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Date palm (*Phoenix dactylifera* L.) biotechnology: a mini-review

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Abstract

Date palm (*Phoenix dactylifera* L.) is the source of fruit and palm oil, which is broadly used in the food industry. The regeneration of recalcitrant date palm genotypes through somatic embryogenesis or organogenesis is difficult to achieve. Micropropagation, however, provides a way to obtain a sufficient number of true-to-type elite, healthy (disease-free) and vigorous plants to satisfy local and international markets. This mini-review highlights some of the main achievements in the tissue culture of date palm. In particular, explant selection and disinfection, shoot induction, multiplication and elongation, root induction and acclimatization are highlighted. In addition to using the *in vitro* tissue culture as the basis for an *in vitro* gene banking, a mid-term low-temperature storage of germplasm is possible by careful selection of the osmotic agent. A long-term storage of date palm using cryopreservation, with or without synthetic seeds is also possible. Molecular markers, as well as sex-specific markers, have been useful to discriminate germplasms and to identify somaclonal variants derived from tissue cultures. The genetic transformation of date palm can be achieved by either particle bombardment or *Agrobacterium*-based protocols. Thus, biotechnology is an important element of date palm germplasm development and its sustainable improvement.

Key words: acclimatization, adventitious shoots, explant, organogenesis, rooting, shoot multiplication

Introduction: economic importance

Date palm (*Phoenix dactylifera* L.) is an important fruit crop that grows in arid, semi-arid and tropical areas in the United States, Europe, Africa and the Middle East with an estimated 150 million trees planted globally (Al-Khalifah et al., 2012). Pakistan was ranked as the sixth largest producer of date palm after Egypt, Saudi Arabia, Iran, Iraq and the United Arab Emirates, which collectively produce about 90% of the world's total production (Aslam and Khan, 2009). Date palm grows in all provinces of Pakistan in an area of 91,355 ha that produces about 535,000 tons of dry weight, with Baluchistan province being the largest producer, followed by Sindh, Punjab and Khyber Pakhtun Khwa (Economic Survey of Pakistan, 2015). Date palm oil, which markets for about US$ 800 a ton, is the cheapest known vegetable oil in the US$ 44 billion global market (Landini and Navach, 2017), made news recently after a potentially carcinogenic contaminant, glycidol, was detected in palm oil by the European Food Safety Authority in 2016 (EFSA, 2016).

Micropropagation: an alternative to sexual reproduction and asexual propagation

Date palm grows readily from seeds that are mostly produced by male plants, which can be distinguished at the flowering stage, and require 6-10 years to reach the fruiting stage (Al-Khalifa, 2000; Othmani et al., 2009). Suckers are also easy to propagate, but are not readily available due to their limited number present on a single plant, whereas propagation by offshoots is a time-consuming process. Micropropagation, the vegetative pro-

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pagation of plant germplasm in a sterile environment that uses artificially created growth conditions, utilizes the *in vitro* tissue culture to mass produce target organs or plants that are genetically identical to the mother plant. It is more efficient than traditional forms of vegetative propagation because it reduces the time required to obtain true-to-type plantlets with genetic fidelity that are disease-free and in quantities that are able to satisfy the demands of farmers and large-scale growers (Abahmane, 2011; Bekheet, 2013). In a bid to establish a production system for the Pakistani date palm market based on micropropagation, this mini-review focuses on the key aspects of date palm tissue culture focusing on selected studies to exemplify core concepts and tissue culture steps and is not meant to be an exhaustive description of the date palm literature. A successful tissue culture procedure involves the selection of a suitable explant, an effective disinfection procedure, and *in vitro* protocols that will result in the desired outcome such as plantlet production, which can be particularly difficult for tree species (Teixeira da Silva et al., 2016a). Applied biotechnologies such as genetic transformation require a well-established tissue culture protocol.

Explant selection

The choice of an explant and its disinfection process can affect the success of micropropagation (Teixeira da Silva et al., 2016b), including the date palm. Shoot tips and adventitious shoots in lateral buds contain more meristematic tissues than other organs, and therefore are frequently used in date palm tissue cultures (Rashid and Quraishi, 1994; Mazri and Meziani, 2015). A successful regeneration of many date palm genotypes has been achieved when shoot tips were used as explants: "Jihel" and "Iklane" (El Hadrami et al., 1995), "Mordarsing" and "Khanizi" (Eshraghi et al., 2005), "Nabout" and "Khasab" (Al-Khayri, 2010), and "Khalasah", "Zardai", "Barhee", "Zart", "Muzati" and "Shishi" (Aslam et al., 2011). Date palm tissue culturing can also be achieved by using explants derived from inflorescences, as was reported for "Barhee" (Bhaskaran and Smith, 1992) and "Gulistan" (Abul-Soad and Mahdi, 2010). Reynolds and Murashige (1979) induced somatic embryogenesis from zygotic embryos obtained from green fruits that were harvested 2-3 months after pollination. Pinker et al. (2009) also used zygotic embryos to induce somatic embryogenesis in "Khistawi", "Zahdi", "Barban", "Asabe" and "Elarous". Somatic embryos are useful for the micropropagation and large-scale production of date palm plants and may also be used to obtain true-to-type genotypes.

Explant disinfection and preparation

The main disinfecting agent that has been used for shoot tips is sodium hypochlorite (NaOCl) at a concentration range from 5% to 25% (Jatoi et al., 2015) and for spikelets, mercuric chloride ($HgCl_2$) at 0.1% concentration (Boufis et al., 2014). In addition, the use of antioxidants such as 150 mg/l ascorbic acid (for 30 min), 4% polyvinylpyrrolidone (Aslam and Khan, 2009), citric acid at a concentration of 150 mg/l with 150 mg/l ascorbic acid (soaked overnight) (Rashid and Quraishi, 1994), or anhydrous caffeine are widely used during shoot tip explant disinfection (Khierallah et al., 2015). Khan and Tabassum (2012) used an effective protocol to eliminate infection from shoot tips: treatment with 5% (w/v) NaOCl containing one drop of a surfactant (Tween-20/100 ml), stirred gently for 30 min, rinsed three times in sterile distilled water (SDW; 5 min each rinse), surface disinfested with 0.2% (w/v) $HgCl_2$ for 10 min and then rinsed three times with SDW. Leaf primordia of 6 cm long shoot tips were removed and used as explants and 2 cm long shoot tips with 2-4 intact primordial leaves also served as explants. A similar protocol has been used by Othmani et al. (2009) for leaves adjacent to the apex of axillary shoots of cv. "Boufeggous". Fki et al. (2003) first washed young leaves with tap water, and surface sterilized them with 0.01% $HgCl_2$ for 1 h, rinsed three times with SDW, then cut them into 5-10, 10-15 and 15-20 mm long explants. Ledo et al. (2002) described a disinfection procedure for zygotic embryos from mature (wine-colored, ~2.17 g) and immature (green, ~1.68 g) fruits from "açai" palm, an *Euterpe* species of palm tree cultivated for its fruit. After being washed under running tap water, fruits were immersed in 40°C water, and seeds were excised on a laminar flow bench, immersed in 70% ethanol for 2 min, then in 2% NaOCl for 20 min under agitation, and finally washed four times with SDW.

Shoot organogenesis

Shoot organogenesis in date palm aims to induce the growth of adventitious shoot buds directly on explants without the formation of callus, and involves three steps:

induction, multiplication and elongation, followed by rooting, as discussed in the following sections.

Shoot induction

Rad et al. (2015) induced shoot growth from shoot tips cultured in Murashige and Skoog's (1962) medium (MS). Regeneration was dependent on the type of plant growth regulator (PGR) present in a medium and on the plant's genotype. The most efficient treatment included 0.5 mg/l 1-naphthaleneacetic acid (NAA), 0.5 mg/l 2-naphthoxyacetic acid (NOA), 1 mg/l N^6 (2-isopentyl) adenine (2iP) and 1 mg/l 6-benzyladenine (BA). Interestingly, in these conditions, "Medjool" produced more vegetative buds (2.62) than "Mazafati". Khierallah and Bader (2007) could not induce the growth of shoot buds in an MS medium in the absence of cytokinins. Most shoot buds, however, formed after 16 weeks in a medium supplemented with 1.0 mg/l BA, 2.0 mg/l 2iP, 1.0 mg/l NAA and 1.0 mg/l NOA from "Maktoom" shoot tips. Khan and Bibi (2012) found that 0.5 mg/l BA, 0.5 mg/l kinetin (Kin) and 1 mg/l NAA induced the highest number of shoots (7.95 shoots/explant) from shoot buds in "Dhakki" after 6 weeks and fewer shoots (2.90 shoots/explant) in a PGR-free MS medium. Jazinizadeh et al. (2015) obtained the highest number of shoots per explant (6.7) in an MS medium with 1.5 mg/l 2iP, 1 mg/l BA and 1 mg/l NAA in "Barhee". Al Khateeb and Alturki (2014) cultured three cultivars ("Sukry", "Medjool", and "Reziz") in a modified liquid and solid MS media, both supplemented with 0.3 mg/l 2iP and 0.3 mg/l BA. For all the three cultivars tested, a higher number of shoots formed in a solid than in a liquid medium (9, 6.5 and 9 vs 6, 5 and 5, respectively). Meziani et al. (2015) found that after 3 months of culturing in a half-strength MS (1/2 MS) medium with 1 mg/l PVP, 0.5 mg/l NOA, 0.2 mg/l indole-3-acetic acid (IAA), 0.4 mg/l Kin and 0.4 mg/l 2iP, a 12.8-fold proliferation rate in shoots was achieved for "Mejhoul", with low levels of hyperhydricity and tissue oxidation (20% each). Also, using "Mejhoul", Mazri et al. (2016) tested the effect of different concentrations of sodium, calcium and potassium salts, noting that a maximum of 18.7 shoots/explant formed in a modified MS basal medium containing 825 mg/l NH_4NO_3, 1900 mg/l KNO_3, 220 mg/l $CaCl_2 \cdot H_2O$, 170 mg/l KH_2PO_4, 370 mg/l $MgSO_4 \cdot 7H_2O$, 1 g/l L-glutamine, 2 g/l *myo*-inositol and 30 g/l sucrose.

Concluding, for shoot induction, an MS medium supplemented with BA or 2iP resulted in the greatest explant (mainly shoot tips) receptivity.

Shoot multiplication

Silver nitrate ($AgNO_3$) is a potent inhibitor of ethylene, which suppresses shoot organogenesis, and therefore plays a major role in a somatic embryogenesis, and shoot and root formation (Bais et al., 2000). When Bekheet (2013) combined 1.5 or 10 mg/l $AgNO_3$ with 5 mg/l 2iP, "Zaghlool" shoot buds could be multiplied compared with a medium without $AgNO_3$. $AgNO_3$ at 1 mg/l induced the formation of 5.5 shoot buds/explant, 5 mg/l resulted in the formation of 6.8 shoot buds/explant, on average 3.3 cm long, whereas 10 mg/l induced the production of 6.2 shoot buds/explant.

Bader and Khierallah (2009) noted that the addition of silver thiosulphate (STS) and glutamine improved the multiplication of "Barhi" and "Maktom" shoot tips when added at 90 μM and 0.7 mM concentrations to an MS medium supplemented with 2.0 mg/l 2iP, 1.0 mg/l BA, 1.0 mg/l NAA and 1.0 mg/l NOA, forming 8.1 and 9.4 shoot buds/explant, respectively, after 16 weeks. When these buds were subcultured in a liquid MS medium shake culture with 90 μM STS and 1.4 mM glutamine, 12.5 and 14.7, shoots formed in "Barhi" and "Maktom", respectively. Using a simpler solution, Aslam and Khan (2009) reported the highest shoot multiplication for "Khalas" when 7.84 mM BA was used. The liquid medium was superior to the solid medium in the shoot multiplication of "Sukry", "Medjool" and "Reziz" (number of shoots = 2.5, 6.8 and 4.0 vs 1.8, 6.0 and 3.8 for the liquid vs solid medium, respectively) (Al Khateeb and Alturki, 2014).

Shoot multiplication was best achieved in plants grown on a BA-based MS medium, but could be improved when $AgNO_3$ or STS was incorporated.

Shoot elongation

Beauchesne et al. (1986) used 1/2 MS medium with 1 mg/l NAA, 0.5 mg/l BA, 0.5 mg/l Kin and 1-3 mg/l gibberellic acid (GA_3) to elongate shoots. El Sharabasy et al. (2001) noted that 0.1 mg/l NAA had a better effect on shoot elongation than IBA or IAA. Mazri (2012) observed maximum shoot elongation (11.3 cm) in "Najda" after three months in an MS medium supplemented with 0.5 mg/l BA and 0.5 mg/l IBA relative to a PGR-free

medium. Khierallah and Bader (2007) also found that GA_3 had a positive effect on shoot elongation in "Maktoom": MS medium supplemented with 0.5 mg/l GA_3 and 0.1 mg/l NAA enhanced the shoot length (5.3 cm), producing the longest shoots (7.42 cm), and inhibited root formation. The shortest shoots (2.23 cm) were obtained in plants grown on a GA_3-free medium. Khan and Bibi (2012) found that MS medium supplemented with 0.5 mg/l BA, 0.5 mg/l Kin and 1 mg/l NAA induced shoots with a suitable length (8 cm) in "Dhakki" after 6 weeks, but stunted shoots (7.5 cm) formed in a PGR-free MS medium. Jazinizadeh et al. (2015) reported an optimum shoot length (8.9 cm) in an MS medium supplemented with 1.5 mg/l 2iP, 1 mg/l BA and 1 mg/l NAA in "Barhee". Elghayaty et al. (2016) found that GA_3 significantly elongated 'Hayani' shoots in an MS medium with 1.0 mg/l NAA (4.5 cm vs 1.5 cm with no GA_3) after 8 weeks. As for shoot multiplication, Al Khateeb and Alturki (2014) found that a liquid medium was superior to a solid medium in the elongation of "Sukry", "Medjool" and "Reziz" shoots (7.8, 8.5 and 9.3 cm vs 6.0, 7.0 and 5.7 cm for a liquid vs solid medium, respectively).

To summarize, GA_3 is necessary for shoot elongation when an MS medium is used.

Reducing hyperhydricity and tissue browning

Hammerschlag (1982) was the first to study a physiological disorder called hyperhydricity as a serious factor inhibiting peach organogenesis, noting that it negatively affected shoot bud proliferation and culture vigor. In date palm "Barhee", hyperhydricity induced the development of tissue with deficient lignification, causing water to accumulate in cultured tissues (Al Khateeb, 2008). Low levels of the gelling agent and high levels of humidity typically cause hyperhydricity; however, an excess of ammonium ions or cytokinins in the culture medium can also increase the process (Bouguerfaoui and Zaid, 1993). Meziani et al. (2015) found that the use of 0.4 mg/l 2iP alone or with 0.4 mg/l Kin could decrease hyperhydricity by 20% in "Mejhoul". Mazri and Meziani (2013) observed low rates of hyperhydricity in "Najda" when Kin concentration did not exceed 0.5 mg/l, although the level of hyperhydricity can vary depending on the cytokinin used (Mazri, 2015).

Al Khateeb (2008) reported that addition of activated charcoal (AC) reduced tissue browning. Zaid et al. (2011) reported that the incidence of tissue browning decreased when date palm axillary shoots were presoaked in an antioxidant solution (150 mg/l citric acid and 100 mg/l ascorbic acid). The use of small explants and frequent subculturing reduce tissue browning in date palm micropropagation. Meziani et al. (2015) noted that a PGR-free medium accentuated tissue browning (95%) in "Mejhoul", whereas the lowest tissue browning (20%) was observed in a medium consisting of 0.2 mg/l NOAA, 0.2 mg/l IAA, 0.4 mg/l Kin and 0.4 mg/l 2iP, when the shoot tips were used as explants.

In date palm, hyperhydricity can thus be controlled by varying the levels of PGRs in the medium.

Rooting

Shaheen (1990) showed that root formation is a crucial stage in date palm micropropagation, as it determines the subsequent success of free-living plants and the continuous formation of lateral roots needed to establish a root system and providing plants with a path of developmental plasticity in changing soil conditions. Khierallah and Bader (2007) studied the response of the NAA concentration (MS medium with 1.0 mg/l) on the rooting percentage, the average number of roots per shoot and root length, obtaining a maximum (90% rooting, 5.4 roots/shoot and 8 cm long roots, respectively) in "Maktoom" after 8 weeks of growth, when the shoots had been transferred to an NAA containing medium. Bekheet (2013) grew "Zaghlool" shoots in an auxin-containing medium to assess the individual effect of IAA, IBA and NAA on the rooting of *in vitro* plantlets. The rooting was achieved in an MS medium with 1 mg/l of IAA, IBA or NAA, but no roots formed on the PGR-free MS medium. NAA was more efficient than IAA and IBA, and 85% of shoots rooted within three weeks. An MS medium supplemented with 1 mg/l IBA produced roots in 40% of shoots, 20% with 1 mg/l IAA or no roots in a PGR-free medium. Al-Kaabi et al. (2001) induced roots with 1 or 2 mg/l NAA. Abo El Nil (1986) rooted plantlets in an MS medium containing 0.2 mg/l NAA. Belal and El-Deeb (1997) induced roots of "Zaghlool" in an MS medium supplemented with 3 mg/l NAA and 0.5 mg/l Kin. Sidky et al. (2007) noted stronger plantlets with a more rapid root formation in 1/2 MS to which 0.1 mg/l NAA, 1 g/l AC, 40 or 50 g/l sucrose, and 4 mg/l paclobutrazol had been added.

Hassan et al. (2008) noted that the sucrose concentration affected root formation of "Bartamouda" after 12 weeks. The average root number and root length increased as the sucrose concentration increased: 2.3 roots/explant of 4.8 cm length in the presence of 15 g/l vs 2.8 roots/explant of 6.1 cm length at 45 g/l. The use of 3/4 MS medium induced 3.0 roots/explant of 4.9 cm length relative to 1/2 MS medium, which induced 1.8 roots/explant of 5.8 cm length. The interaction between abscisic acid (ABA) and sucrose was also assessed by Hassan et al. (2008), who obtained the highest number of roots/explant (2.8) using 1.0 mg/l ABA and 45 g/l sucrose versus the longest roots in 1/2 MS medium supplemented with 15 g/l sucrose, but without ABA (2.1 roots/explant). In addition, 3/4 MS medium containing 0.25 mg/l ABA induced 4.1 roots/explant, but the longest roots were obtained in 1/2 MS medium without ABA (1.0 root/explant). Jatoi et al. (2015) reported best rooting in 1/4 MS medium with 0.1 mg/l NAA but without AC, in Pakistani cultivars "Gajar", "Kashoowari", and "Dedhi". Elghayaty et al. (2016) studied the rooting in "Hayani" after 8 weeks, noting that a combination of 1.0 mg/l each of IBA and NAA in MS medium significantly enhanced the number of root formations and root length when one shoot was cultured per test tube. Al Khateeb and Alturki (2014) observed a better root formation and elongation in a solid medium than in a liquid medium: 6.2 roots and 3.8 cm, 11.0 roots and 5.5 cm, and 3.8 roots and 4.5 cm for "Sukry", "Medjool" and "Reziz" in a solid medium (vs 6.0 roots and 5.0 cm, 9.8 roots and 4.8 cm, and 6.3 roots and 4.5 cm, respectively, in a liquid medium). Meziani et al. (2015) observed a precocious rooting in "Mejhoul" when shoots were cultured in 1/2 MS medium with 0.4 mg/l NOA, 0.4 mg/l Kin and 0.4 mg/l 2iP, whereas a culture carried out in the dark improved root formation (95% vs 80% in light) and root number per shoot (6.9 vs 4.2 in light).

As demonstrated above, rooting is affected by the concentration of sucrose and is best achieved when NAA is used.

Acclimatization

Abul-Soad (2011) and Jatoi et al. (2015) described detailed procedures on how to acclimatize date palm plants derived from tissue cultures. The roots of plantlets removed from test tubes were washed in SDW and then immersed in a 0.5% (w/v) fungicide for 5 min. The plants were potted (250 mm diameter pots) into a substrate of sand and peat moss (1:1, v/v) and kept under natural light and 90-95% relative humidity, watered weekly, covered by a white polyethylene sheet for one week and gradually uncovered to allow plants to acclimatize to greenhouse conditions. Othmani et al. (2009a) observed 60% and 80% survival in "Boufeggous" and "Deglet Nour", respectively. 5-Aminolevulinic acid-based fertilizer (Awad, 2008), or ammonium nitrate combined with GA_3 (Darwesh et al., 2011) promoted date palm plantlet acclimatization. Khierallah and Bader (2007) observed 85% survival of plantlets after 3 months of growth when potted in a mixture of peat moss and perlite (2:1). Sharma et al. (1990) described a successful acclimatization protocol for "Khadrawi" and "Medzool". The regenerated plants were washed for 30 min under running tap water and then rinsed once or twice with distilled water. The plants were then soaked for 30 sec in a fungicide (0.5% Bavistin), then transferred to a field soil, sand and farm yard manure (1:1:1) mixture in 15-cm diameter earthen pots. They were watered with 1/10 MS salts, covered with a beaker and then exposed to direct sun light, for a few hours in winter and 30-60 min/day in summer. Plants were kept under laboratory conditions for 15 days and then transferred to a shaded place. After removing the beaker, new leaves formed after 1-2 months.

General procedures for *in vitro* plantlet acclimatization can be successfully applied to date palm (Hazarika et al., 2006).

Other biotechnological approaches for conserving date palm germplasms

Low-temperature storage and cryopreservation

Date palm germplasms can be maintained under ideal *in vitro* conditions. Consequently, tissue cultures serve only as a temporary storage method, as an *in vitro*-based gene bank, which allows for immediate multiplication and distribution. Low temperature storage (typically 4-10 °C) as well as cryopreservation (between −79 using solid CO_2 or −196 °C using liquid N_2) are other techniques used to store date palm germplasms. Bekheet et al. (2001b) and Hassan (2002) established protocols for *in vitro* preservation of Egyptian date palm by adding osmotic agents (pyridoxine or nicotinic acid) to tissue cul-

ture media. Bekheet et al. (2007) and El-Dawayati (2008) developed cryopreservation protocols for date palms, which allowed for long-term preservation. To achieve this, undifferentiated tissues (i.e., callus) and somatic embryos were cryopreserved and from them plantlets could be regenerated. However, details of the protocols were not provided. Date palm germplasms have also been preserved in the form of synthetic seeds (Sharma et al., 2013). Bekheet et al. (2005) encapsulated somatic embryos derived from shoot tips in sodium alginate, stored them at 5°C for 12 months and then regenerated the plantlets, thus allowing for an appropriate conservation strategy for date palm germplasms.

Molecular characterization

Molecular techniques allow to analyze, characterize and discriminate date palm germplasms and to assess variations among them. Using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers, Moghaieb et al. (2010) identified an unknown date palm cultivar grown in Egypt. RAPD and amplified fragment length polymorphism (AFLP) markers have been useful to identify somaclonal variations in the tissue culture-derived Egyptian date palm (Saker et al., 2000; El-Assar et al., 2005; Moghaieb et al., 2011). Five ISSR anchored primers were used to assess the genetic stability of micropropagated date palms: the micropropagated plantlets of the var. "Karama" showed a high similarity to one another and with the mother plant but a dissimilarity was detected among several samples, possibly due to the somaclonal variation that occurred during micropropagation (Abd-Alla, 2010). Ahmed et al. (2006) used a differential display PCR (DD-PCR) and an RAPD analysis to identify three unknown Egyptian male date palm from 4 known females (cvs. "Sakkoty", "Malkabi", "Bartamoda" and "Dagana"). RAPD only used five 10-mer primers and one 20-mer primer. The highest percentage similarities were observed in males 1, 2, 3 and 4, with 91.2, 86.5, 81.2 and 79.0% genetic similarity to "Malkabi", "Bartamoda", "Sakkoty" and "Dagana", respectively, but a differential display using the 18S rRNA gene showed no difference between the tested females and males. Al-Ameri et al. (2016) screened 200 ISSR primers among 10 male and 10 female date palm plants to identify a putative sex-specific markers, among which only two primers (IS_A02 and IS_A71) were associated with sex. The IS_A02 primer produced a unique band of 390 bp in size that was found in all female plants, but was absent in all tested male plants. The IS_A71 primer produced a unique band (380 bp) that was found in all male plants, but was absent from all female plants. Subsequently, these specific fragments were excised from gels, purified and sequenced to develop sequence-specific markers. Such an approach allows for the use of specific DNA markers in breeding programs that need to determine sex at the early stages of the breeding process such as at the early seedling stage.

Proteomics techniques can also be used to profile the data palm germplasms (Rasool et al., 2015).

Genetic improvement by transformation

Bekheet (2013) described genetic transformation as a way to increase yield and induce resistance to biotic and abiotic stresses in date palm plants. Particle bombardment was used to transform the Egyptian date palm "Sewi" (Saker et al., 2007). In their protocol, the embryogenic callus induced from shoot tips was bombarded with gold particles covered with a construct harboring the cholesterol oxidase gene, intended to render a plant insect resistant. The *Agrobacterium*-mediated transformation of the *GUS* gene into the embryogenic callus (obtained from shoot tip explants) was achieved by Saker et al. (2009). El-Rakshy et al. (2011) transformed the date palm with the endotoxin *Cry3Aa* gene, which relies on the toxicity of *Bacillus thuringiensis* proteins to control a pest, red palm weevil. The yield and fruit quality of these palms did not differ significantly from those of non-transgenic plants. Jain (2012) developed a dwarf date palm hybrid by a zygotic embryo rescue after interspecific hybridization using *P. dactylifera* × *P. pusilla*.

Conclusions

Date palm has been cultured from antiquity for its oil and fruits. Currently, there are several protocols that allow the clonal production of date palms *in vitro* and a long-term conservation through cryopreservation. The existence of a genetic map (Mathew et al., 2014) makes it possible to choose useful genes, clone them and transform them into date palms to increase the yield or to improve agronomic qualities. Targeted genetic improvement programs will allow increasing the quantitative and specific secondary metabolites (Hamad et al., 2015) *in planta*. The *in vitro* culture of date palm is well establi-

shed, and shoot tips are primarily used as explants for micropropagation. BA or 2iP are good inducers of shoots. Shoot multiplication best occurs when $AgNO_3$ or STS is incorporated into a BA-containing MS-based medium. GA_3 is an effective shoot elongation agent, whereas NAA is the most effective for rooting *in vitro*-induced shoots. The Hazarika et al. (2006) protocol can be successfully applied for the acclimatization of *in vitro* plantlets. Date palm germplasms can be preserved by storing them in low temperatures or by cryopreservation, whereas molecular markers serve as useful tools for identifying and discriminating germplasms. The embryogenic callus obtained from shoot tips (Tisserat, 1982), axillary or terminal, is useful for genetic transformation.

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