquid nitrogen). A genuine micropropagation method is the primary requirement of cryopreservation of germplasm and in vitro conservation. Over decades researchers have standardized date palm micropropagation and started the cryopreservation of date palm tissue (Bekheet et al., 2007). Embryogenic calli of date palm were treated with cryogen and stored for several months under ultra-low temperature (-196 ºC). Tisserat (1982) reported date palm regeneration from latent calli revived after 4-8 weeks. The successful cryopreservation of date palm callus after 4 months at -25 ºC was achieved by Mater (1987) by treating with cryoprotectant mixture. The normal growth of date palm embryos was achieved by Mycock et al. (1997) by pre-treating the embryos with glycerol and sucrose and dried to a water content of 0.4-0.7 g g^{-1}. Bekheet, Taha and Saker (2002b) reported a long-term preservation method for in vitro shoot bud and callus culture with optimum percent of viability after 12 months treatment at 5 ºC. Al-Bahrany and

Al-Khayri (2012) described the germplasm conservation by providing reliable cryoprotectant solution for date palm (cv. Khalas) cell suspension. Fki et al. (2011b) reported the successful cryopreservation method for proembryogenic masses (PEMs) of date palm variety Barhee and the morphology of the regenerated plants confirmed the stability of the clonal material. Furthermore, Fki et al. (2013) reported the regeneration of cryopreserved callogenic meristems of date palm cv. Khenizi. Cryopreserved date palm tissue with optimum regeneration capacity can boost the micropropagation process.

COMMERCIAL PRODUCTION

Date palm micropropagation is an economical means to achieve rural development (Rajmohan, 2011). It has been proven that date palm micropropagation protocols have been developed and achieved commercial production (Hoop, 2000). Numbers of cultivars are being developed and multiplied throughout the world. It has been reported that United Arab Emirates University developed micropropagation protocol for about 50 date palm cultivars (Rajmohan, 2011). In India Atul Rajasthan Date Palms Ltd, has mounted a project to grow tissue cultured plants in the desert area using appropriate cultivars. As commercial production of date palm involves considerable profit, a number of private and public-sector agencies are involved in developing protocols for micropropagation of date palm. Some of the countries and their leading agencies/companies are producing millions of micropropagated plants annually are as follows: Marionnet G.F.A. in France, Palmdat in France and Namibia, Atul Rajasthan Date Palms Ltd. in India, Rahan Meristem in Israel, Domaine Agricole El Bassatine in Morocco, Al Rajhi Tissue Culture Laboratory in Saudi Arabia, Sapad Tissue Culture Date Palm Co. in Saudi Arabia, United Arab Emirates University - Date Palm Development Research Unit in U.A.E. and Date Palm Developments in United Kingdom. Most of the institutions and laboratories are focused on cv. Medjool because of its high economic importance; recently cv. Barhee also has been a focus for micropropagation, selling for USD 24-26 per plant. The price of the plant depends on the growth stage, cultivar and quantity ordered.

CONCLUSIONS AND FUTURE PROSPECTS

Date palm is a traditional crop in the Arab world which has the capacity to withstand adverse climatic conditions. For thousands of years date pam was propagated through conventional breeding which is a time-consuming and tedious process. The application of the tissue culture techniques gave date palm an improved efficiency compared to other crops. Micropropagation of date palm through somatic embryogenesis and organogenesis reduces labor cost and time. Date palm micropropagation depends on various factors which include tissue browning, hyperhyricity, explant source, age of explant, size of explant, cultivars, intensity and quality of light, temperature, pH of the medium, plant hormones, culture medium composition and age of culture. The various researchers all over the world are working on the factors which control date palm micropropagation via somatic embryogenesis and organogenesis. Optimization of all these factors in a particular elite cultivar will enhance the chances of biotechnological application such as somaclonal variation selection, cryopreservation, synthetic seeds, cell culture, protoplast cultures, haploid production, mutation studies, secondary metabolite production, use of bioreactor, assessment of genetic fidelity and genetic transformation (Al-Khayri; Jain; Johnson, 2015a, 2015b; Aslam; Khan; Azad, 2015; Jain; Al-Khayri; Johnson, 2011). Thus, there is a need for the optimization of date palm micropropagation in elite cultivars to encourage the development of commercial production of date via tissue culture laboratories in various parts of the world.

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