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MINI-REVIEW

The retrospect and prospect of the applications of biotechnology in *Phoenix dactylifera* L.

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Abstract
Date palm (*Phoenix dactylifera* L.) is one of the most important fruit trees that contribute a major part to the economy of Middle East and North African countries. It is quintessentially called "tree of life" owing to its resilience to adverse climatic conditions, along with manifold nutritional-cum-medicinal attributes that comes from its fruits and other plant parts. Being a tree with such immense utility, it has gained substantial attention of tree breeders for its genetic advancement via in vitro biotechnological interventions. Herein, an extensive review of biotechnological research advances in date palm has been consolidated as one of the major research achievements during the past two decades. This article compares the different biotechnological techniques used in this species such as: tissue and organ culture, bioreactor-mediated large-scale propagation, cell suspension culture, embryogenic culture, protoplast culture, conservation (for short- and long-term) of germplasms, in vitro mutagenesis, in vitro selection against biotic and abiotic stresses, secondary metabolite production in vitro, and genetic transformation. This review provides an insight on crop improvement and breeding programs for improved yield and quality fruits; besides, it would undeniably facilitate the tissue culture-based research on date palm for accelerated propagation and enhanced production of quality planting materials, along with conservation and exchange of germplasms, and genetic engineering. In addition, the unexplored research methodologies and major bottlenecks identified in this review should be contemplated on in near future.

Keywords Clonal fidelity · Date palm · Genetic transformation · Germplasm conservation · Micropropagation · Secondary metabolite · Somatic embryogenesis

Introduction

Phoenix dactylifera L., universally identified as date palm, is acknowledged to be one of the most ancient tree species in the world. This majestic crop is cultivated in the Middle East and in some South African countries for more than 5000 years (Zohary and Hopf 2000). Date palm tree is perennial and diploid ($2n = 2\times = 36$) in nature. It belongs to Spermatophyta-Angiospermae-Monocotyledonae-Arecales-Arecaceae families that possess ~200 genera, comprising of more than 2500 species. *Dactylifera* species of date palm belongs to Phoenix genera. The scientific name of the date palm species "*dactylifera*"

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originated from the Greek word "dactylus" meaning "finger," and in Latin the word "ferrous" meaning "bearing"; together, the word "finger-bearing" refers to the plant, producing fruits in clusters. However, the name "*dactylifera*" could have also originated from the Hebrew word "dachel", which describes the fruit's shape. In old saying, the name "Phoenix" refers to the legendary Egyptian bird that had lived for ~ 500 years and possessed the miracle ability to regenerate itself from its own ashes after dying in a show of flames. This phenomenon to some extent resembles the lifecycle of the date palm tree that also can resume its growth even after damage from fire (Popenoe 1938; Al-Alawi et al. 2017). This tree has an important place in Indian mythology and has special spiritual value in religious ceremonies of Muslims, Christians, and Jews. Christians use date palm fronds for the celebration of "Palm Sunday." Since Jewish people consider the date fruit as one of the seven holy fruits, they also use date palm fronds for celebrating the "Feast of Tabernacles." Date fruits also form an important part of the Muslims' diet since it is their ritual to break their fast with its sweet and nourishing flesh during their holy month of fasting. Date palm plays a significant part in improving the socio-economic and environmental status in the areas where it is cultivated, especially in the dry and semi-arid regions, thus forming an integral component of the farming system. Moreover, date palm has a vital role in soil improvement, erosion control, and windbreak. It can also develop efficient agroforestry systems by encouraging sustainable agricultural development in areas affected by salinity and drought, by the way of providing balanced microclimatic conditions within oasis ecosystems (Jain 2012). Date palm ensures the food security and income of the people of the Middle East and North Africa (Johnson 2011; Rajmohan 2011).

Numerous selections and cultivars of date palm have been reported in several countries, specifically where date palm trees are intensively grown. Genetic associations amid the date palm cultivars were studied with the aid of multiple genetic marker systems. In recent studies, the whole genome of date palm tree was re-sequenced to understand the divergence of the fruit tree (Hazzouri et al. 2015; Gros-Balthazard et al. 2016).

An array of research studies on successful in vitro biotechnological interventions in date palm has been reported till date. However, limited numbers of standard review reports have been published on this particular topic so far (Abahmane 2013; Al-Khayri and Naik 2017). The present review article provides an exhaustive report on research achievements on various facets of in vitro propagation via cell, tissue, and organ culture; bioreactor-mediated large-scale propagation; cell suspension culture; protoplast culture; conservation (for short- and long-term) of germplasms; in vitro mutagenesis; in vitro selection against biotic and abiotic stress; secondary metabolite production in vitro; and genetic transformation in date palm, including its distribution, description, industrial use, and conventional propagation as well.

Geographical distribution

Hot and arid areas of the Arabian Peninsula, Middle East, and North Africa are the native regions where date palm trees are extensively grown. Cultivation of date palm has now been spread to countries like Mexico, South America, the USA, Southern Africa, and Australia by means of germplasm exchange (Jain et al. 2011; Tengberg 2012). The particular place of origin of date palm is unknown; however, there is archeological evidence suggesting its cultivation to originate near Mesopotamia (presently, Iraq), furthermore extending to Egypt and also to the Indus Valley and Pakistan (Tengberg 2012). Presently, Egypt is considered as the number one date-producing country, followed by Iran, Algeria, Saudi Arabia, Iraq, Pakistan, Sudan, Oman, UAE, and Tunisia. Around 1.3 million ha area of the earth is covered by > 100 million date palm trees (http://www.fao.org). Middle Eastern countries have contributed the largest area cultivating date palm (833,351 ha), followed by Africa (416,695 ha), out of which 392,200 ha area is cultivated by North Africa alone (Al-Shahib and Marshall 2013). According to the recent statistics (http://www.fao.org), the world production of dates (involving the top 20 date-producing countries) increased from ~ 3.5 million metric tons (in 1990) to ~ 7.5 million metric tons (in 2014), which is almost double the initial amount (Al-Alawi et al. 2017).

Botanical description

Date palm tree, being a monocotyledon, carries several morphological characteristics such as a vertical trunk devoid of cambium layer, without any ramification. Its stem can reach a height up to 30 m at maturity. The trunk (stem) of date palm is brown in color, lignified, with a diameter of 40–50 cm, cylindrical in shape, and of uniform girth all the way up to the crown. The existing bases of all the dried up old fronds (rachis base) cover the entire trunk (Fig. 1a). The upper part of the trunk of a mature tree is called "crown zone" that ranges from 6 to 10 m in length (Fig. 1b, c), with moderately dense (60–80) leaves (depending on agricultural practices) ~ 3–6 m in length. Each leaf has a life span of 3–7 years and around 12 fresh leaves emerge annually (Zaid and de Wet 2002a). The shape of date palm leaves is pinnate in nature. The rachis (the midrib of date palm frond) holds numerous (~ 150) stiff leaflets that are 2 cm wide and 30 cm long. Sharp spines, also identified as modified leaflets, are located on each side (at about 10–15 cm) from the base of the rachis. The number of spines per rachis can be used as a parameter for morphological characterization of date palm cultivars. Date palm trunk bear offshoots or suckers near its base during its early stage of growth, wherein the numbers of emerging offshoots is dependent on cultivars (Zaid and de Wet 2002a).

Fig. 1 Salient botanical features of date palm. **a** Date palm trunk. **b**, **c** Pinnate and erect date palm leaves with numerous stiff leaflets; the rachis is armed with broad sharp spines on each side. **d** Inflorescence of date palm consists of many unbranched rachillae, known as "strands," arranged in spirals on the rachis, only the portion of the rachillae that bears flowers are exposed. **e** Sheaths crack longitudinally (spath of male inflorescences), fruits of date palm cultivars: **f** Zhaglool Egyptian cultivar (soft), **g** Barhy Arabian cultivar in Egypt (considered as semi-dry), **h** date palm seed, **i** Sewi Egyptian cultivar (semi-dry), and **j** Malakabe Egyptian cultivar (dry). **k** Offshoots or suckers around mother plant: the most common conventional procedure for date palm propagation. **l** Planting via date palm offshoots (photographs are not in scale; source: photos of MM El-Dawayati)

Roots of date palm are fibrous in nature and can spread up to 25 m from the main tree, at a depth of > 6 m. It has a primary root that emerges directly from its monocotyledonous seed. Secondary roots appear on the primary root, and these in turn produce lateral roots that are of comparable nature, with nearly similar diameter all through their length. Because of the presence of pneumatics in its roots, the date palm roots are considered as respiratory organs (Munier 1973; Dowson 1982).

In date palm, the female trees are different from the male trees owing to its dioecious nature. The hard fibrous cover over the inflorescence is called spathe that protects tiny male

or female flowers during the early stage of flower development. The male spathe is different than female spathe in terms of its length and width. The male one is shorter and wider than the female one. Usually, both the female and male flowers comprise of three sepals and three petals (Fig. 1d). Inflorescences of female and male trees display distinct morphological variations, wherein the female flowers are typically yellowish green, but the male flowers are waxy-white in appearance. The flowers are born on an acuminate and flat peduncle (inflorescence strand), which is commonly known as the "fruit stalk" (for the female trees) (Vandercook et al. 1980). On the onset of flowering period, inflorescence emerges in the axis of the leaves pushing through the longitudinally cracked spathe (Fig. 1e). The parts of the strands that carry the flowers remain the only exposed section. It takes ~ 200 days from pollination to the development of mature fruits (Chao and Krueger 2007). Remarkable changes are observed on date palm fruits (including external and internal characteristics as color, sweetness, texture, and chemical composition) during the stages of growth and development. Date palm fruits are significantly influenced by their region of cultivation, leading to variation in their organoleptic, physical, and chemical characteristics (Fig. 1f–j). *P. dactylifera* fruits are considered to be larger (4–7 × 2–3 cm) than any other *Phoenix* species. The fruits are ovoid to oblong in shape, berry in nature, with smooth fruit skin (pericarp), and a fleshy mesocarp, also containing a single elongate seed, enclosed by a fibrous parchment (endocarp) (Fig. 1h) (Al-Mssallem et al. 2013).

Industrial use

Date fruits are valuable for their high nutritional importance, since the fruit contains several vital minerals such as iron, potassium, calcium, chlorine, phosphorous, copper, magnesium, silicon, sulfur, and low quantity of sodium. Multiple vitamins, including thiamine, riboflavin, biotin, and folic and ascorbic acid are also found in the date fruit. Carbohydrates are the dominant component, as sugar contributes to 60–65% in the flesh of dates, whereas fiber (2.5%), protein (2%), and fat (2%) are present in low ratios (Al-Harrasi et al. 2014). The associated water content in date fruits and the harvesting time determine the classification of date cultivars into soft, semi-dry, or dry type (Fig. 1f–j); this explains why the date fruit can be eaten fresh or in dry form and can also be used as storage-food round the year (Rajmohan 2011). Most of the date palm cultivars are harvested and consumed when the fruits are high in sugar content but low in moisture and tannin levels. However, some date cultivars in North Africa and the Middle East are harvested and consumed when the fruits are still very firm, containing high amounts of tannin (Chao and Krueger 2007). Important cultivars of (semi-dry and dry category) date fruits are usually used in several food industries as pressed and easily-transportable date cake, cookies, candy bars, ice cream, pudding, and date pastes (for different uses in bakery and confectionary), etc. (Ashraf and Hamidi-Esfahani 2011). The date fruit concentrates can be further processed to manufacture syrup, spreads, liquid sugar, chutney, pickle, dip, and food flavoring. Several by-products such as wine, alcohol, vinegar, and organic acids can be obtained through fermentation of date fruits. Date extracts possess high remedial importance with substantial antioxidant, antibacterial, antifungal, and anti-proliferative properties, which recently gained special importance in pharmaceutical and nutraceutical industries to develop natural compound-based industrial products (Al-Alawi et al. 2017).

Wood from date palm tree trunk is used as timber, making furniture, or as fuel. Date palm leaves also have special industrial importance, wherein the ribs of the leaves are used to build small fishing boats and dried bundles of leaves are used in making shades and roofs. Fibers of trunk and leaves are utilized for manufacturing of mats, papers, bags, baskets, hand-made fans, ropes, camel saddles, cords, and twine. Date palm seed oil is utilized in the manufacture of soap and the seeds are used in livestock fodder industry (Chao and Krueger 2007). Date palm inflorescences and trunks also are tapped for sap collection and the collected sap is further processed into a mild alcoholic beverage (Johnson 2011).

Growing condition and conventional propagation of date palm

Date palm adapts well to saline conditions and can withstand long drought spells making this tree best suited for semi-arid and arid climates. It can tolerate very high temperature up to 50 °C in the summer and it can withstand very cold winters as well. Date palm tree has the ability to sustain short bouts of frost (where temperatures gets as low as − 5 °C). Temperature ranging from 21 to 27 °C is considered as the most suitable one for pollination and ripening of fruits in date palm (Yaish and Kumar 2015).

Long spell of hot summer with very low humidity index and scanty rain are preferred from pollination to harvesting period, but irrigation facility or availability of underground water near the surface is obligatory. Flowering is normally annual and the optimum temperature for date palm flowering is > 18 °C, and temperature > 25 °C is favorable for fruit formation. Usually, application of fertilizers is recommended in date palm plantations (Zaid and de Wet 2002b; Johnson 2011), although in many instances the application is limited to nitrogen only. Factors such as cultivar type and/or soil type influence the results of fertilization (Chao and Krueger 2007; Al-Kharusi et al. 2009). Though date palm has the ability to withstand harsh conditions of long drought spells and high temperatures throughout its growth period, however, to assure

vigorous growth, high yield, and best-quality fruit, large amounts of water are required (Zaid and de Wet 2002c; Carr 2013). Date palm is naturally wind pollinated, but for commercial production of high-quality fruits, the pollination is done manually by skilled human pollinator, who can pollinate as many as 100 females using a single male. Date palm plantation needs a number of cultural practices that involve skilled climbers to reach to the crown of the tree for pollination, bunch tie-down, covering, harvesting, and pruning. Date palm cultivation can be continued with assured economical productivity till 65 years with limited inputs (Chao and Krueger 2007; Johnson 2011). Substantial differences among the yields of adult date palm trees ranging from 80 to 200 kg/tree, influenced by cultivar, environmental factors, and farming approaches were reported. Date palm tree needs specific propagation technique, cultural practice, and tree management owing to its unique biological and developmental characteristics (Jain et al. 2011; Mazri and Meziani 2015).

The oldest as well as conventional method of date palm propagation is sexual propagation via seeds. Nonetheless, this traditional approach of propagation is unsuitable to meet the commercial requirements. In addition, the commercial cultivars do not remain identical to mother plant due to the heterozygous nature of seed-derived propagules. Moreover, owing to the dioecious nature, there is 50% probability that new seedling might bear male characters, which do not produce any fruits. It is not possible to detect male seedling (in the nursery) to eliminate those prior to the field transplantation. The growth of seedling is extremely slow and it may take 7 to 10 years or even more to attain maturity, which makes the date palm breeding quite challenging. Hence, the seed-mediated propagation method is not practiced (apart from exceptional cases when supplies of offshoots are unavailable) by date palm growers or by the plant breeders. Instead, the usage of offshoots for date palm propagation is the most common way (Fig. 1k, l), which ensures the development of new progenies having the genetic identity of maternal cultivars. Fruiting of offshoots plants occurs earlier (2–3 years) than seed-derived plants. Although, propagation via offshoots is comparatively safe; however, it comes with some restrictions. Herein, the offshoots, attached to the mother plant have a very slow growth and hence they fail to survive in the field unless they are about 10–15 kg in weight with well-developed rooting system. Moreover, the number and season of offshoot production remain limited (20 to 30 depending on the cultivar), while some cultivars could not even produce offshoots at all and some may produce up to three offshoots during their entire lifecycle. Offshoot-mediated propagation faces the attacks of pests and diseases and hence the substantial improvement of plantation quality gets hindered (Chao and Krueger 2007; Johnson 2011; Khierallah et al. 2017). As a lucrative alternative, in vitro propagation proved to be efficient and appropriate for rapid propagation of date palm rather than conventional methods. Additionally, in vitro propagation can ensure production of true-to-type plants of elite cultivars (Al-Khayri 2007; Abahmane 2017).

In vitro approaches for propagation

For the enhancement of large-scale propagation and to guarantee the sustainable supply of elite quality propagules of date palm, in vitro regeneration protocols from plant cell, tissue, and/or organs were reported by many scientists since 1970s till date. Apart from mass propagation, in vitro interventions proved to be very effective in paving ways for genetic transformation, synthetic seed production, cryopreservation, etc., overcoming the low in vitro response of this recalcitrant species. The subsequent sections in this review deliver an exclusive outline on earlier achievements of past two decades, current status, and imminent scopes of in vitro regeneration of date palm.

Adventitious shoot culture

Direct adventitious shoot and root regeneration in date palm was first reported by Reuveni et al. (1972), who observed initiation of leaves, shoots, and roots from shoot tip and lateral bud explants. Since then, a number of successful attempts have been made to improve the efficiency of in vitro regeneration of date palm through direct organogenesis approach. In due course, an array of factors was optimized for the enhancement of adventitious shoot initiation and proliferation frequency, as well as subsequent rooting efficiency of in vitro-regenerated shoots. Such key factors that played the vital role during the course of adventitious shoot and root development, along with their influence on the in vitro response of date palm, have been listed in Table 1.

Different types of explants were tested for their morphogenetic competence to develop adventitious shoot buds and their subsequent proliferation-cum-elongation. Shoot tip (excised from offshoots) (Fig. 2a–c), lateral shoot, juvenile leaf, and inflorescences were used for the establishment of adventitious shoot culture (Fig. 2d) (Bekheet 2013a; Al-Mayahi 2014; Jazinizadeh et al. 2015; Al-Mayahi 2016a; Abahmane 2017; Gadalla 2017). Among these explants, shoot tip was the preferred choice, since it comprised of highly regenerative meristematic tissue that eventually induced the multiple bud initiation. According to Gantait and Kundu (2017), regeneration of plants via direct organogenesis (like multiple shoot formation) essentially depends on the activity of axillary meristems that are mostly synchronized by morphogenetic competence of explants with the assistance of optimized basal media, carbon source, plant growth regulators (PGRs) (particularly cytokinins), additives, and favorable culture conditions;

Table 1 In vitro propagation via organogenesis in date palm

Cultivar	Explant	Basal medium	PGR (type and concentration, mg/l or μM*)	Carbon source	Other media additives (mg/l or g/l*)	Culture conditions [temp.; photoperiod; light intensity (lux or PPFD); relative humidity)	Response	Acclimatization [substrate used (*v*/*v*); % survival]	Reference
Zaghlool	Shoot tip	MS	3 2iP + 0.5 NAA PGR-free	3% sucrose	None	Not mentioned	Mult Sht Rt	Not carried out	Taha et al. (2001)
Khanezi	In vitro shoot	MS	0.2 NAA	6% sucrose	170 NaH_2PO_4 + 100 inositol + 1 thiamine	Not mentioned	Rt	Not carried out	Al-Khateeb (2002)
Maktoom	Shoot tip	MS	2 2iP + 1 BA + 1 NAA + 1 NOA 4 2iP + 2 BA +1 NAA 1 NAA + 1 GA_3 0.5 NAA	3% sucrose	2* PVP	27 ± 1 °C; 16 h; 1000 lx; unspecified	Bud break Mult Sht Sht Elong Rt	Peat moss: perlite (1:2); 80%	Khierallah and Bader (2007)
Khanezi	Shoot tip	MS	0.2 kinetin + 0.1 2iP + 0.1 NAA + 0.1 NOA + 0.1 IAA	3% sucrose 3% fructose	None	25 ± 2 °C; 16 h; unspecified; unspecified	Mult Sht Sht Elong	Not carried out	Al-Khateeb (2008)
Barhee	Juvenile leaf	MS	0.2 2,4-D 4 IBA	5% sucrose	None	28 °C; 16 h; 30 μE/m^2/s; unspecified	Mult Sht Rt	Peat: vermiculite (1:3); 83.3%	Fki et al. (2011)
Dhakki	Shoot tip	MS	1 NAA + 3 2iP + 3 BA 1 NAA + 0.5 kinetin + 0.5 BA 1.5 NAA	4% sucrose 3% sucrose	75 Ascorbic acid + 75 Citric acid + 0.3* AC 1 Biotin	25 ± 2 °C; 16 h; unspecified; unspecified	Bud break Mult Sht Rt	Sand: peat moss (2:1); 83%	Khan and Bibi (2012)
Najda	Shoot	MS	0.5 BA + 0.5 IBA	3% sucrose	1* PVP	25 ± 1 °C; 16 h; unspecified; unspecified	Mult Sht, Rt	Peat: gravel (1:1); > 73%	Mazri (2012)
Elsafada	Lateral shoot	MS	10 2iP	6.5% sucrose	0.75* AC + 40 AdS	27 ± 1 °C; 16 h; 1000 lx; unspecified	Mult Sht Rt	Not carried out	Abdulwahed (2013)
Zaghlool	Young off-shoot	MS	2 2iP + 1 NAA 5 2iP + 1 NAA 1 NAA	3% sucrose	 10 $AgNO_3$	25 ± 3 °C; 16 h; 3000 lx; unspecified	Bud break Mult Sht Rt	Peat moss: vermiculite (1:1); unspecified	Bekheet (2013a)
16-bis	Shoot tip	MS, WPM, NM	0.5 NAA + 0.5 kinetin PGR-free	3% sucrose	1* PVP	25 °C; 16 h; unspecified; unspecified	Mult Sht Rt	Peat: gravel (1:1); 80%	Mazri (2013)
Najda	Shoot tip	½ MS	0.5 NOA + 0.5 kinetin 0.5 NOA + 0.5 kinetin	3% sucrose	1* PVP	25 °C; 16 h; unspecified; unspecified	Mult Sht Rt	Peat: gravel (1:1); unspecified	Mazri and Meziani (2013)
Gajar, Kasho Wari, Gulistan	In vitro shoot	MS	0.1 GA_3 + 0.1 NAA	4% sucrose	3* AC	Not mentioned	Rt	Sand: peat moss (1:1); 90%	Abul-Soad and Jatoi (2014)
Hillawi	Shoot tip	MS	1 BA +0.5 TDZ 0.5 GA_3 + 0.1 NAA 0.2 NAA	3% sucrose	2* AC	27 ± 2 °C; 16 h; 40 μmol/m^2/s; 55–60%	Bud break Sht Elong Rt	Peatmoss: perlite (2:1); 80%	Al-Mayahi (2014)
16-bis	Shoot tip	MS	2.5* IBA + 2.5* BA PGR-free	2% sucrose 4% sucrose	None	25 °C; 16 h; 40 μmol/m^2/s; unspecified	Mult Sht Rt	Peat: gravel (1:1); 92.5%	Mazri (2014)
Barhee	Shoot tip	MS	4 2iP 1.5 2iP + 1 BA + 1 NAA 1 NAA	3% sucrose	2* AC	24 ± 1 °C; 16 h; unspecified; unspecified	Bud break Mult Sht Rt	Not carried out	Jazinizadeh et al. (2015)
Boufeggous	Shoot tip	½ MS	3* IBA + 3* BA PGR-free	3% sucrose	1* PVP	25 °C; 16 h; unspecified; unspecified	Mult Sht Rt	Peat: gravel (1:1); 92.5%	Mazri (2015)
Mejhoul	Shoot tip	½ MS	0.2 NOA + 0.2 IAA + 0.4 2iP + 0.4 kinetin	3% sucrose	None	25 ± 1 °C; 16 h; 1000 lx; unspecified	Mult Sht	Not mentioned; 88%	Meziani et al. (2015)

Table 1 (continued)

Cultivar	Explant	Basal medium	PGR (type and concentration, mg/l or µM*)	Carbon source	Other media additives (mg/l or g/l*)	Culture conditions [temp.; photoperiod; light intensity (lux or PPFD); relative humidity)	Response	Acclimatization [substrate used (*v*/*v*); % survival]	Reference
Alshakr	Shoot tip	MS	PGR-free 2 2iP + 1 NAA	3% sucrose	100 Glutamine + 5 thiamine HCl + 1 biotin + 3* AC	27 ± 1 °C 14 h; 80–100 µmol/m^2/s; unspecified	Rt Mult Sht	Not carried out	Al-Mayahi (2016a)

2iP 6-g,g-dimethylallylaminopurine, *AC* activated charcoal, *BA* N^6-benzyladenine, *GA$_3$* gibberellin A$_3$, *IAA* indole-3-acetic acid, *IBA* indole-3-butyric acid, *kinetin* 6-furfurylaminopurine, *MS* Murashige Skoog medium (Murashige and Skoog 1962), *Mult Sht* Multiple shoot, *NAA* α-napthalene acetic acid, *NM* Nitsch and Nitsch medium (Nitsch and Nitsch 1969), *NOA* 2-naphthoxyacetic acid, *PGR* plant growth regulator, *PVP* polyvenylpyrrolidone, *Rt* root, *WPM* Woody Plant Medium (Lloyd and McCown 1981)

nonetheless, the supplementary inference of auxins continued to be influential as well. Among the other explants, offshoots remained the next preferred and effective choice, following shoot tip explants (Bekheet 2013a; Al-Mayahi 2014; Jazinizadeh et al. 2015; Al-Mayahi 2016a). Interestingly, the usage of juvenile leaf explant was the sole exception, wherein no apical meristem was involved, yet it induced multiple shoots in date palm (Fki et al. 2011).

Full-strength Murashige and Skoog (MS) medium (Murashige and Skoog 1962) was the widely used basal nutrient medium for initiation of adventitious shoot buds and their subsequent proliferation in date palm. However, the single exception was reported by Mazri (2013), who studied the comparative effectiveness of MS, Woody Plant Medium (WPM) (Lloyd and McCown 1981), and Nitsch and Nitsch medium (NM) (Nitsch and Nitsch 1969) during multiple shoot induction from shoot tip explants. It was observed that MS medium outperformed WPM and NM, resulting in maximum frequency of multiple shoot formation. In few other instances, the use of ½ MS medium was the preferred choice instead of full strength, during multiple shoot and root development (Mazri and Meziani 2013; Mazri 2015; Meziani et al. 2015); however, such results were reported by a specific group of authors and interestingly, not adopted by other scientists in last two decades.

Even though source of carbon and its concentration usually play a major role during in vitro regeneration of any explant tissue, for date palm, it was the single source (sucrose) but in variable concentrations that influenced the regeneration efficiency of explants to develop multiple shoots, or roots from in vitro shoots (Fig. 2e) (Table 1). Majority of the available reports suggested that the use of 3% (*w*/*v*) sucrose was found to be the optimal concentration for multiple shoot culture; conversely, there are other reports that exhibited the requirement of higher concentrations (4–6.5%) of sucrose to attain comparable results (Al-Khateeb 2002; Fki et al. 2011; Khan and Bibi 2012; Abdulwahed 2013; Abul-Soad and Jatoi 2014). On the other hand, use of as low as 2% sucrose was reported to be effective for induction of multiple shoots (Mazri 2014). However, to induce roots from the in vitro developed shoots, the concentration of sucrose was increased to 4% to attain higher number and length of roots. The only exceptional study on successful use of fructose (3%), as an alternative to sucrose, for elongation of in vitro shoots was reported by Al-Khateeb (2008).

The most critical component of adventitious shoot culture of date palm was the categories and concentrations of PGRs that influenced the morphogenetic pathway to induce either multiple shoot or root primordia. Combinations of cytokinin-auxin in variable levels were preferred over individual use of auxins or cytokinins, where cytokinins were used in comparatively higher concentrations than that of the auxins (Table 1).

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◀ **Fig. 2** In vitro regeneration and conservation of date palm. **a**, **b** Preparation of shoot tip explants. **c** Inoculation of shoot tip explants in nutrient medium supplemented with optimized high cytokinin and low auxin concentrations. **d** Direct adventitious shoots from shoot tip explant. **e** Further multiplication and proliferation of shoots (without any interference of callus phase). **f** Indirect regeneration procedure starting with swilling of explant segments. **g** Embryogenic calli formation. **h** Induction of somatic embryos that later in turn developed to multiple shoots and roots. **i** Immature female inflorescence spath. **j** Indirect induction of somatic embryos female inflorescence explants via callus. **k** Direct somatic embryogenesis from shoot tip explant. **l** Regeneration of somatic embryos into shoots, its proliferation and rooting. **m** Mass multiplication and proliferation of shoots. **n** Root initiation from in vitro shoots. **o** Acclimatization of in vitro-derived plantlets on peat moss and vermiculite substrates. **p** Date palm farm planted with tissue culture-derived plantlets. **q**, **r** Short-term storage of shoot clusters of date palm in modified culture medium and under low temperature inside programmable incubator, where all growth conditions are controlled for different period. **s** Date palm synthetic seeds developed from microshoots and their germination into complete plantlets. **t** Developing cryopreservation method for conservation of embryonic calli (photographs are not in scale; source: photos of MM El-Dawayati)

Yet there are few reports on the use of equal ratio of cytokinin-auxin to attain optimum regeneration frequency (Mazri 2012; Mazri and Meziani 2013; Mazri 2014, 2015). Out of the reported successful cytokinin-auxin combinations, the combinations of 6-g,g-dimethylallylaminopurine (2iP) and α-napthalene acetic acid (NAA) at different levels were found to be very effective to induce multiple shoots (Taha et al. 2001; Bekheet 2013a; Al-Mayahi 2016a). The enhancement in the frequency of multiple shoot formation was observed, when 2iP-NAA combinations were supplemented with N^6-benzyladenine (BA) alone (Khan and Bibi 2012; Jazinizadeh et al. 2015) or combinations of BA and 2-naphthoxyacetic acid (NOA) (Khierallah and Bader 2007), or 6-furfurylaminopurine (kinetin)-NOA-indole-3-acetic acid (IAA) (Al-Khateeb 2008). Apart from 2iP-NAA, the other effective cytokinin-auxin combinations were BA-indole-3-butyric acid (IBA) (Mazri 2012, 2014, 2015) or kinetin-NAA (Mazri 2013) or kinetin-NOA (Mazri and Meziani 2013), during shoot bud break and multiple shoot initiation process. There are merely a couple of instances wherein 2–4 mg/l 2iP as the single cytokinin source was used for successful adventitious shoot formation (Abdulwahed 2013; Jazinizadeh et al. 2015). Auxins, especially 2,4-dichlorophenoxy acetic acid (2,4-D) that is frequently used for callus induction in plants, were employed in a very low concentration (0.2 mg/l) to induce multiple shoots from juvenile leaf of date palm (Fki et al. 2011). Following initiation of multiple shoots buds, the next crucial developmental event was elongation of juvenile shoots. To recover multiple shoots with optimal length (which is a prerequisite for enhanced root formation), usually, subculture or continuous culture in the same shoot initiation media was preferred, as reflected in the available reports. However, to facilitate the elongation process in a short span of time, use of GA_3 in combination of NAA (in 1:1 ratio) was found to be very effective (Khierallah and Bader 2007; Abul-Soad and Jatoi 2014).

To ensure the success of adventitious shoot culture, initiation and elongation of in vitro roots remains the key. High frequency of root formation was recorded in date palm, when the in vitro shoots were separated from clumps and cultured individually on MS media, supplemented with various levels of auxins (Table 1). Use of NAA at a range of 0.2–1.5 mg/l (Al-Khateeb 2002; Khierallah and Bader 2007; Khan and Bibi 2012; Bekheet 2013a; Al-Mayahi 2014; Jazinizadeh et al. 2015) was proved to be effective for in vitro rooting of shoots. As an alternative (to NAA), IBA (the other root-inducing PGR) was successfully used at a much higher concentration (4 mg/l) by Fki et al. (2011) to induce frequent root formation. Interestingly, there are a number of reports wherein it was observed that in vitro roots could be induced in MS medium without fortification of any PGR (Taha et al. 2001; Mazri 2013, 2014, 2015; Meziani et al. 2015).

To improve the efficiency of in vitro adventitious shoot and root development in date palm, supplementation of other chemical components were found to be effective. Activated charcoal (AC) (at the concentrations of 0.75 to 3 mg/l) was one of these additives that alone (Abul-Soad and Jatoi 2014; Al-Mayahi 2014; Jazinizadeh et al. 2015) [or in combination with adenine sulfate (AdS) or ascorbic acid and citric acid] (Khan and Bibi 2012; Abdulwahed 2013) significantly enhanced the morphogenetic competence of explants through removal (or absorbance) of phenolic compounds that critically hinders the development of plant cells, tissue, or organs. Polyvenylpyrrolidone (PVP) (1–2 mg/l) was the other effective additive that assisted in bud break, shoot multiplication, and rooting from shoot tip explants of date palm (Khierallah and Bader 2007; Mazri 2012, 2013; Mazri and Meziani 2013; Mazri 2015). Bekheet (2013a) used high level of silver nitrate ($AgNO_3$) (10 mg/l) as an additive for proliferation of multiple shoots, induced from young offshoot explants.

During the course of adventitious shoot culture and root formation, the culture room condition was set with distinct temperature (24 to 28 °C), photoperiod (16 h), light intensity (1000 to 3000 lx or 30 to 100 μmol/m^2/s), and relative humidity (55–60%) (Table 1). In most of the reports, relative humidity was not mentioned (apart from the report of Al-Mayahi 2014), even though it is a major component of microenvironment. As an exception to conventional 16 h photoperiod, Al-Mayahi (2016a) reported 14 h photoperiod to be the most effective one for the induction and proliferation of multiple shoots.

Callus culture, somatic embryogenesis, and its regeneration

Plant cells, which multiply in nonsynchronized way and ultimately result in unstructured form of tissue, are characterized

as "callus" ("calli" in plural). However, this event is not spontaneous and occurs only if the explant tissue is exposed to specific PGRs at specific concentrations, accompanying favorable culture conditions (Gantait and Kundu 2017). Callus can be either organogenic or embryogenic in nature, depending on the culture medium and PGR formulations it is treated with (Gantait et al. 2015a). Friable callus is usually recognized to be the preferred source for cell suspension culture, whereas organogenic and embryogenic callus yield multiple shoots and/or roots, and somatic embryos, respectively. Somatic embryogenesis is mostly involved for extensive production of plant propagules and for the development of genetically transformed plants. Somatic embryogenesis trails two distinct routes: one involves the "intermediate callus stage" (Fig. 2f–j) and the other one is initiation of somatic embryos straight from the explant tissue (Fig. 2k, l). In date palm, majority of the available reports portray that the induction and conversion of callus were chiefly aimed at somatic embryogenesis (Table 2). Only three reports have been found since past two decades (Asemota et al. 2007; Gueye et al. 2009b; Kurup et al. 2014) that described the induction of direct somatic embryogenesis or organogenic calli and their regeneration into multiple shoots and/or roots. In addition, there is merely a lone report on description of callus induction (Gueye et al. 2009a), without any regeneration study.

An array of morphogenetic, chemical, and physical components was considered for the standardization of callus induction and its successive transformation either into plantlet or into somatic embryos in date palm. These components and their significant results have been displayed in Table 2. The key component that affected the callus induction of date palm at its preliminary phase was the type of explants used to establish in vitro culture. Shoot tip (Fig. 2f), lateral/axillary bud, primordial leaf, juvenile/immature leaf, female inflorescence (Fig. 2i), and even in vitro adventitious buds were employed as explants to induce indirect embryogenic (Fig. 2g, h, j) or organogenic callus under optimized in vitro condition, and among these explants, shoot tip was the most preferred one followed by juvenile leaf (Table 2). The only study of callus induction from adventitious buds carried out recently by Mazri et al. (2017, 2018) where there are several reports on callus induction and subsequent indirect somatic embryogenesis using shoot tip and inflorescence explants in date palm.

The next most crucial component that played the major role in the success of callogenesis was the type of basal media [Gamborg's (B5) medium (Gamborg et al. 1968), EM Eeuwen's medium (EM) (Eeuwen 1976), MS, NM, Schenk and Hildebrandt (SH) medium (Schenk and Hildebrandt 1972), White's (W) medium (White 1963), and WPM], their state (liquid or semi-solid), and their concentrations (½ or ¾ or full-strength). The details of such basal media and their concerned role over callus induction, regeneration, somatic embryogenesis (Fig. 2h), and its conversion into multiple shoots (Fig. 2m) and shoots with roots (Fig. 2n) have been mentioned in detail in Table 2. Among all these seven types of basal media, their state, and concentrations, the use of full-strength semi-solid MS medium was prevalent for induction of callus or somatic embryo in date palm. To enhance the callogenic efficiency, the combined use of MS and B5 salts to induce embryogenic calli from inflorescence explants was reported by Abul-Soad and Mahdi (2010). Al-Khayri (2011a) studied the comparative effect of SH, W, MS, WPM, and NM media during callus induction, somatic embryogenesis and its conversion in three popular cultivars of date palm. It was found that the optimum callus induction and proliferation was attained using WPM and W media in cv. Khusab; using NN and SH media in cv. Berny; and using MS, SH, and W media in cv. Barhee. This report suggests that response of basal media towards morphogenetic response in date palm can be cultivar-specific. There are few reports on use of liquid MS medium over semi-solid one, for proliferation of embryogenic calli (Boufis et al. 2014), induction of somatic embryos from calli (Othmani et al. 2009b; Sané et al. 2012; Al-Mayahi 2015), and regeneration (or conversion) of somatic embryos into shoot and root (Othmani et al. 2009b). On the other hand, ½ strength (Bhargava et al. 2003; Al-Khayri 2010; Kriaâ et al. 2012) or ¾ strength (Abul-Soad and Mahdi 2010; Abdolvand et al. 2014) semi-solid MS medium was used only a few instances, specifically for induction of somatic embryos from calli or for root induction during conversion of somatic embryos. The exceptional experiment on the use of EM to induce organogenic green-compact calli and their subsequent regeneration from young leaves of date palm was reported by Asemota et al. (2007).

Apart from of explant sources and basal media, the type and concentration of carbon source rendered a substantial function in the success of callus culture, regeneration, and callus-mediated or direct somatic embryogenesis in date palm wherein the source of carbon continued to be the same (i.e., sucrose) but the concentrations of sucrose varied based on cultivars used and in vitro response required (Table 2). The large section of the available reports on date palm callogenesis and somatic embryogenesis suggested that 3% sucrose was ideal for high frequency callus induction and subsequent somatic embryogenesis. However, use of lower level of sucrose (2%) (Bekheet et al. 2001a, b; Gueye et al. 2009a) or 4–4.5% (Asemota et al. 2007; Hegazy and Aboshama 2010; Al-Khayri 2011a; Abd El Bar and El-Dawayati 2014; Boufis et al. 2014) or 5% (Othmani et al. 2009b; Abul-Soad and Mahdi 2010; Kriaâ et al. 2012; Sidky and El-Dawyati 2012) or even as high as 6% (Alkhateeb 2006; Sané et al. 2012; Abdolvand et al. 2014) sucrose were supplemented to basal media for callus or somatic embryo induction and ensuing regeneration/conversion of date palm. Based on the available reports (Table 2), it can be postulated that concentration is more important than the source of carbon for desirable

Table 2 Plant regeneration via somatic embryogenesis in date palm

Cultivar	Explant	Basal medium	PGR (type and concentration, mg/l or µM*)	Carbon source	Other media additives (mg/l or g/l*)	Culture conditions [temp.; photoperiod; light intensity (lux or PPFD); relative humidity)	Response	Acclimatization [substrate used (v/v); % survival]	Reference
Zaghlool	Shoot tip	MS	10 2,4-D + 3 2iP	3% sucrose	None	Not mentioned	SE	Vermiculite: peat moss (1:1); 93%	Bekheet et al. (2001a, b)
			PGR-free	2% sucrose			Sht Rt		
Not mentioned	Shoot tip	MS	100 2,4-D + 3 2iP	3% sucrose	1.5* AC	24 ± 3 °C; dark; unspecified	E Ca	Soil: peat moss: vermiculite (1:1:1); unspecified	Al-Khayri (2001)
			10 NAA + 30 2iP		1.5* AC	24 ± 3 °C; 16 h; 50 µmol/m²/s; unspecified	Ca Pro		
			PGR-free				SE		
			PGR-free				Sht		
			0.1 NAA				Rt		
Barhee	Shoot tip	MS	100 2,4-D + 3 2iP	3% sucrose	1.5* AC	24 ± 3 °C; dark; unspecified	E Ca	Soil: peat moss: vermiculite (1:1:1); unspecified	Al-Khayri and Al-Bahrany (2001)
			10 NAA + 6 2iP		1.5* AC	24 ± 3 °C; 16 h; 50 µmol/m²/s; unspecified	Ca Pro		
			0.5* 2iP		25 µM $AgNO_3$		SE		
			PGR-free				Sht		
			0.1 NAA				Rt		
Estamera, Kabkab	Lateral bud, shoot tip	MS	100 2,4-D	3% sucrose	3* AC	28 °C; 16 h; 2000 lx; unspecified	Ca	Not carried out	Davoodi et al. (2002)
			PGR-free				SE		
Zaghlool	Shoot tip, primordial leaf	MS	10 2,4-D + 3 2iP	Not mentioned	None	25 ± 2 °C; dark ➔ 16 h; unspecified; unspecified	E Ca	Vermiculite: peat moss (1:1); unspecified	Taha et al. (2002)
			1 2iP.				SE		
			PGR-free				Sht Rt		
Medzool, Sayar, Samran, Jaglool	Shoot tip	MS	2.5–15 2,4-D + 0.1 IAA	3% sucrose	50 Ascorbic acid + 50 citric acid + 100 PVP + 25 AdS	26 ± 1 °C; dark; unspecified	Ca	Soilrite: farmyard manure: sand (1:1:1); unspecified	Bhargava et al. (2003)
			2.5 2,4-D + 0.25 kinetin +0.1 IAA			26 ± 1 °C; 14 h; 14 µmol/m²/s; unspecified	E Ca		
			2.5 2,4-D + 0.1 IAA ➔ 0.1 BA			26 ± 1 °C; 14 h; 28 µmol/m²/s; unspecified	SE		
		½ MS	PGR-free			26 ± 1 °C; 14 h; 68 µmol/m²/s; unspecified	Sht		
							Rt		
Barhee, Nabout Saif, Ruzaiz, and Hillali	Shoot tip	MS	452.5* 2,4-D + 14.7* NAA	3% sucrose		24 ± 3 °C; dark; unspecified	E Ca	Not carried out	Al-Khayri and Al-Bahrany (2004a)
			53.7* NAA + 7.4* 2iP		50 µM $AgNO_3$	24 ± 3 °C; 16 h; 50 µmol/m²/s; unspecified	Ca Pro		
			PGR-free		50 µM $AgNO_3$		SE		

Table 2 (continued)

Cultivar	Explant	Basal medium	PGR (type and concentration, mg/l or µM*)	Carbon source	Other media additives (mg/l or g/l*)	Culture conditions [temp.; photoperiod; light intensity (lux or PPFD); relative humidity)	Response	Acclimatization [substrate used (v/v); % survival]	Reference
Sewei	Shoot tip, axillary bud, leaf primordia	MS	100 2 4-D + 3 2iP	3% sucrose	9* AC	Not mentioned	Ca	Not carried out	Badawy et al. (2005)
			0.1 NAA + 2 2iP				SE		
			0.1 NAA + 3 2iP				Sht		
			1.5 NAA				Rt		
Khanizi and Mordarsing	Shoot tip	MS	453* 2,4-D + 15* 2iP + 13* BA	Not mentioned		24 ± 3 °C; dark; unspecified	E Ca/SE	Not carried out	Eshraghi et al. (2005)
			54* NAA + 148* 2iP		1.5* AC		Sht Rt		
Barhy	Shoot tip, flower rachis	MS	100 2,4-D + 3 2iP + 3 kinetin	Not mentioned	1.5* AC + 75 Ascorbic acid + 75 citric acid	26 ± 1 °C; 16 h; 2500 lx; unspecified	Ca	Not carried out	Al-Khalifah et al. (2006)
			2.5 NAA + 3 2iP + 3 kinetin				E Ca/SE		
			PGR-free				Sht Rt		
Sukary	Shoot tip	MS	100 2,4-D + 3 2iP	3% sucrose	1.5* AC	25 ± 2 °C; dark; unspecified	Ca	Not carried out	Alkhatee (2006)
			10 NAA + 30 2iP			25 ± 2 °C; 16 h; unspecified; unspecified	Ca Pro		
			10 NAA + 6 2iP				E Ca/SE		
			PGR-free	6% sucrose	5% PEG		Sht Rt		
Not mentioned	Young leaf	EM	15 NAA	3% sucrose	None	27 °C; dark; unspecified	O Ca	Not carried out	Asemota et al. (2007)
			10–15 NAA	4.5%			Sht		
			5–15 NAA	4.5–9%			Rt		
Bartamoda, Sakkoty, Malkaby	Shoot tip, primordial leaf	MS	10 2,4-D + 3 2iP	3% sucrose	3* AC + 170 NaH_2PO_4 + 200 glutamine	Unspecified; dark	E Ca	Not carried out	Taha et al. (2007)
		¾ MS	10 2,4-D + 3 2iP			3000 lx; unspecified	SE		
		MS	PGR-free				Sht Rt		
Ahmar	Immature leaf	MS	9* 2,4-D	2% sucrose	None	28 °C; dark; unspecified; 60%	Ca	Not carried out	Gueye et al. (2009a)
Ahmar	Immature leaf	MS	54* NAA	3% sucrose	None	Not mentioned	Ca	Not carried out	Gueye et al. (2009b)
			1* NAA				Rt		
Boufeggous	Immature leaf	MS	10 2,4-D	3% sucrose	0.3 AC	28 ± 2 °C; dark; unspecified	Ca	Peat moss: sand (2:1); 60%	Othmani et al. (2009a)
			1 ABA			28 ± 2 °C; 16 h; 80 µmol/m^2/s; unspecified	SE		
			1 NAA		0.1 AC		Sht Rt		
Deglet Bey	Immature leaf	MS	10 2,4-D	5% sucrose	0.3 AC	28 ± 2 °C; dark; unspecified	Ca	Peat moss: sand (2:1); 96%	Othmani et al. (2009b)
		MS(L)	0.1 2,4-D			28 ± 2 °C; 16 h; 80 µmol/m^2/s; unspecified	SE, Sht, Rt		

Table 2 (continued)

Cultivar	Explant	Basal medium	PGR (type and concentration, mg/l or µM*)	Carbon source	Other media additives (mg/l or g/l*)	Culture conditions [temp.; photoperiod; light intensity (lux or PPFD); relative humidity)	Response	Acclimatization [substrate used (v/v); % survival]	Reference
Gulistan	Inflorescence	MS + B5	0.1 2,4-D + 0.1 IAA + 5 NAA	3% sucrose	40 AdS +170 KH_2PO_4 + 100 glutamine	25 ± 2 °C; dark; unspecified	Ca	Unspecified	Abul-Soad and Mahdi (2010)
		MS	0.1 NAA + 0.1 kinetin				SE		
			0.1 NAA + 0.05 BA				Sht		
		¾ MS	0.1 NAA	5% sucrose	3* AC		Rt		
Khasab, Nabout Saif	Shoot tip	MS	100 2,4-D + 3 2iP	3% sucrose	1.5* AC + 10–15% CW	24 ± 3 °C; dark; unspecified	Ca	Soil: peat moss: vermiculite (1:1:1); 70–84%	Al-Khayri (2010)
			10 2,4-D + 6 2iP			24 ± 3 °C; 16 h; 50 µmol/m^2/s; unspecified	Ca Pro		
			10 2,4-D + 1.5 2iP				SE		
			PGR-free				Sht		
		½ MS	0.1 NAA				Rt		
Mishrig wad Khateeb, Mishrig wad Laggi	Shoot tip	MS	100 2,4-D + 3 2iP	3% sucrose	3* AC	27 ± 2 °C; dark; unspecified	Ca	Not carried out	El Fatih and Hind Badereldin (2010)
		MS	0.1 NAA			27 ± 2 °C; 16 h; 1000 lx; unspecified	SE		
Medjool	Shoot tip	MS	1 2iP + 1 kinetin + 1 BA + 0.5 NOA	4% sucrose	1.5* AC	25 ± 1 °C; dark; unspecified	Bud	Compost: perlite (1:1); 80%	Hegazy and Aboshama (2010)
			-do-		150 putrescine	25 ± 1 °C; 16 h; 1000 lx; unspecified	SE		
			1 2iP + 1 kinetin + 0.5 NOA		150 putrescine		Sht		
			1 IBA		None	25 ± 1 °C; 16 h; 3000 lx; unspecified	Rt		
Seven genotypes (DDP)	Shoot tip	MS	50* 2,4-D	Not mentioned	250 AC	29 ± 1 °C; dark; unspecified	Ca	Not carried out	Sani et al. (2010)
			200* 2,4-D				Ca Pro		
Khusab, Berny, and Barhee	Shoot tip	SH, W, MS, WPM, NM	10 NAA + 30 2iP	4% sucrose	1.5* AC	24 ± 3 °C; dark; unspecified	Ca	Soil: peat moss: vermiculite (1:1:1); unspecified	Al-Khayri (2011a)
			10 NAA + 6 2iP			24 ± 3 °C; 16 h; 50 µmol/m^2/s; unspecified	E Ca/SE		
			PGR-free				Sht		
			0.1 NAA				Rt		
Nabout Saif	Shoot tip	MS	100 2,4-D + 3 2iP	3% sucrose	1.5* AC + 1* YE + 1* CH	24 ± 3 °C; dark; unspecified	Ca		Al-Khayri (2011b)
			10 NAA + 6 2iP		1.5* AC + 1* YE + 1* CH	24 ± 3 °C; 16 h; 50 µmol/m^2/s; unspecified	Ca Pro		

Table 2 (continued)

Cultivar	Explant	Basal medium	PGR (type and concentration, mg/l or µM*)	Carbon source	Other media additives (mg/l or g/l*)	Culture conditions [temp.; photoperiod; light intensity (lux or PPFD); relative humidity)	Response	Acclimatization [substrate used (*v/v*); % survival]	Reference
			10 NAA + 1.5 2iP		1* YE + 1* CH		SE		
Barhee, Zardai, Khalasah, Muzati, Shishi, Zart	Shoot tip	MS	1.5 2,4-D 0.5 NAA 0.75 BA	Not mentioned	None	25 ± 2 °C; 16 h; 100 µmol/m^2/s; unspecified	E Ca SE Sht, Rt	Unspecified	Aslam et al. (2011)
Gajar, Kashoo-Wari, Khar, Karbline, Khormo, Dedhi, Aseel	Inflorescence	MS	100 2,4-D + 3 2iP 10 2,4-D + 3 2iP	3% sucrose	1.5* AC 1.5* AC	25 ± 2 °C; dark; unspecified	Ca SE	Unspecified	Abul-Soad (2012)
Nabout Saif	Shoot tip	MS	100 2,4-D + 3 2iP	3% sucrose	1.5* AC	24 ± 3 °C; dark; unspecified	Ca	Not carried out	Al-Khayri and Al-Bahrany (2012)
			10 NAA + 6 2iP		1.5* AC + 10% PEG +1–10 µM ABA	24 ± 3 °C; 16 h; 50 µmol/m^2/s; unspecified	SE		
Deglet Noor, Hamma Rgaya, Menakher, Gondi, Makni, Barhee, Blombek, DNm1, DNm2	Female inflorescence	MS	10 2,4-D	5% sucrose		28 °C; dark; unspecified	Ca	Not carried out	Kriaâ et al. (2012)
		½ MS	1 2,4-D 0.5 BA + 0.5 NAA 2 IBA		0.3* AC	26 ± 2 °C; 16 h; unspecified; unspecified	SE Sht Rt		
Ahmar, Amsekhsi, Tijib, and Amaside	Juvenile leaf	MS	2 2,4-D	3% sucrose	40 AdS	27 ± 2 °C; dark; unspecified	Ca Pro	Not carried out	Sané et al. (2012)
		MS(L) MS	PGR-free or 0.5 BA PGR-free or 1 NAA	6% sucrose		27 ± 2 °C; 12 h; 80 µE/m^2/s; unspecified	SE Sht Rt		
Not mentioned	Female inflorescence	MS	5 2,4-D + 1 2iP	5% sucrose	40 AdS + 0.5 AC + ABA + ancymidol	27 ± 2 °C; dark; unspecified	Ca	Not carried out	Sidky and El-Dawyati (2012)
			0.05 BA + 0.1 NAA + 0.25 ABA	2% sucrose	0.3 AC	27 ± 2 °C; unspecified; 2000 lx; unspecified	SE		
Medjool	Shoot tip	MS	100 2,4-D + 3 2iP	3% sucrose	3* AC		E Ca	Not carried out	

Table 2 (continued)

Cultivar	Explant	Basal medium	PGR (type and concentration, mg/l or µM*)	Carbon source	Other media additives (mg/l or g/l*)	Culture conditions [temp.; photoperiod; light intensity (lux or PPFD); relative humidity)	Response	Acclimatization [substrate used (*v/v*); % survival]	Reference
			70 2,4-D			24 ± 3 °C; dark; unspecified	Ca Pro		Abdolvand et al. (2014)
		¾ MS	1 2,4-D	6% sucrose			SE		
Degla Beïda	Shoot tip	MS	1 2,4-D + 1 BA	4.5% sucrose	3* AC	25 °C; dark; unspecified	Ca	Not carried out	Boufis et al. (2014)
		MS(L)	1 2,4-D	3% sucrose	300 AC		E Ca Pro		
		MS	PGR-free		300 AC		SE		
Sakoty	Leaf	MS	2 BA	4% sucrose	None	27 ± 2 °C; dark; unspecified	SE (direct)	Not carried out	Abd El Bar and El-Dawayati (2014)
Kheneizi	Juvenile leaf	MS	100 2,4-D	3% sucrose	None	25 ± 2 °C; dark; unspecified	O Ca	Peat: sand: dehydrated cow manure (1:1:1); 67%	Kurup et al. (2014)
			0.5 NAA + 0.25 BA		200 CH + 3* AC	28 ± 2 °C; 16 h; 45 $\mu mol/m^2/s$; unspecified	Sht Rt		
Quntar	Leaf	MS	5 2,4-D + 1 BA	3% sucrose	2* AC + 40 AdS	27 ± 2 °C; dark; unspecified	Ca	Peat moss: perlite (2:1); 80%	Al-Mayahi (2015)
		MS(L)	1 2,4-D		500 AC	27 ± 2 °C; 16 h; 30 $\mu mol/m^2/s$; unspecified	SE		
		MS	3 2iP + 1 NAA				Sht		
			0.5 GA_3 + 1 NAA				Rt		
Barhi	Shoot	MS	0.05 BA + 0.1 NAA	3% sucrose	30% CW + 2.5* CH	27 ± 2 °C; 16 h; 1500 lx; unspecified	SE	Not carried out	Hosny et al. (2016)
Halawy, Medjool	Shoot tip	MS	100 2,4-D + 3 BA	3% sucrose	3* AC	25 ± 2 °C; dark; unspecified	Ca	Not carried out	Bhati et al. (2017)
			PGR-free			27 ± 2 °C; 24 h; 1000 lx; unspecified	SE		
Deglet Nur, Takerbucht	Inflorescence	MS	2 2,4-D + 3 IPA	3% sucrose	None	27 ± 1 °C; dark; unspecified	Ca	Not carried out	Bouguedoura et al. (2017)
			0.5 BA			25–27 °C; 12 h; 3000 lx; unspecified	SE		
Najda	Adventitious buds	MS	45* 2,4-D + 4.5* 2iP	3% sucrose	1* AC	25 °C; dark; unspecified	Ca	Peat: gravel (1:1, *w/w*); 80%	Mazri et al. (2017, 2018)
			4.5* picloram + 0.45* 2iP		1* AC	25 °C; dark; unspecified	SE		
			PGR-free		1* AC	25 °C; 16 h; unspecified	Sht Rt		

Table 2 (continued)

Cultivar	Explant	Basal medium	PGR (type and concentration, mg/l or µM*)	Carbon source	Other media additives (mg/l or g/l*)	Culture conditions [temp.; photoperiod; light intensity (lux or PPFD); relative humidity)	Response	Acclimatization [substrate used (v/v); % survival]	Reference
Medjool	Shoot tip	MS	100 2,4-D + 3 2iP	3% sucrose	3* AC	24 ± 3 °C; dark; unspecified	Ca	Not carried out	Abdolvand et al. (2018)
			PGR-free		4 $AgNO_3$	24 ± 3 °C; dark; unspecified	SE		
			PGR-free		8 $AgNO_3$		Sht Rt		
Bartamuda, Shamia, Gondeila, Malakaby, Sakkoty	SE cluster	MS	0.4 2iP	3% sucrose	2* AC	27 ± 2 °C; 16 h; 3000 lx; unspecified	SE	Not carried out	Gadalla et al. (2018)
			0.4 BA				Sht		
			0.05 BA-ribozide				Rt		

2,4-D 2,4-dichlorophenoxy acetic acid, *2iP* 6-g,g-dimethylallylaminopurine, *ABA* abscisic acid, *AC* activated charcoal, *AdS* adenine sulfate, *B5* Gamborg's medium (Gamborg et al. 1968), *BA* N^6-benzyladenine, *Ca* callus, *Ca Pro* callus proliferation, *CH* casein hydrolysate, *CW* Coconut water, *E Ca* Embryogenic callus, *EM* Eeuwen's medium (Eeuwen 1976), *IAA* indole-3-acetic acid, *IBA* indole-3-butyric acid, *MS* Murashige Skoog medium (Murashige and Skoog 1962), *MS(L)* Murashige Skoog liquid medium, *NAA* α-napthalene acetic acid, *NM* Nitsch and Nitsch medium (Nitsch and Nitsch 1969), *O Ca* organogenic callus, *PEG* polyethelene glycol, *PGR* plant growth regulator, *Rt* root, *SH* medium (Schenk and Hildebrandt 1972), *Sht* shoot, *TDZ* *N*-phenyl-*N'*-(1,2,3-thidiazol-5-yl)urea or thidiazuron, *W* medium (White 1963), *WPM* Woody Plant Medium (Lloyd and McCown 1981), *YE* yeast extract

morphogenetic response of date palm under in vitro condition. In addition, the concentration of any effective carbon source should be optimized considering both the cultivar type and essential in vitro response.

The most predominant element that eventually regulated the frequency of callogenesis and somatic embryogenesis was the type of PGRs and their concentrations. Quite a few auxin and cytokinin sources were used, either alone or in combinations to ensure effective morphogenetic response. Be it induction of organogenic or embryogenic callus, or even callus-mediated somatic embryo development, of 2,4-D (ranged from 1 to 100 mg/l) was the most efficient auxin that was supplemented with basal media, either alone (Davoodi et al. 2002; Gueye et al. 2009a; Othmani et al. 2009a, b; Sani et al. 2010; Aslam et al. 2011; Kriaâ et al. 2012; Sané et al. 2012; Kurup et al. 2014) or (a) in combination with comparatively low concentrations of cytokinins, such as 2iP (Al-Khayri 2001; Al-Khayri and Al-Bahrany 2001; Taha et al. 2002; Badawy et al. 2005; Alkhateeb 2006; Taha et al. 2007; Al-Khayri 2010; El Fatih and Hind Badereldin 2010; Al-Khayri 2011b; Abul-Soad 2012; Al-Khayri and Al-Bahrany 2012; Sidky and El-Dawyati 2012; Abdolvand et al. 2014; Mazri et al. 2017, 2018; Abdolvand et al. 2018), or (b) BA (Boufis et al. 2014; Al-Mayahi 2015; Bhati et al. 2017; Zayed 2017), or (c) both 2iP and BA/kinetin (Eshraghi et al. 2005; Al-Khalifah et al. 2006), or (d) in combination with comparatively low concentrations of auxins, such as NAA (Al-Khayri and Al-Bahrany 2004a), or (e) both NAA and IAA (Abul-Soad and Mahdi 2010). Among all these tested and successful combinations, 10 mg/l 2,4-D plus 3 mg/l 2iP was the most adopted PGR-combinations that yielded maximum frequency of embryogenic callus induction (Table 2). As an alternative of 2,4-D, NAA (15 mg/l) (Asemota et al. 2007) or NAA (10 mg/l) plus 2iP (30 mg/l) (Al-Khayri 2011a) proved to be effective during induction of embryogenic calli. Apart from callus-mediated pathway, somatic embryos of date palm were reported to be induced directly from leaf (Abd El Bar and El-Dawayati 2014) or shoot (Hosny et al. 2016) explants when these were inoculated in MS medium supplemented either with BA (2 mg/l) alone or BA (0.05 mg/l) in combination with NAA (0.1 mg/l). For the induction of somatic embryos, several reports exhibited the transfer of proliferating calli into nutrient media, fortified with 2iP or NAA or BA alone (Al-Khayri and Al-Bahrany 2001; Taha et al. 2002; Aslam et al. 2011; Bouguedoura et al. 2017) or fortified with the combinations of NAA-2iP (Badawy et al. 2005; Alkhateeb 2006; Al-Khayri 2011a, b; Al-Khayri and Al-Bahrany 2012) or NAA-kinetin (Abul-Soad and Mahdi 2010) or NAA-2iP-kinetin (Al-Khalifah et al. 2006). Most recently, Gadalla et al. (2018) reported concurrent (secondary) somatic embryogenesis induced from somatic embryonic clusters in MS medium supplemented with 0.4 mg/l 2iP. The same was converted into multiple shoots using 0.4 mg/l BA and shoots were

rooted in 0.05 mg/l BA-ribozide. Most interestingly, it was observed from an array of reports (Table 2) that no PGR was required in several instances, wherein somatic embryos were induced from embryogenic callus (Al-Khayri 2001; Davoodi et al. 2002; Al-Khayri and Al-Bahrany 2004a; Sané et al. 2012; Boufis et al. 2014; Bhati et al. 2017; Abdolvand et al. 2018). Similar efficiency of PGR-free MS medium was observed during subsequent conversion of somatic embryos into plantlets (Fig. 2m), and in those cases, PGR-free MS basal medium was adequate to induce multiple shoots and roots, concurrently (Bekheet et al. 2001a, b; Taha et al. 2002; Bhargava et al. 2003; Al-Khalifah et al. 2006; Alkhateeb 2006; Taha et al. 2007; Sané et al. 2012; Mazri et al. 2017, 2018; Abdolvand et al. 2018). On the other hand, some of the date palm cultivars, that induced multiple shoots from somatic embryos in PGR-free MS medium, exceptionally required supplementation of auxin source in a very low concentration (0.1 mg/l NAA) for rooting of shoots (which were regenerated from somatic embryos) (Al-Khayri 2001; Al-Khayri and Al-Bahrany 2001; Al-Khayri 2010). However, such low auxin supplementation facilitated the enhanced root formation (Fig. 2n) (in terms of number and length) in comparison to that of the PGR-free medium.

To enhance the frequency of callus induction, callus regeneration, somatic embryogenesis and its conversion, multiple additives were supplemented with mainstream PGRs in basal media. AC (in a range of 0.1 to 3 g/l, or exceptionally 9 g/l) was the most preferred additive as reflected in most of the reports. The preference of AC over other additives might be attributed to its unique attributes of absorbing polyphenol produced across multiple biochemical activities during morphogenesis and in addition, it eradicates the luminosity for optimum callogenic, embryogenic, and rhizospheric atmosphere (Gantait et al. 2009; Suranthran et al. 2011). Apart from AC, the second most utilized additive in date palm callus culture and somatic embryogenesis was $AgNO_3$. In a number of reports, addition of $AgNO_3$ was described to be effective to facilitate callus-mediated somatic embryogenesis and their conversion in some specific media formulations such as in MS medium in supplementation with 2iP (Al-Khayri and Al-Bahrany 2001) or MS medium without any PGR (Al-Khayri and Al-Bahrany 2004a; Abdolvand et al. 2018). Supplementation of $AgNO_3$ was equally effective during induction of callus in MS medium supplemented with NAA plus 2iP (Al-Khayri and Al-Bahrany 2004a). Casin hydrolysate (CH) and yeast extract (YE) are the other two important additives that improved the callogenesis and somatic embryogenesis of date palm (Table 2). CH in combination with CW (Hosny et al. (2016) assisted in induction of somatic embryos from embryogenic calli or CH in combination with AC (Kurup et al. 2014) improved the conversion of somatic embryos into multiple shoots. Use of CH and YE in combination with AC aided in improved performance of PGRs during induction of embryogenic calli (Al-Khayri 2011b), and when AC was withdrawn from this combination, CH-YE resulted in somatic embryo development. Polyethylene glycol (PEG) (Al-Khayri and Al-Bahrany 2012; Alkhateeb 2006; Mazri et al. 2018) and polyamines like putrescine and spermidine (Hegazy and Aboshama 2010; El-Dawayati et al. 2018b) and AdS (Bhargava et al. 2003; Abul-Soad and Mahdi 2010; Sidky and El-Dawyati 2012; Al-Mayahi 2015; El-Dawayati et al. 2018b) are three the other mentionable additives that played crucial roles in assisting the high frequency callus induction and succeeding somatic embryogenesis.

Nonetheless, culture condition remained equally significant for fruitful induction of calli, their regeneration, and/or direct or callus-mediated somatic embryogenesis in date palm. Irrespective of cultivars, basal medium, PGRs, additives used, the explants were incubated in continuous dark condition for friable or embryogenic callus induction (Table 2). In the callus induction phase, the explants must not be exposed to light at all. The somatic embryos were reported to be induced in dark too, but for succeeding shoot and root development, the somatic embryos were exposed to light (with 12–16 h photoperiod provided by 28–100 μmol/m^2/s or 1000–3000 lx light intensity). The temperature of the culture room followed a range of 24–28 °C throughout the culture period. There is specific instance wherein culture room temperature kept higher (28 °C) during callus induction and later that was reduced (26 ± 2 °C) for somatic embryogenesis (Kriaâ et al. 2012). A reverse instance to this was reported where callus induction was observed at 25 ± 2 °C and subsequent regeneration was observed only at 28 ± 2 °C (Kurup et al. 2014).

Even though multiple factors have been assessed for establishment and improvement of callus culture and callus-mediated somatic embryogenesis, yet ample scope prevails to explore some other critical factors like thin cell layer and leaf disc explants, meticulous use of different kinds of basal media (apart from overexploited MS medium), as well as PGRs like meta-topolin, *N*-phenyl-*N*′-(1,2,3-thidiazol-5-yl) urea (thidiazuron) or zeatin, etc. In addition, focus should be given to induce somatic embryos directly from explants without any intervention of callus phase in near future.

Cell suspension culture

Cell suspension culture and subsequent somatic embryogenesis deliver various new opportunities for the genetic improvement date palm, creating the foundation for in vitro mutation and selection promoting mutants (Naik and Al-Khayri 2016). Yet, relatively limited number of attempts has been made in last two decades to establish cell suspension culture and its consequent conversion to somatic embryo in date palm.

Fki et al. (2003) achieved the initiation of cell suspension culture when the small fragmented friable calli,

induced in a very high level of (10 mg/l) 2,4-D-supplemented semi-solid MS medium, were transferred in to a much lower (1 mg/l) 2,4-D- and 300 mg/l AC-supplemented MS liquid medium. Sané et al. (2006) established suspension culture of cells from friable granular calli, induced in 2,4-D-supplemented MS medium, and successively, the suspension increased the number of somatic embryos when 2,4-D was withdrawn. Saker et al. (2007a) used fragments of friable and loose embryogenic calli of Egyptian semi-dry date palm cultivar "Sewi" in liquid MS medium (supplemented with 170 mg/l KH_2PO_4, 10 mg/l thiamine HCl, 200 mg/l glutamine, and 3% sucrose) for the initiation of cell suspension cultures. Later, the cell suspension stimulated somatic embryo production (~ 120 embryos/flask) when the same was subcultured in liquid MS medium, fortified with 0.2 mg/l NAA. Such high frequency of somatic embryogenesis might be attributed to the liquid state of basal medium that provides necessary nutrients to the proliferating suspension of cells much easily, owing to the large surface areas of the cells directly in contact with the nutrient medium. Zouine and El Hadrami (2007) assessed the efficiency of an important additive glutamine to develop embryogenic cell suspension and subsequent somatic embryogenesis in Bousthami Noir and Jihel cultivars of date palm. The fragments of granular embryogenic calli, developed from shoot tip explants, were sieved (with a 500-mm mesh size) and transferred to liquid ½ MS medium, supplemented with optimized levels of 2,4-D, BA, and glutamine and cultured on a rotary shaker (at 100 rpm and 25 ± 2 °C) that successfully induce cell suspension culture and ensuing somatic embryogenesis, its maturation and conversion. Following the protocol of Zouine and El Hadrami (2007), Abohatem et al. (2011) established cell suspension culture from shoot tips of Boufeggouss and Bouskri cultivars of date palm and reported an enhanced globular somatic embryogenesis in 0.3 mg/l BA-enriched liquid MS medium with weekly subculturing, to reduce oxidative browning of cells or tissues. Al-Khayri (2012) effectively established the growth curve of cell suspension, optimal "plating efficiency" (of 14%), and cell density (10,000 cells/ml medium), besides standardization of liquid medium and PGR formulations (MS medium with 10 mg/l NAA and 1.5 mg/l 2iP) for somatic embryogenesis. Ibraheem et al. (2013) reported an effective protocol for production of high quantity embryogenic callus mass (4 g) within 16 weeks of culture of the cell suspension. That embryogenic callus mass later developed into proliferating somatic embryos when the same was transferred to liquid MS medium with 0.1 mg/l NAA, 0.05 mg/l BA, and 1.5 g/l AC. Lately, the exploration of suspension cell culture became scarce, which need to be addressed in near future.

In vitro flowering

In the life cycle of date palm, the flowering stage is one of the utmost important characterizing phase that transforms its vegetative phase to reproductive one. Flowering is a complicated developing stage, which can be influenced by an array of physical and chemical factors. Apart from an efficient approach to understand the flowering physiology and the genetic mechanism involved in sex organ development (Masmoudi-Allouche et al. 2009), in vitro induction of flowers have manifold advantages, such as blooming of season-independent flower development, year-round production of secondary metabolites (for the plants with pharmaceutical importance), source of biotic contamination-free explants, and source of haploid production (Gantait et al. 2012; Panigrahi et al. 2018). Since 1987 (Ammar et al. 1987), there are merely four attempts (three reports since the past two decades) have been successfully made to induce in vitro flowering in date palm. Masmoudi-Allouche et al. (2009) studied in vitro flowering through assessment of floral developmental phases and the associated PGR combinations and their concentrations during in vitro flower induction from female inflorescence explants, collected in different developmental stages. A combination of IBA (4.92 μM) and BA (4.44 μM), supplemented in MS medium, induced as high as 90% hermaphrodite flowers with significant proliferation of stamens under continuous dark condition at 27 °C. However, the frequency of hermaphrodite flower induction is influenced by cultivars of date palm too. In this study, the authors further observed that differential developmental stages of female inflorescence played the most vital role in induction of hermaphrodism, wherein, a distinct growth stage of the explant ("staminodes' proliferation at the inner whorl") induced highest frequency of hermaphrodite flowers. Later on, Masmoudi-Allouche et al. (2010) reported in vitro flowering in some elite cultivars of date palm (such as Arichti, Bousthammi Noir, Boufeggous, and Deglet Nour), from epical vegetative meristem through optimization of specific components that were associated with photoperiodism. Alternate culture of explants in specific medium formulation with comprising MS basal medium and ½ strength Quoirin and Lepoivre's (QL) nutrient solution (Quoirin and Lepoivre 1977), fortified with [NAA (2.68 μM), BA (4.44 μM), kinetin (4.64 μM), and IPA (5.28 μM)] of without any PGR was the most efficient nutrient system that induced highest frequency of in vitro flowers under 16 h photoperiod in a low light (15 $\mu mol/m^2/s$) condition at 28 °C (day) to 24 °C (night). Jazinizadeh et al. (2015) observed in vitro flower induction during multiple shoot culture from young offshoot explants of Barhee cultivar of date palm. During their study, it was observed that a surge in the 2iP concentration from 4 to 12 mg/l resulted in voluminous expansion of explants with reduction in regular shoot bud formation; instead, indication of flowering was recorded. Well-developed inflorescence was observed in the MS nutrient medium, supplemented with 12 mg/l 2iP and higher sucrose level (6%).

Ex vitro acclimatization

An in vitro regeneration protocol is deemed complete only when the in vitro-derived plantlets undergo successful acclimatization. There are several crucial factors such as, potting mixture or substrate, humidity, and light intensity, which determine the survival of plantlets during acclimatization. "Rapid dehydration of plantlets and vulnerability to bacterial and fungal attack hampers the acclimatization process. For the proper hardening of *in vitro* plantlets, it usually takes about 4–6 weeks to survive under normal environmental conditions" (Gantait and Kundu 2017). In date palm, the influence of major factors and the consequent survival rates recorded in several reports are briefly highlighted in Tables 1 and 2. There are multiple reports available on in vitro plant regeneration via direct organogenesis (Al-Khateeb 2002, 2008; Abdulwahed 2013; Jazinizadeh et al. 2015; Al-Mayahi 2016a) or via callus-mediated somatic embryogenesis and subsequent plantlet development (Davoodi et al. 2002; Al-Khayri and Al-Bahrany 2004a; Badawy et al. 2005; Eshraghi et al. 2005; Al-Khalifah et al. 2006; Alkhateeb 2006; Asemota et al. 2007; Taha et al. 2007; Gueye et al. 2009a, b; El Fatih and Hind Badereldin 2010; Sani et al. 2010; Al-Khayri and Al-Bahrany 2012; Kriaâ et al. 2012; Sané et al. 2012; Sidky and El-Dawyati 2012; Abdolvand et al. 2014; Boufis et al. 2014; Abd El Bar and El-Dawayati 2014; Hosny et al. 2016; Bhati et al. 2017; Bouguedoura et al. 2017; Abdolvand et al. 2018; Gadalla et al. 2018) in date palm; however, none of them demonstrated acclimatization of in vitro regenerants. It is evident from Tables 1 and 2 that the majority of the protocols on adventitious shoot and root regeneration described the acclimatization methods, while almost two-thirds of the reported protocols on indirect organogenesis and/or somatic embryogenesis did not implement acclimatization process. Most of the researchers who carried out acclimatization of multiple in vitro developed plantlets via adventitious shoot culture observed that peat moss was almost indispensible as a part of acclimatization substrate (Fig. 2o). Use of peat moss in combination (1:2 or 2:1; *v*/*v*) with perlite (Khierallah and Bader 2007; Al-Mayahi 2014) or sand (1:2) (Khan and Bibi 2012) or vermiculite (Bekheet 2013a) or use of peat in combination with vermiculite (1:3) Fki et al. (2011) or gravel (1:1) (Mazri 2012, 2013; Mazri and Meziani 2013; Mazri 2014, 2015) ensured high survival (73–92.5%) of in vitro-regenerated plants following their acclimatization and field transfer (Fig. 2p). Comparable efficiency of peat moss was demonstrated in several reports on acclimatization of in vitro-regenerated plantlets derived via indirect organogenesis and somatic embryogenesis too (Table 2). For the improved post-acclimatization survival of plantlets, supplementation of soil and vermiculite in peat moss substrate (1:1:1) proved to be successful (Al-Khayri 2001; Al-Khayri and Al-Bahrany 2001; Al-Khayri 2010; Al-Khayri 2011a; Kurup et al. 2014). As an alternative to peat moss-based ex vitro acclimatization, use of soilrite/farmyard manure/sand (1:1:1) was of reported (Bhargava et al. 2003). There are quite a few reports that suggested the necessity of specified treatment, like fungicide or insecticide spraying for eradication of pathogen attacks during acclimatization. Apart from that, the other specifications of acclimatization process implemented were not mentioned in most of the reports. For the enhancement of the survival rate during acclimatization, the techniques like "increased aeration and carbon dioxide (CO_2) intensification in gas-permeable vessels" could be fruitfully applied because this technique improves the pre-acclimatization survival of in vitro plantlets.

Clonal fidelity assessment

Clonal fidelity of in vitro-regenerated plants is important for quality control. True-to-type and genetically similar plantlets are vital in ensuring successful production of in vitro plantlets identical to its parent source. There are several ways to access clonal fidelity including morphological identification, molecular markers, and protein markers. Among these, molecular markers offer a quick and accurate identification of clonal fidelity. A number of molecular markers have been developed for clonal fidelity assessment in tissue-cultured date palms (Al-Khalifah and Askari 2003; El-Assar et al. 2005; Saker et al. 2006). Among these markers that have previously been used for date palm in vitro-regenerated plantlets include random amplified polymorphic DNA (RAPD) markers, inter-simple sequence repeats (ISSR) markers, restriction fragment length polymorphism (RFLP), amplification fragment length polymorphism (AFLP), microsatellite markers, and start codon targeted (SCoT) markers. RAPD analysis is simple and rapid method for clonal fidelity assessment. In date palm, RAPD analysis was first reported by Corniquel and Mercier (1994). Three RAPD primers OPE01, OPB05, and OPE06 were used to differentiate between individuals from the cultivars Barhee, Deglet Noor, and Medjool. The use of RAPD markers for clonal fidelity assessment of in vitro-regenerated date palm plantlets is well studied. Early reports showed that RAPD markers successfully detected 4% variation in 70 date palm regenerants after 6–12 moths of in vitro culture (Saker et al. 2000). Thirty-seven random RAPD primers also showed no variations in tissue culture-derived date palm offshoots (Saker et al. 2006). Subsequently, nine RAPD primers were used to amplify 180 plantlets and showed identical banding patterns with their original mother plant (Ahmed et al. 2009). A sum of 30 RAPD primers successfully showed high similarity to mother plants in micropropagated date palm plants (Kumar et al. 2010). Thereafter, 180 in vitro plantlets were

analyzed with nine RAPD markers that amplified a total of approximately 60 bands with similarity to mother plants (Othmani et al. 2010). More recently, ten random RAPD primers produced amplification products ranging in size from 200 to 2600 bp in regenerated plants from an unknown cultivar and Ferhi cultivar of date palm. High similarity was observed between the cultivars with their mother plants (Moghaieb et al. 2011). Ten RAPD markers also showed a low level of polymorphism reflecting high genetic similarity between regenerated date palms cv. Zaghlool and their mother plant (Bekheet 2013a). Most reports on RAPD markers showed high success to determine clonal fidelity of in vitro-regenerated date palms. Some studies also reported the use of AFLP markers. AFLP banding patterns generated using 13 primer combinations showed that tissue culture-derived date palm offshoots belonging to cultivars Sakkoty, Gandila, and Bertamoda had genetic variations of 2.6, 0.79, and 1%, respectively (Saker et al. 2006). In another study, six primer pairs tested for their ability to generate AFLP banding patterns from DNA corresponding to the plant mother together with all the derived in vitro plantlets (Othmani et al. 2010). ISSR primers have also been employed to study clonal fidelity in in vitro studies of date palm. Twelve ISSR primers successfully produced 347 clear and reproducible monomorphic amplicons for micropropagated date palm plants (Kumar et al. 2010). Sixteen microsatellite markers [simple sequence repeat (SSR) markers] were employed to examine the genetic stability of 27 randomly chosen date palm plants from micropropagated date palms. No microsatellite DNA variation was observed among all micropropagated plants indicating high clonal fidelity in cultures (Kumar et al. 2010). More recent investigation used 18 SCoT primers to assess clonal fidelity in regenerated plantlets after cryopreservation. Out of 118 amplicons produced 114 were monomorphic suggesting low genetic variation and high genetic stability of regrown plantlets post-cryopreservation treatment (Al-Qurainy et al. 2015). Previous investigations have shown high success rates in using molecular markers to determine clonal fidelity of in vitro-regenerated date palm plantlets. RAPD markers however have been used more extensively due to its rapid, simple, and cost-effectiveness compared to other markers. Clonal fidelity assessment is an important prerequisite for in vitro production and successful methods of identification are essential to assure authenticity and true-to-type plantlets for commercial propagation of date palms.

Germplasm storage and conservation

There is a basic necessity in preserving the germplasm of date palm to achieve sustainable availability of commercial cultivars and protect the endangered cultivars (El-Dawayati et al. 2018a, b). Safe conservation of the genetic resources of date palm is facing various problems under field conditions or as seeds storage. The advantages of in vitro storage can be explored even in very limited space for germplasm conservation and for high potential of in vitro regrowth. No genetic erosion could be occurred, and under controlled conditions, the attacks of pests and diseases could be escaped. All these advantages give the superiority to in vitro preservation over the conventional germplasm conservation methods. Date palm germplasm can be stored in vitro in variety of forms including callus cultures, meristem tips, somatic embryos, shoot tips, and multiple shoot cultures (Bekheet and Taha 2013; El-Dawayati 2017). Technology of in vitro plant cell, tissue, and organ culture paves the way for three approaches of germplasm conservation:

Short-term storage via slow growth

Growth reduction studies for date palm germplasm with the aid of "slow growth storage" offer the solution for short- and medium-term storage, which can be achieved by reducing the culture temperature, modifying the environmental conditions and formulation of the culture medium (with supplementation of osmotic agents or/and growth inhibitors) (El-Dawayati 2017). Progress and prospect in vitro storage of date palm under low temperature has been reviewed by several researchers (Bekheet et al. 2001b; Hassan 2002; El-Dawayati 2008; El-Ashry et al. 2013; El-Dawayati et al. 2018a). Shoot tip and callus tissues of micropropagated Egyptian cultivars of date palm have been preserved for short term through protocol of low temperature at 5 or 15 °C, combined with optimized culture medium, supplemented with osmotic agents (Fig. 2q, r) (Bekheet 2017b; El-Dawayati 2017) or ABA growth inhibitors (El-Dawayati 2017). It was found that incubation temperature of 15 °C during slow growth storage of shoot tip, callus (El-Dawayati et al. 2011; El-Dawayati et al. 2013), and somatic embryo cluster explants of date palm exhibited the most adequate results for ensuing survival and recovery of conserved explants (especially conserved callus tissues). In addition, sucrose is found to be sufficient source of energy and osmotic agent, supplemented in conservation medium as reflected by the highest survival rates and effective regeneration after different storage periods (El-Bahr et al. 2016; El-Dawayati et al. 2018a). The supplementation of exogenous ABA also induces the adaptive response of plant cells and tissue to various environmental stresses during in vitro conservation of date palm (El-Dawayati 2017). Slow growth storage maintains the conserved explants quite conveniently to return to their normal growth conditions and give the facility to be transported among users.

Short-term storage via synthetic seeds

Synthetic seed system presents multiple applications for in vitro tissues, wherein it has great value of efficient in vitro short- or long-term storage and propagation of valuable germplasm (Gantait et al. 2015b). This approach offers easy handling, exchange, and storage of alginate-encapsulated germplasms under room temperature, or under low and ultra-low-temperature conditions for months or for several years. Unfortunately, limited number of studies have been conducted for development of synthetic seed and synthetic seed-mediated storage of date palm germplasms (Fig. 2s). In this concern, an initial study on encapsulation of somatic embryos of date palm was conducted by (Ibrahim et al. 2003). Subaih et al. (2007) studied cryopreservation of date palm embryonic callus via encapsulation-dehydration-vitrification and encapsulation-vitrification approach. Bekheet et al. (2005) and Bekheet (2017a) presented a valid method for induction of somatic embryogenesis, development of synthetic seeds via encapsulation of induced somatic embryos and their preservation in date palm. Bekheet et al. (2005) reported maximum conversion of stored synthetic seeds when those were developed with 3% (*w*/*v*) sodium alginate. An effective system for preservation of germplasm by artificial seeds was developed by (Fki et al. 2011, 2017). Encapsulated embryos of date palm could have been explored further, considering its benefits and hence this approach needs more intensive studies in date palm.

Long-term conservation via cryopreservation

Cryopreservation is an in vitro storage technique under ultra-low temperature for extended period of time, where all the physiological process of living cells are stopped and which ensures the genetic structures of conserved germplasm remains unchanged (Gantait et al. 2015c).

Studies for long-term germplasm preservation in date palm by using liquid nitrogen (LN) at − 196 °C have been described since the beginning of reports by Tisserat et al. (1981), Mater (1987), and MyCock et al. (1997). More recently, cryopreservation procedures of in vitro-established shoot tip, callus, and cell suspension explants of date palm were optimized by El-Dawayati et al. (2007), Bekheet et al. (2007), and Al-Bahrany and Al-Khayri (2012a). Several protocol of date palm cryopreservation were reported to be conducted through a number of procedures including cryoprotectant pre-treatments (such as preculturing the explants on media, enriched with osmotic active compounds), use of cryoprotectant chemical solutes, as well as dehydration treatments (Fig. 2t), followed by reliable post-freezing thawing and regrowth of conserved explants (Mandumpal et al. 2011; Engelmann 2014). Using air dehydration or/and sucrose sugars as osmotic agent added to pre-culture medium was found to be effective cryoprotectant pre-treatment for successive cryopreservation of shoot tip and embryonic callus explants (El-Dawayati et al. 2007; Bekheet 2017b). With the aid of cryoplate technique, cryopreservation of date palm pro-embryonic masses protocol was established that ensured high post-conservation recovery rates (Salma and Engelmann 2017). This technique facilitated the manipulation of explants, which were kept in the cryoplate (Niino et al. 2013). Fki et al. (2017) described the effectiveness of typical vitrification and droplet-vitrification, for cryoconservation of date palm meristems. They did not found any morphological variation between the regenerated plants from cryopreserved and non-cryopreserved explants, without any adverse effect of cryogenic treatment on upholding genetic integrity. With such varieties of cryopreservation techniques, it is possible to preserve the bank of viable tissues of highly exploited or endangered cultivars or species in vitro, with minimal maintenance effort and the possibility of obtaining complete plants as and when required.

Protoplast culture

The sexual incompatibility between equitably distant species makes it impossible for the development of hybrids that leads to a major hindrance in crop improvement program. In such cases, the isolated protoplasts (the cell without cell wall) and their fusion product, i.e., somatic hybrid, make it convenient for the improvement in the quality of crops. The explants for protoplast culture involve cotyledons, leaves, shoots, flowers, and roots. The best explant for protoplast isolation would be the embryogenic cell suspension in case of monocotyledons. But in rye, the immature inflorescence has been chosen as inducing friable callus established under suspension culture was taken as protoplast. Merely two cases of induction of callus from protoplast in date palm have been reported to date. Chabane et al. (2007) reported of protoplast culture via nurse technique from difficile date palm. Two genotypes of date palm, one from southeast of Algeria, i.e., Deglet Nour from Biskra, and Takerboucht from Adrar have been used. The former one is sensitive to fusariose pathogen but with good fruit quality, whereas the latter one is resistant to this pathogen while with lower fruit quality. The authors used young leaves and the shoot apical tip as their experimental material. Two culture systems were accessed: nurse culture and liquid culture. The culture density was 10^6. Viable protoplasts were obtained from both the genotypes. The incubation time, genotype, and the donor material were also affected the protoplast isolation. The nodular callus was the best donor material with a protoplast yield of 5.6×10^5 g per fresh weight in Deglet Nour while 4.95×10^5 g per fresh weight in Takerboucht. Importantly, the protoplast viability percentage was 80% at the time of introduction but this gradually

perished and 65% on the feeder layer whereas 60% in the liquid culture in Deglet Nour, likewise, with 57% survivability on feeder layer and 49% in liquid culture in Takerboucht. In the same year, the protoplast isolation study was reported by Rizkalla et al. (2007). They have isolated protoplast from young leaves of offshoots in Zaghloul and Barbee genotypes with the advent of 4% cellulase, 2% pectinase, and 1% hemicellulase. The best results obtained in the case of Barbee where the protoplast yield was 6.4×10^5 per gram. The use of 45 m stainless steel sieve was helpful in producing 5.8×10^5 protoplast with an effective removal of clumps. Later on, Titouh et al. (2015) reported a protocol on isolation and culture of protoplasts to be applied in somatic hybridization program, eventually for the advancement of date palm cultivars. As much as 57, 70, and 62% viable protoplast were isolated from Deglet Nour, Akerbouch, and Degla Beida cultivars of date palm, respectively. Such high viability of protoplast were attributed to the use of optimized maceration enzyme combination (comprising 1.5% cellulase, 1% macerozyme R10, and 0.5 M mannitol) for > 16 h with mild vibration. For protoplast purification, removal of fragments of cells and for ensuring highest protoplast recovery, the use of 21 or 25% sucrose mat was proved to be most efficient. Subsequently, for preservation and cell wall regeneration of isolated viable protoplasts, use of semi-solidified MS medium, fortified with 2,4-D (2 mg/l) and BA (0.5 mg/l), was reported to be highly effective.

Consequently, the combinations of these protoplasts can produce a superior quality of date palm that can enhance disease resistance capability along with higher yield. Also, the perfect combination of plant growth regulators for shoot induction from callus obtained from protoplasts and further conversion of these protoplasts into synthetic seeds with the fortification of alginate may boost germplasm conservation-related research, even though substantial development has been recorded in protoplast culture since the past 50 years in brassica, citrus, eggplant, potato, and tobacco, etc., to yield novel commercial varieties. On the other hand, being a recalcitrant species, only a limited advancement has been reported on isolation, purification, and regeneration of protoplast in date palm, and in addition, no successful somatic hybridization in this crop has been described yet. Generally, the optimization of regeneration protocol remains crucial for any protoplast culture system. Certain resemblances exist between plant protoplasts and mammalian stem cells in terms of their responses towards particular growth regulators. Hence, animal and plant scientists are encouraged to jointly tackle the challenges that arise during protoplast culture and its regeneration system (Assani et al. 2011).

Genetic transformation

Most of the researches on date palm have been concentrated on in vitro propagation and molecular characterization. However, this economically valuable plant is a target for various insects and diseases (Al-Khayri and Niblett 2012). For this reason, there is a great demand for a powerful tool to produce new date palm cultivars that could display resistance to insect pests and other diseases. Plant biotechnology offers a reliable and stable approach to develop new improved date palm cultivars (Jain et al. 2011). Gene silencing or RNA interference has great potential to control disease and insect pest infestation in date palm (Niblett and Bailey 2012). In addition, several gene transformation methods such as *Agrobaterium*-mediated technique and particle bombardment approach have been used in date palm species to address the issue of disease pest infestation (Izawati et al. 2015; Parveez et al. 2015). However, the successful application of this technology requires the availability of a reliable and easy transformation system that could provide efficient gene expression following transformation (Solliman et al. 2017). Since the past two decades, significant effort has been implemented on transformation techniques mainly focused on African oil palm (Abdullah et al. 2003, 2005). However, research attempts to develop genetically transformed date palm is still lacking finesse (Saker 2011).

According to available reports on date palm, regarding *Agrobacterium*-mediated transformation methods, no conclusive report is available so far. However, Saker et al. (2009) successfully developed effective *Agrobacterium*-mediated transformation methods using date palm embryogenic callus that led to the establishment of its gene transfer system. Another study reported the optimization of direct gene transformation by particle bombardment method in date palm cells (Habashi et al. 2008; Saker et al. 2006, 2007b). In this study, gene construct was developed (harboring a cholesterol oxidase gene), which provided the transgenic plants with resistance from insect attacks. This gene construct was introduced into embrogenic callus and successful transgenic plants were developed. The significant factors that affected gene transfer by biolistic approach were the flight distance of microprojectiles and their size and applied pressure, type of cell and tissue (Iida et al. 1990).

Reverse genetics (from gene to phynotype) is a powerful tool in fuctional genomics investigation and molecular breeding of crops, which is mainly based on gain and loss of gene function theory (Colbert et al. 2001). This method could be useful to study salt tolerance in date palm or related species. Diverse approaches are available to create random point mutations, like the use of ionizing radiation therapy, ethyl mesylate ($CH_3SO_3C_2H_5$), transposable elements or jumping genes, RNA interference (RNAi), and transfer DNA (T-DNA) mutagenesis, which could additionally be

helpful to recognize the particular genes related to salt tolerance in mutants. This method along with next-generation sequencing technologies can create a powerful toolset for finding the genes of interest. Nevertheless, these approaches need a well-established and effective in vitro regeneration and gene transformation (*Agrobacterium*-mediated) system for date palm (Mousavi et al. 2014).

Secondary metabolite production in vitro

Particular consideration has been given to secondary metabolites of date palm and their health-benefit claims and potential use in the booming industries of functional foods and nutraceuticals (Biglari et al. 2008; Bekheet 2013b). In vitro cell culture technology has become a prosperous approach for the biomass production and generation of bioactive compounds (Naik and Al-Khayri 2017). Secondary metabolites of date palm involve several phytochemicals including, essential oils, flavonoid, polyphenols, steroids, and tannin. Steroids, cholesterol, and β-sitosterol were separated from the in vitro tissues of two cultivars of date palm (Zaghloul and Sewy) and were identified using thin layer chromatography (TLC) by El-Sharabasy (2000). Flavoniod content in multiple date palm cultivars was determined by Saker et al. (2002) from in vitro multiple shoot culture (in MS media contained 10 mg/l 2,4-D plus 3 mg/l 2iP). The antioxidant and estrogen-like activities were determined from the extracts of Egyptian date palm seeds (Ammar et al. 2009). This finding showed that polar and non-polar extracts have estrogen-like and antioxidant activity although in different degree. From another standpoint, accumulation and production of peroxidase and phenolics from suspension cultures of Egyptian date palm (Samany and Zaghlool) was studied (Taha et al. 2010). The leaf primordial and shoot tip were cultured on modified MS medium supplemented with 5 mg/l BAP plus 3 mg/l 2,4-D and 3 mg/l 2iP for callus inducation and transferred in suspension culture for further growth. Well-developed cells were collected from suspension culture for determined activities of peroxidase and phenolic compounds. In addition, a favorable and effective method for augmented peroxides and total phenolics production from Zaghlool (Egyptian date palm) cells in reactor (stirred tank) was developed (Taha et al. 2012). In another study by El-Sharabasy et al. (2012), high-frequency embryogenic callus having high callus volume and callus weight, subsequent embryoids formation (1.73 g/embryo), and shoot regeneration (2.33 shoots/embryo) was reported following the use of amino acids [glutamine, spermidine, and asparagine (50, 250, and 500 mg/l)]. In addition, as high as 366% higher quantity of total steroid production was observed in 250 mg/l glutamine-supplemented medium than that of the control medium. Enhancement of steroid production from calli was reported by the application of osmoticum (mannitol, sorbitol, and sucrose) also (El-Dawayati et al. 2012). An important and reproducible protocol was described by El-Sharabasy and El-Dawayati (2017) for steroid production (and its analytical steps) from date palm embryonic callus using a bioreactor technique. Al-Khayri et al. (2017) described a protocol to evaluate proline accumulation under salinity stress [calcium chloride (11.96 $CaCl_2$ g/l), potassium chloride (12.06 KCl g/l), and sodium chloride (9.45 NaCl g/l) and drought stress [polyethylene glycol (0–30%, *w/v*)] with different exposure time (1–12 days). The authors concluded that drought stress was increased the proline accumulation in all concentrations; however, the salt stress dependent on exposure time up to 6 days increased while extending further 9 days decreased the proline accumulation (Al-Khayri et al. 2017). Naik and Al-Khayri (2017) described step-by step procedures for cell suspension culture on MS medium supplemented with NAA, 2ip, and 2,4-D. After 11- and 12-week-old cultures were extracted, different secondary metabolites such as catechin (10.611 and 14.091 mg/100 g DW), caffeic acid (0.491 and 0.792 mg/100 g DW), apigenin (0.155 and 0.248 mg/100 g DW), and kaempferol (0.02 and 0.04 mg/100 g DW) respectively were estimated (Naik and Al-Khayri 2017). The embryogenic callus contained the lowest concentration of phenols (36.50 mg/100 g FW) and flavonoids (23 mg/100 g FW); however, zygotic embryo contained the maximum amounts of free amino acid (620.5 mg/100 g FW), flavonoids (341.25 mg/100 g FW), phenols (258.4 mg/100 g FW), protein (273 mg/100 g FW), and malondialdehyde (MDA; 2.9×10^{-3}) were recorded. In date palm, polyamines (an organic compound having two or more amino groups) are often detected in callus and other in vitro cultures organs and tissues (El-Hadrami 1995).

Bioreactor: large-scale propagation

Bioreactor for large-scale propagation of date palm could improve multiplication rate and weaning success, reduce micropropagation time, and reduce the cost (Almusawi et al. 2017). Othmani et al. (2009b) reported the propagation of date palm plantlets in TIB-bioreactors. In their experiment, they developed temporary immersion cultures by dipping the cultures for 5 min every 8 h for 6 weeks. Almusawi et al. (2017) described a novel date palm micropropagation protocol via somatic embryogenesis and organogenesis using Plantform bioreactor contained mixed system (liquid and semi-solid medium). This system achieved reduction in costs and reduced the time frame for plantlet production (less than 3 years). Taha et al. (2012) investigated the effect of biotic elicitors (*Aspergilus niger* and methyl-jasmonate) on cell growth patterns of date palm and subsequent accumulation of bioactive compounds using a bioreactor. The maximum value of cell growth parameters and the highest content of bioactive compounds were obtained from modified MS medium contained with *A. niger* (0.1%) combination with

methyl-jasmonate (100 μM) after 10 days of the cultured (Taha et al. 2012). A simple and effective in vitro protocol was developed by El-Sharabasy and El-Dawayati (2017) for large-scle production, identification, and determination of steroids in date palm culture. For this, shoot tip was cultured on modified MS medium supplement with 100 mg/l 2,4-D plus 3 mg/l 2iP and 3 g/l AC (for callus induction); the induced calli were transferred to the same medium with reduced level (10 mg/l 2,4-D) of 2,4-D for the development of embryogenic calli, which were then cultured in 0.1 mg/l NAA-supplemented liquid MS medium to develop cell suspension culture in large scale. Different culture stages (especially embryogenic callus formation and cell suspension) pyruvic acid (0.01 mg/l) was added as a precursor to induce the production and accumulation of the total steroids in a submerged stirred bioreactor. Finally total sterol was estimated by gas chromatography-mass spectrometry (GC-MS) and gas-liquid chromatography (GLC). A crucial problem in the successful commercial tissue culture of date palm is the undesirable decrease in shoot multiplication rate in agar solidified-medium. This can be corrected by the usage of liquid cultures that are more competent for automation technique, which is indispensable for commercial scaling-up production systems. For instance, the production of cotyledonary somatic embryos in suspension cultures were 17-fold higher than that on the semi-solid medium. The subculture of multiple shoots in temporary immersion bioreactor evidently performed better (5.5-fold increase in shoot regeneration) in comparison to the ones on semi-solid medium.

In vitro selection against biotic and abiotic stress

Date palm plants generally faced the problem of salinity and drought stress particularly in arid zones. The modern biotechnological techniques such as in vitro studies may be promising in surpassing these circumstances. Few significant studies on influence of stress such as drought stress and salinity stress on date palm have been reported.

The initial study of water stress on two varieties of date palm (cv. Barbee and Hillali) was reported by Al-Khayri and Al-Bahrany (2004b). As the water stress increased a progressive reduction in growth, increase in proline accumulation was observed. Between these two varieties, cv. Barbee was observed to show more tolerant capacity to drought stress. The PEG concentration was also accompanied with enhancement in endogenous proline content. The other report on screening of date palm against salinity (Rizkalla et al. 2007) involved the protoplast culture, wherein the isolated and screened protoplasts were cultured in 100 mg/l 2,4-D and 3.0 mg/l 2iP supplemented with 0 to 300 mM KCl or NaCl with a difference in range of 50 mM. After 2 weeks of incubation, the protoplasts number and division were significantly increased at 100 mM KCl or NaCl in Zaghloul genotype. But as the dose increased, i.e., at 250 and 300 mM NaCl the protoplasts number and division drastically decreased. A similar report on in vitro screening of some salinity tolerant indigenous date palm cultivars (namely, Khlass, Khnazy, Merzaban, and Khasbat Ausfoor) testified the influence of NaCl on immature embryos-derived “dedifferentiating” and “fast-growing” phases calli (Al-Mansoori et al. 2007). It was observed that “dedifferentiating” phase of calli were more competent in ascertaining the difference in salinity tolerance amid the date palm cultivars in comparison to that of the “fast-growing” phase. The latter phase was incapable to distinguish substantial variance in response against salinity stress, while the “dedifferentiating” phase was efficient to identify that disparity amid the cultivars. According to Al-Mansoori et al. (2007), the superior dedifferentiation procedure might be associated to proline level that regulates the intracellular osmotic force between cytoplasm and vacuole, because proline (believed to be a similar osmoticum) level was raised substantially in both phases of calli, responding against salinity. It was further noted that Na^+ concentration in calli was considerably elevated with the rising of NaCl level, while K^+ concentration lessened triggering a substantial drop in K^+/Na^+ status of calli. Selection from three date palm cultivars, namely, Samani, Sewy, and Bartamuda against salinity stress was reported using exposures of 0, 4000, 8000, and 12,000 ppm NaCl for a period of 12 weeks (El-Sharabasy et al. 2008a). Notably, significant shoot length was obtained irrespective of all the three species up to 4000 ppm and thereafter decrease in 8000 and 12,000 ppm, although insignificant difference in shoot length under 0 ppm was observed in all three varieties. In the subsequent study of El-Sharabasy et al. (2008b), it was observed that isozyme polymorphism could be an efficient tool (biochemical marker) to characterize specific embryogenic calli and in vitro-regenerated plantlets of date palm (cv. Sakkoti, Zaghloul, and Sewy) that exhibited tolerance against water stress. The authors identified quite a few cultivar-specific as well as cultivar-independent peroxidase and esterase bands, associated with embryonic callus and plantlets that displayed distinct response to drought stress (based on degree of response to PEG). The use of sodium chloride with different concentrations (0 to 2%) as stress and proline (0 to 100 mg/l) as antagonist of stress has been used in the development of callus and somatic embryogenesis of date palm cv. Ashkar (Jasim et al. 2010). The use of proline in presence of NaCl encouraged the synthesis of more metabolites such as total soluble carbohydrates and proteins in both the callus and somatic embryos. Therefore, the stress retarder such as proline could nullify the role of the salt stress. In another study, two date palm genotypes (Shamia and Amri) have been investigated based on their morpho-physiological response towards the drought stress during in vitro conditions (Helaly and El-Hosieny 2011). As a result, reduced root length, plantlet height, and swelled embryos along with less number of embryos were observed. Though the photosynthetic rate, stomatal conductance, and transpiration

decreased quite significantly in both the genotypes, the water use efficiency was enhanced with increase in the water stress. Likewise, the quantum yield was also decreased under water stress condition. Thus, the chlorophyll fluorescence study could be an effective investigation used by plant breeders to know the quality of date palm effective towards water stress. In a following report, it was documented that date palm (cv. Barbee) calli, derived from shoot tips of offshoots were exposed to salinity stress with a concentration of 0 to 225 mM NaCl (Al-Khayri 2011c). At 25 mM NaCl, the callus weight was increased after a gap of 6 weeks. But in 125 mM NaCl and higher than that, callus growth was hampered and almost inhibited. Physiologically, proline accumulation was also enhanced as correlated with callus growth inhibition. As well as the Na^+/K^+ ratio increased along with callus growth inhibition and proline accumulation. A similar report of assessment of salinity tolerance of calli was reported by Al-Bahrany and Al-Khayri (2012b) in a cell suspension culture, treated with NaCl, KCl, and $CaCl_2$. It was observed that callus waight and proline content started to decline with the increased concentrations of salts used. Likewise, the simultaneous interaction of sodium and proline caused remarkable reduction in the germination time, percentages, and lengths of plumules and radicals observed in date palm cv. Barhee (Al-Zubaydi et al. 2013). The pretreated (KCl, $CaCl_2$ 0.2 M, or oleic acid 100 ppm) Ruzaiz date palm could increase the thermo-tolerance capacity (Al-Busaidi and Farag 2015). Similarly, pretreated 12 salt treatments added to MS medium and found out that retention of Na^+ in roots and excluding the Na^+ from the leaves of in vitro date palm (Al-Khateeb et al. 2015). It was evident from a study that there was DNA polymorphism observed when callus of Hillawi cv. of date palm were fortified with *A. niger* culture and NaCl (68.45, 137, and 205.34 M) as biotic and abiotic stresses respectively (Abass 2016). In the recent study on cv. Nersy of date palm, the use of salicylic acid and ascorbic acid once introduced along with two stress levels of 75 and 150 mM NaCl, there was significant increase in the antioxidant enzymes such as superoxide dismutase and ascorbate peroxidase alongside synthesis of additional protein bands (Al-Mayahi 2016b). Thus, these studies related to the date palm varieties provide an understanding and developing strategies to select the somaclonal variants to tolerate these stress conditions.

In vitro mutagenesis

The date palm faces various diseases such as Bayoud (whitish discoloration of diseased fronds) in North African Saharan and sub-Saharan regions caused by a soil-borne fungus *Fusarium oxysporum* f. sp. *albedinis* (FOA). This leads to severe economic loss to the growers. In this backdrop, it needs the exploitation of genetic variability that creates the opportunity for the development of new cultivars. Induction of genetic variability demands the support of somaclonal variation derived from tissue culture along with physical and chemical mutagens and T-DNA insertional mutagenesis. There are two reports of in vitro mutagenesis in date palm. As reported by Omar and Novak (1990), the germination of the somatic embryos was persuaded by the treatment with ethylmethanesulfonate (EMS), a mutagen. The higher concentration (49.38 and 98.76 mM) of EMS was predominantly influenced the germination of seed and subsequently developed into plants. But the uptake of EMS required dimethyl sulfoxide (DMSO) as a carrier agent. According to a project study by Jain (2006), the gamma-irradiated in vitro propagated date palm has shown better adaptation towards the Bayoud disease as compared to the control. He had chosen three in vitro cultivars of date palm, i.e., Deglet Nour, Mejhool, and Tegaza and irradiated with gamma ray. He proposed that somatic embryogenesis technology can be opted for isolation of mutants and recommended for the clonal propagation of date palm in huge numbers by using the bioreactor. The toxin derived from Bayoud disease-causing fungus FOA recommended for in vitro selected date palms. He observed that these toxin-treated in vitro plants survive significantly in countries like Morocco, Algeria, and Tunisia. Thus, the use of in vitro mutagenesis may be helpful in the induction of dwarfism, faster growing plants along with disease resistance capacity. In addition, there is an ample prospective of induced mutations for the upgrading of the varietal of date palm in support of traditional practices and biotechnology. It is also highly desirable to choose from the selected in vitro propagules and modify the micropropagation protocols for each of the elite genotype and figure out the upper limit of subcultures before the somaclonal variations appeared and fixed the new fresh cultures.

Conclusion and future

Specification of suitable, consolidated, and comprehensive information concerning the optimized protocols, such as basal medium, PGR formulations, carbon source, additives, and growth room conditions should be furnished so that the same could be immediately practiced in multiple other cultivars of the same species or the other critically endangered species of the same genus for the standardization of appropriate in vitro protocols for micropropagation, conservation, and genetic transformation. Recent progress has been made to enhance the initiation of multiple shoot culture, callus culture, and somatic embryogenesis to ensure sustainable supply of quality propagules, in order to promote in vitro-mediated advanced crop improvement and germplasm conservation in date palm. Use of cell suspension culture, protoplast culture, in vitro mutagenesis, and in vitro selection of plant tissue and organs, along with the development of transgenic date palm plants are the evidences of substantial achievements in the past two decades. Several pertinent technological improvements have

been reported so far; however, it remains unexplored till date in date palm. The application of thin cell layer culture, photoautotrophic micropropagation, sonication and ultrasound, or magnetic fields to adjust or enhance in vitro growth of tissue in date palm, are all techniques that warrant exploration.

Utilization of an array of unexploited explants like thin cell layer and leaf disc could be collected, sterilized, and assessed for their possible efficiency to induce adventitious buds, callus, and somatic embryogenesis. Potential use of other basal media, a range of potential cytokinin and auxin sources, along with newly introduced additives are yet to be explored in date plam. Multiple critical factors like meticulous use of different kinds of basal media (apart from the overexploited MS medium), as well as PGRs like meta-topolin, *N*-phenyl-*N′*-(1,2,3-thidiazol-5-yl) urea (thidiazuron) or zeatin should be assessed for their efficiency to achieve enhanced regeneration frequency. In addition, focus should be given on to induce somatic embryos directly from explants without any intervention of callus phase in near future. Lately, the exploration of suspension cell culture and in vitro flowering became insubstantial, which requires urgent attention. Special issues such as recalcitrancy, phenolic exudation, tissue browning, hyperhydricity, undesirable adventitious root development during shoot multiplication, and abnormal and asynchronous differentiation of somatic embryos need to be prioritized during the establishment of date palm plant tissue culture and the other approaches that rely on in vitro regeneration system.

The utilization of molecular marker techniques (chiefly involving RAPD and ISSR and limited number of AFLP markers) successfully determined the genetic uniformity or somaclonal variation status anomg in vitro regenerants of date palm; however, there is ample scope of using other dominant and co-dominant markers like SCoT, SSRs, EST-SSRs, and RFLPs to bring further improvements in the results. Involvement of nanotechnology is another booming option that can be exercised for biotechnological improvement in date palm. Considering the immense commercial stature of date palm, the in vitro-based biotechnological approaches explored in the present review provide a platform to further both fundamental and fresh cutting-edge researches in near future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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