

Review

Worldwide status of micropropagation in date palm (*Phoenix dactylifera* L)

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ABSTRACT

Date palm (*Phoenix dactylifera* L) is a most potential fruit crop having high nutritional value for the arid and semi-arid regions of the world and India. The slow nature of traditional vegetative method of propagation limits the expansion of cultivation of date palm on commercial scale. For commercial expansion there is a need for rapid mass multiplication of date palm which can be achieved by micropropagation techniques. In micropropagation there are two methods viz organogenesis and somatic embryogenesis. In these two methods organogenesis is dependent on potentiality of meristematic tissue to form direct organs whereas in somatic embryogenesis somatic cells develop into somatic embryos and their germination. The present article summarizes the literature of micropropagation through organogenesis and somatic embryogenesis work in date palm.

Keywords: *Phoenix dactylifera*; micropropagation; organogenesis; somatic embryogenesis

INTRODUCTION

Date palm (*Phoenix dactylifera* L) is a most potential fruit crop belonging to family Arecaceae for the arid and semi-arid regions of the world and India. Date palm fruit is a nutritious one having high calorific value (3,150 calories/kg) and is a good source of iron and potassium (Al-Shahib and Marshall 2002, 2003; Elleuch et al 2008). It is distributed in Middle East and Arab countries, North Africa and South Sahel areas of East and South Africa, Asia and even in parts of Europe and USA. In India, Thar desert fully and partially meets the climatic conditions of date palm. Thar covers maximum area in Rajasthan followed by Gujarat, Haryana and Punjab.

Date palm cultivation has been recorded for thousands of years and over that time selection procedures have developed for more than 3,000 known varieties of date palm around the world (Johnson 2011). Date fruits possess important nutritional and medicinal values, are used as a part of religious practice and are consumed by people all over the world (Vayalil 2012).

In these recent years, the diversity of the date palm has declined due to its over-exploitation. The production and utilization of the date fruits vary with the countrys' individual demand. There are various factors which affect or lower the production of date palm like major pests and diseases, salinity, drought, poor harvest and postharvest practices (Al-Khayri et al 2015a, 2015b) but the major element is heterozygous nature. It 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 its life span is only about 20-30 (Zaid et al 2011) and their survival rate is also low (Abahmane 2011). To meet the industrial high demand it is necessary to propagate the date palm on mass level which is only possible through in vitro propagation of it. It produces disease free, high yielding and true to type plants.

Since from the early reports of date palm in vitro regeneration (Tisserat 1979, 1982) many researchers described different approaches to achieve

date palm micropropagation such as somatic embryogenesis (Al-Khayri 2003, 2005; Fki et al 2003, 2011; Mazri et al 2017, Naik and Al-Khayri 2016, Roshanfekrrad et al 2017) and organogenesis (Bekheet 2013, Jazinizadeh et al 2015, Khan and Bi 2012, Khierallah and Bader 2007, Meziani et al 2015, 2016). In addition for providing a means for rapid clonal propagation of date palm (Khierallah and Bader 2007) tissue culture techniques can be utilized for the production of synthetic seeds (Bekheet et al 2002), cell suspension culture (Othmani et al 2009), cryopreservation (Fki et al 2013), somaclonal variation introduced stress tolerant, disease resistance and with high quality fruits (El Hadrami and El Hadrami 2009, Jain 2001).

The aim of present article is to give a status and summarize the literature of micropropagation through organogenesis and somatic embryogenesis work in date palm throughout the world.

Planting material and disinfection

In the beginning era of date palm tissue culture researchers used different kinds of explants such as embryos, immature fruits, roots, leaf petioles, lateral buds, shoot tips, pieces of stem and rachilla tissue (Sharma et al 1986, Tisserat 1979).

Shoot tips obtained from offshoots are the most used explants for somatic embryogenesis and organogenesis. The use of shoot tip explants has resulted in successful regeneration in many date palm genotypes such as Iklane and Jihel (El Hadrami et al 1995), Khanizi and Mordarsing (Esharaghi et al 2005), Khasab and Nabout (Al-Khayri 2010), Barhee, Zardai, Khalasah, Muzati, Shishi and Zart (Aslam et al 2011). But shoot tip requires the sacrifice of offshoots. Many researchers used inflorescence as explant (Abul-Soad 2012, Bhaskaran and Smith 1992) but there responsiveness rate was very low. Similarly other explants' responsiveness was also not successful.

Cultivars response towards micropropagation also varies (Bhati et al 2014). Zygotic embryos derived from green fruits can be used to induce somatic embryogenesis in date palm (Reynolds and Murashige 1979). Pinker et al (2009) also used zygotic embryos to induce somatic embryogenesis in cultivars Zahdi, Khistawi, Asabe Elarous and Barban. Nevertheless such explants are not appropriate for true-to-type propagation.

Different methods have been used for explants' disinfection like sodium hypochlorite, mercuric chloride and sodium hydroxide (Al-Khayri 2010, Khierallah et al 2015, Bhati et al 2014, Naik and Al-Khayri 2016). In addition to it many researchers used antioxidants to prevent oxidation activity of phenolic compounds present in date palm. Antioxidants such as ascorbic acid, citric acid, polyvinylpyrrolidone (PVP), anhydrous caffeine and/or sodium diethyldithiocarbonate have been widely used during disinfection in order to reduce explant browning (Al-Khayri 2010, Aslam et al 2011, Khierallah et al 2015).

Organogenesis

Organogenesis is the process by which explants undergo changes which lead to the formation of a unipolar structure (shoot or root primordium) with a vascular connection to the parent tissues (Thrope 1993). In the case of date palm the purpose of this technique is the formation of adventitious buds directly on the explant without the callus formation step. The regeneration of date palm plantlets through organogenesis was achieved by many countries but for commercial production the multiplication rate is very low. This process involves initiation of meristematic shoot buds, shoot multiplication, shoot elongation and rooting (Loutfi and El Hadrami 2005).

Initiation of meristematic shoot buds: Most of the meristematic shoot bud initiation from date palm cultivars has been established from the internal face of young bases taken from offshoots (Balal and El-Deep 1997, Mater 1987), petiole of female date palm and male inflorescence. However shoot tip is also used for in vitro plantlet generation to avoid the contamination (Tisserat 1982). Meristematic bud induction takes 5-6 months under dark conditions to avoid browning and phenolic compounds having temperature $25 \pm 2^\circ\text{C}$. Meristematic induction from explants depends on several factors like hormone combination, explants preparation, light intensity, mother plant genotype, age and stage of offshoots. Various researchers used different combinations of auxin and cytokinin ratio with MS media with full and half strength. The hormones: auxins like 2,4-D (Bhati et al 2014), NOA (Beauchesne et al 1986), IBA (Balal and El-Deep 1997, Mater 1987), NAA (Asemota et al 2007) and cytokinins like 2-iP, kinetin, BA (Balal and El-Deep 1997, Mater 1987) combinations and concentrations were used. The variations were observed in same medium by different cultivars response. Induction requires the low light intensity (1000-1400 lux) with temperature $27 \pm 2^\circ\text{C}$.

and sub-culturing once in a month. According to Al-Khateeb (2006) low PGRs concentrations promote the formation of buds while high concentrations induce abnormal growth without bud formation. Salama (1990) reported that the explants taken between October and February show the highest bud formation rate whereas Zaid et al (2011) reported that the best period for the in vitro culturing of offshoot-derived explants is from the onset of flowering.

Shoot multiplication: Shoot bud multiplication in date palm is affected by especially the basal formulation of the culture medium, the genotype and PGRs. Abahmane (2011) mentioned that the main basal formulation used is MS at full or half-strength supplemented with PGRs at low concentrations as compared with the bud initiation stage. Zaid et al (2011) reported that for shoot bud multiplication NAA, NOA, IAA, BAP and kinetin might be used at 0.5-5 mg/l. Beauchesne et al (1986) suggested half-strength MS medium supplemented with 2 mg/l NOA, 1 mg/l NAA, IAA 1 mg/l, BAP 0.5 mg/l, 2iP 1 mg/l and kinetin 1-5 mg/l. For cultivar Khalas, Aslam et al (2011) used 7.84 μ M BAP for high shoot bud multiplication. Khierallah and Bader (2007) recommended MS medium with a combination of 1 mg/l NAA, 1 mg/l NOA, 4 mg/l 2iP and 2 mg/l BAP for date palm cv Maktoom while Khan and Bi (2012) found that MS medium containing 0.5 mg/l BAP and 0.5 mg/l kinetin yields the highest number of shoots per explant in cv Dhakki.

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 that 1 mg/l NAA induces better and optimum rooting at the same concentration of IAA or IBA. Meziani et al (2015) reported that cv Medhjool shoots grew to 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.

Somatic embryogenesis

Somatic embryogenesis is a developmental process where a plant somatic cell can dedifferentiate to a totipotent embryonic stem cell that has the ability to give rise to an embryo under appropriate conditions.

This new embryo can further develop into a whole plant (Guan et al 2016). The somatic embryogenesis involves the phases induction of callus from explants, somatic embryo development, maturation and germination.

Induction of callus from explants: This phase involves the conversion of differentiated tissue to dedifferentiated tissue which is a group of cells known as callus. Various explants have been used to initiate callus. The explants used were shoot tip (Bhati et al 2014, Mater 1987, Tisserat 1979, Yadav et al 2001, Asemota et al 2008), inflorescence (Tisserat 1979, Fki et al 2003, Abul-Soad 2012), mature and immature zygotic embryos (Bekheet et al 2002), leaf segments and meristematic tissues (Asemota et al 2007), axillary buds (Jain 2001) and roots (Roshanfekr et al 2017). The most frequently explants used are apical shoot tips for good results (Jain 2001).

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 concentration (Al-Khayri 2005, 2010; Eshraghi et al 2005, Naik and Al-Khayri 2016). Fki et al (2011) reported that high concentrations of 2,4-D cause somaclonal variations. Many researchers applied low concentration of 2,4-D such as 1.5, 5 and 10 mg/l to induce somatic embryo (Aslam et al 2011, El Hadrami et al 1995, Othmani et al 2009). Depending on the genotype, the somatic embryo formation period also varies from a few to several months (Eshraghi et al 2005, Hassan and Taha 2012, Othmani et al 2009). 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.

Somatic embryo development and maturation

Development of somatic embryo from pro-embryogenic calli is the important and crucial phase in somatic embryogenesis. The large scale multiplication of somatic embryos decides its commercialization. Many factors are involved in somatic embryo development and maturation like hormone composition, media, cultural conditions, sub-culturing and genotypes. Many researchers used MS media (Eshraghi et al 2005, Hassan and Taha 2012, Fki et al 2003, Abul-Soad 2012,

Aslam et al 2011, El Hadrami et al 1995) as a base with additives whereas many used hormone-free media for somatic embryo development and maturation (Al-Khayri 2003, Eke et al 2005, Othmani et al 2009).

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

Somatic embryo germination: Mature somatic embryos can germinate on the same medium used for its development and maturation. The germination can also occur on hormone-free medium with very low success rate. It is also reported that hormone NAA (Fki et al 2003, Eke et al 2005), IBA (Al-Khayri 2003), 2,4-D (Othmani et al 2009) and BA (Fki et al 2003) induce germination. Ibrahim et al (2012) reported that the germination frequency of somatic embryos varies with the genotype used.

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