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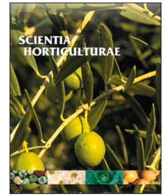

The potential to propagate coconut clones through direct shoot organogenesis: A review

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ABSTRACT

The production of a single stem, which is a characteristic of most members of the Arecaceae family, is a distinctive feature of the coconut palm (*Cocos nucifera* L.), oil palm (*Elaeis guineensis* Jacq.) and date palm (*Phoenix dactylifera* L.). This feature has been a constant for many palm species since the late Cretaceous period, but rare cases of shoot branching in coconut do occur. This original feature leads to multiple stem formation and is considered to be a novel trait. An understanding of the role of plant growth regulators in shoot branching offers a new pathway to produce multiple stem buds in a palm through *in vitro* culture. As the world's coconut crop is now facing a decline in productivity due to palm aging, extreme climate events, and biotic and abiotic stresses, this approach could help to meet the increasing demand for elite coconut seedlings. Hence, this review examines the potential for direct shoot organogenesis to be used as an option for the clonal propagation of coconut. Cytokinins are essential to induce direct shoot formation. Although more research is needed to confirm the best method to produce coconut clones, based on the literature, for direct shoot organogenesis, it is expected that a treatment of thidiazuron only (for embryo) or in combination with picloram (for immature inflorescence) in Eeuwens Y3 medium would produce the best likelihood of success. The feasibility of this pathway of plantlet production in coconut is bolstered by the relatively easy direct shoot organogenesis now available as an alternative clonal propagation protocol for oil palm and date palm.

1. Introduction

The three major crops from the Arecaceae family are coconut (*Cocos nucifera* L.), oil palm (*Elaeis guineensis* Jacq.) and date palm (*Phoenix dactylifera* L.) (Xiao et al., 2019). These species account for the majority of the family's economic importance, which comes in third after the Poaceae and Fabaceae (Meerow et al., 2012). Some members of this family are unique among plants as they are the longest living plants with "stem cells" (shoot and root apical meristems) that stay active throughout the palm's lifetime. These stem cells, which are responsible for the primary developmental processes, also lead to the distinctive features of palms as being tall and long-lived plants in general. This feature is particularly unique to coconut (Nayar, 2018).

About half of the coconut palm populations in commercially producing countries are facing senility and declining productivity. The traditional replanting approach using seed is not producing sufficient elite seedlings to meet the demand for high-quality planting material (Kong et al., 2019). It has been proposed that traditional breeding could be replaced by generating high quantities of coconut plantlets from tissue culture via somatic embryogenesis (SE) (Kong et al., 2020).

Over the past 60 years, SE was generally considered the only biotechnology to produce clonal coconut palms (Nguyen et al., 2015). However, direct shoot organogenesis, a less studied plant regeneration pathway, could be an alternative method for the clonal propagation of coconut. Direct shoot organogenesis involves the formation of shoots directly form a tissue without an intermediate callus phase (Bhatia and Bera, 2015), regardless of the origin (axillary or adventitious) in this context. This is followed by rooting of the regenerated shoots (Abahmane, 2011). So far, very little research has been undertaken on the direct shoot organogenesis of coconut (Chandrakala et al., 2019; Raju, 2006; Vidhana Arachchi and Weerakoon, 1997). The reason for this is most likely because coconut shoot tips do not have an apparent capacity

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to produce vegetative lateral bud outgrowths (Tisserat, 1997), as the axillary meristem differentiated from the indeterminate shoot apical meristem transforms into reproductive meristem at each leaf axil of coconut (Perera et al., 2010).

Hence, clonal propagation of coconut was thought to be achievable only through SE (Tisserat, 1997). However, there must be exceptions, as there have been reports of vegetative branching in coconut palms and seedlings under both natural and tissue culture conditions (Fisher and Tsai, 1979; Mondal, 2014). Multiple *in vitro* shoots, which have been reported by Romyanon et al. (2015) and Raju (2006), induced directly from organized tissue, such as shoot tips (axillary origin) of oil palm and immature inflorescence (adventitious origin) of coconut, without callus intervention.

Regeneration through direct shoot organogenesis may be a more secure pathway as compared to SE in coconut, because the risk of somaclonal variation is thought to be lower (Abahmane, 2011), due to the elimination of the callus phase, which has higher potential for somaclonal variation (Su, 2002). Callus is unorganized tissue which is vulnerable to instability (Pasqual et al., 2014), and stress imposed by callus formation (a differentiation phase with uncontrolled cell division) can lead to higher somaclonal variation (Krishna et al., 2016). Somaclonal variation in oil palm propagated via SE that resulted in floral abnormality known as mantled (*ca.* 9.7% occurrence) is a notable example. It causes a significant impact on oil yield (Weckx et al., 2019), and occurrence of the abnormality is spatially heterogeneous between clones as well as within the inflorescences and flowers of the same clone (Jaligot et al., 2011). The mantled abnormality is epigenetic and thus, cannot be identified with conventional structural molecular markers (Morcillo et al., 2006). Therefore, direct shoot organogenesis could be a better solution for cloning coconuts.

In addition, direct shoot organogenesis (in 8 to 10 months) has been shown to be a faster way to produce plants compared to SE (in 9 to 12 months) in oil palm. As for the production rates for oil palm, five plantlets per explant were formed from direct shoot organogenesis (Romyanon et al., 2015), while 8 to 15 plantlets per explant were regenerated via SE depending on the system used (Gomes et al., 2016). Although the production rates for oil palm via SE may be higher, and likely similar for coconut, it is worthwhile investigating direct shoot organogenesis, with potentially lower risk of somaclonal variations, as an alternative clonal propagation pathway to contribute to the generation of elite coconut planting material.

2. Non-branching coconut and shoot branching potential

Most of the members of the Arecaceae have only one apical bud with an unbranched stem. They are among the least branched of all terrestrial plants, perhaps because the fibrous monocot stem is relatively weak and unable to support branches. However, the non-branching characteristic of palms can be advantageous, as it allows the energy produced to be distributed for the development of the trunk, fruit, leaves and roots rather than lateral branching. The single stem feature provides extreme physical flexibility to withstand external forces occurring within the environment such as cyclonic winds. As adaptation is the fundamental key to evolution, retaining an apparently simple unbranched structure of the palm over millions of years is of significance (Windsor-Collins et al., 2007).

Coconut is a single-stemmed, non-branching palm which does not produce vegetative buds, such as the offshoots (of limited number) seen in date palm during the juvenile phase (Blake, 1983; Tisserat, 1997). The shoot meristem is connected to the young stem made up of larger cells, with large starch granules and sugar reserves, and contains numerous procambial strands. These procambial strands are responsible for the formation of xylem and phloem cell bundles which are scattered throughout the palm stem (Niral and Jerard, 2018), there being no peripheral vascular cambium like that found in dicotyledonous species. Hence, stem thickening in palms depends on primary growth close to the growing tip (Windsor-Collins et al., 2007), and the palm will die if the coconut apical bud is destroyed.

Although coconut is recognized as a non-branching palm (Edelman and Richards, 2019), there are rare exceptions such as shoot branching (Fig. 1) and the formation of suckers from the underground portion of the bole under field conditions. These are economically interesting phenomena which reveal the possibility of vegetative propagation of coconut (Niral and Jerard, 2018). Some branching events in coconut can be the result of mechanical injury at the growing point or terminal bud (Fisher and Tsai, 1979; Mondal, 2014), observed occasionally as a result of damage from storms and cyclone. Injury leads to the formation of a neomeristem (newly developed meristem), of adventitious origin, that forms a new growing point. However, branching in coconut can also be due to dichotomy and stem fasciation (Niral and Jerard, 2018). Dichotomy is the division of shoot apical meristem (Edelman and Richards, 2019), while stem fasciation is an enlargement of the shoot or floral meristem resulting in a change of stem shape and leaf number and a disturbance in organotaxis (Choob and Sinyushin, 2012).

Although shoot branching is generally linked with the spatio-temporal regulation of axillary bud outgrowth (Rameau et al., 2015), branching in coconut at the shoot tip is thought to correlate with the development of neomeristems which are of adventitious origin (Niral and Jerard, 2018). Nevertheless, axillary branches are generally produced at the shoot tip (when the shoot meristem is intact) to form multiple shoots. This phenomenon can be observed in the *in vitro* regeneration of wheat (*Triticum aestivum* L.), as both axillary and adventitious shoots are produced from the shoot apex (Ahmad et al., 2002). Thus, the shoots produced from the shoot tip can be of axillary or adventitious origin and the precise origin can only be confirmed through anatomical examination (George and Debergh, 2008).

Shoot branching, brought about by axillary bud outgrowth is regulated by multiple pathways which include plant growth regulators (PGRs) (auxins, cytokinins, gibberellins and strigolactones), signals such as sugars and molecular actors of plant phase transition. Environmental signals, such as shade, can also influence shoot branching (Rameau et al., 2015). Strigolactone, a carotenoid derivative, has an emerging role as a PGR (Pandey et al., 2016). It has been demonstrated to regulate shoot growth redistribution by rapid modulation of auxin transport and to control the depletion of the auxin efflux protein, PIN-FORMED 1 from the plasma membrane of xylem parenchyma cells in the stem. Consequently, it can affect shoot branching as the shoot apices compete for the same auxin transport pathway to the root (Shinohara et al., 2013).

Similarly, organogenesis is influenced by the genotype, the physiological state of the explant and its size, the PGRs applied and the environmental conditions. The exogeneous auxin and cytokinin ratio applied

Fig. 1. Branched coconut palm growing in natural conditions in (**a**) Bali and (**b**) Banyumas, Indonesia. Photos courtesy of Sisunandar Sudarma.

is a crucial factor for *in vitro* differentiation via organogenesis (Priyadarshini and Pandey, 2019). The factors affecting axillary shoot formation and adventitious organogenesis are similar. The similarity infers the possibility of evaluating these factors for the success in establishing a direct shoot organogenesis system for coconut.

3. Organogenesis

Organogenesis is generally defined as the formation of shoots or roots differentiated from a single cell or a cluster of cells (Su, 2002). As organogenesis literally means the formation of organ (Priyadarshini and Pandey, 2019), *in vitro* shoots can therefore arise from axillary or adventitious buds. When axillary shoots originate from pre-existing meristems, this is commonly known as enhanced axillary branching (Phillips and Garda, 2019). Pre-existing meristems in this context are apical or axillary meristems. In contrast, the development of new organs (shoot or root) from cells lacking preformed meristem is regarded as adventitious origin (Gahan and George, 2008). Adventitious organogenesis usually arises from organized tissues at an unusual location of the plant with or without being surrounded by the attendant callus. Apart from the two origins mentioned earlier, shoots and roots can also arise anew from unorganized proliferating callus which is regarded *de novo* origin and this development pathway is known as *de novo* organogenesis (Phillips and Garda, 2019). Hence, *in vitro* organogenesis in coconut can theoretically occur from three origins: axillary, adventitious or *de novo* origins.

Generally, organogenesis can be categorized into direct or indirect organogenesis. Direct organogenesis allows the shoot or root to form directly from the tissue without a callus phase (Bhatia and Bera, 2015; Cardoza, 2008; Su, 2002). This happens when the cells undergo a trans-differentiation process where one cell type is transformed into another cell type without dedifferentiation occurring (Rosspopoff et al., 2017). Thus, direct shoot organogenesis in coconut is defined as shoot organogenesis arising from both axillary and adventitious origins without callus intervention for the purpose of this review. Whilst, indirect organogenesis normally involves a dedifferentiation stage which involves a callus phase. Commonly used system for *de novo* organogenesis involve a two step-procedure (Sang et al., 2018), where a true callus is first induced, and shoots or roots are then developed from the true callus (Phillips and Garda, 2019).

Generally, *in vitro* plant regeneration by an organogenic pathway often begins with shoot formation from the explants in one medium, then induction and development of the root in another medium (Su, 2002). The process is dependent on the exogenous PGRs applied, particularly the concentration of auxins and cytokinins, and the ability of cell or tissue to respond to such treatments during culture (Cardoza, 2008; Sugiyama, 1999). Generally, the growth and proliferation of axillary shoots is associated with the incorporation of cytokinins in the medium to remove the apical dominance of the shoot tip, which consequently induce the production of multiple axillary shoots, however, this can also result in the frequent occurrence of adventitious shoots (George and Debergh, 2008).

Adventitious organogenesis can be divided into three stages. The cells will first acquire cell competency (the ability to respond to the phytohormonal signals for organ initiation), then cell dedifferentiation will take place (the process of acquiring organogenic competence). In the second phase, the competent cells, influenced by the balance of PGRs, form a specific organ. Finally, morphogenesis continues without being affected by exogeneous PGRs (Sugiyama, 1999). In short: 1) Regeneration-initial cells are activated, 2) they acquire competency, and 3) they establish apical meristems to form organs. Procambium, cambium, pericycle, and pericycle-like cells are examples of the regeneration-initial cells which function as the origin for the initial cell divisions during organogenesis (Sang et al., 2018).

Evidence to date indicates that direct shoot organogenesis, from axillary or adventitious origin, is the preferable plant regeneration pathway for palms. Axillary shoot formation (axillary origin) has been preferred because it is the system with the lowest spontaneous mutation occurrence (Phillips and Garda, 2019), and it is a reliable true-to-type system for *in vitro* clonal propagation (George and Debergh, 2008). Adventitious shoot organogenesis (adventitious origin) is also preferable compared to SE because it eliminates the intervention of a callus phase, which has the potential to cause somaclonal variation particularly after culturing the callus for a long period of time. It also has the potential to be a more time-efficient pathway because the time required to induce callus is saved (Su, 2002). Hence, the application of direct shoot organogenesis system could benefit recalcitrant species like coconut in similar ways.

3.1. Direct shoot organogenesis

Plant regeneration by direct shoot organogenesis involves four stages *viz.* vegetative shoot bud induction/ initiation, bud proliferation/ multiplication, shoot elongation and finally root organogenesis as shown in Fig. 2 for oil palm. The first stage is critical (Abahmane, 2011), as the induction of a shoot bud is highly dependent upon the plant genotype and the nature of the explant tissue. The capability of a plant to produce new organs is dependent upon the continually dividing cells (stem cells) in the meristem. Almost all nuclear and cell divisions take place in the meristem. Shoot and root apical meristems are localized in the region at the tip of both shoots and roots that perform cell division and elongation leading to primary plant growth. Thus, they are called primary meristems, while, the inflorescence, floral, intercalary and lateral meristems are considered secondary meristems (Pavlović and Radotić, 2017). Therefore, the shoot tips may be a suitable explant for direct shoot organogenesis to produce either axillary or adventitious shoot buds or both. The origin of the buds formed can only be determined through anatomical study. This is because the existing shoot apical meristem has a central zone of stem cells at the tip of the dome, which leads to the formation of leaves, stems, flowers (Traas, 2018), as well as axillary meristem (Yang and Jiao, 2016). For this reason, the shoot tip has been used as the common explant for direct shoot organogenesis in the micropropagation of oil palm (Romyanon et al., 2015), and date palm (Khierallah and Bader, 2007; Sidky, 2017), as shown in Table 1. Although, the immature female inflorescence has been demonstrated as an alternative explant source. Adventitious buds can be formed directly from the inflorescence tissue of date palm, via direct shoot organogenesis (Gadalla, 2017). The formation of an adventitious shoot has been demonstrated to be closely linked with the ease of its formation from explants under natural conditions (Gahan and George, 2008).

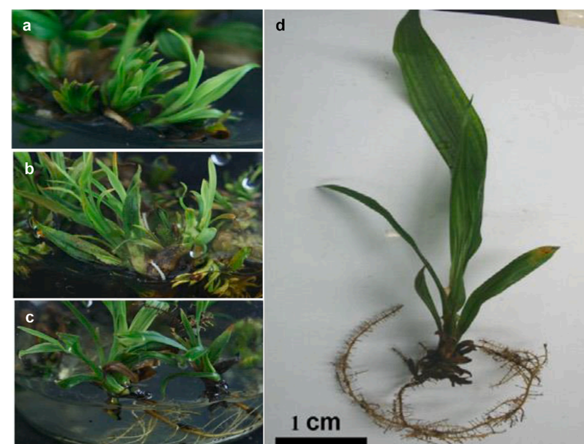

Fig. 2. Direct shoot organogenesis of oil palm using *in vitro* shoots as explants to (**a**) induce multiple shoots, (**b**) elongate the shoots, and (**c**) produce plantlets. **d** The well-rooted plantlet is used for acclimatization. The photos are reprinted from Romyanon et al. (2015) with permission.

Table 1
Plant regeneration of coconut, oil palm and date palm via direct organogenesis.

Palm/Explant	BM/ Pre-culture	SIM	SMM	SEM	RFM/PRM	Outcome	Reference
Coconut cv. **Sri Lanka Tall** Immature inflorescence (8 to 10 cm outer spathe length) from 12 years old palm •Middle portion (3 cm in length) •Section into 0.1 to 0.15 cm long for culture	Modified Y3 medium from Verdeil, et al. (1994) + 200 μM 2,4-D + 2 g L^{-1} AC	8 μM BAP + 10 μM 2,4-D Dark conditions; T: 30 ± 1°C	-	-	Based on Fernando and Gamage (1994-1995) Pre-treatment in 100 μM IAA for 3 days and then cultured in rooting medium (Y3 + 10 μM BAP)	•Formation of translucent structure from floral bud directly in 6 weeks. •Directly developed into shoot after subculture into shoot induction medium but at a low frequency. •Roots formed after 3 days.	Vidhana Arachchi and Weerakoon (1997)
Coconut cv. **West Coast Tall** Immature inflorescence (10 to 18 cm inner spathe length) from 15 to 20 years old palm •Section into 0.5 to 0.7 cm long for culture	**Pre-condition medium:** Modified ½ MS medium (200 mg NaH_2PO_4 + 1 mg Calcium pantothenate + 0.05 mg Biotin + 0.01 mg folic acid + 30 g L^{-1} sucrose + 1 g L^{-1} AC + 5.5 g L^{-1} agar) **Initiation medium:** 41.41 μM picloram + 4.54 μM thidiazuron under dark conditions for 32 weeks and subcultured once after 16 weeks **Maintenance medium:** 20.71 μM picloram + 4.54 μM thidiazuron under dark conditions for 16 weeks	8.28 μM picloram + 4.54 μM thidiazuron Diffused light conditions (less than 100 lux) for 16 weeks; T: 27 ± 2°C	-	-	PGR-free L/D: 16:8 h; L: 6800K white fluorescent light; T: 27 ± 2°C	•Shoot induction in spadices of 10 to 12 cm inner spathe length. •Callus formation in spadices of less than 10 cm inner spathe length. •Floral formation in spadices of more than 13 cm inner spathe length. •Best precondition media for shoot formation was the combination of 41.41 μM picloram + 4.54 μM thidiazuron. •2,4-D in combination with TDZ resulted in callus formation followed by root formation. •Vegetative shoots formed under diffused light and the shoots formed per explant ranged from 1 to 14. •Vegetative shoots transferred as a small group for plantlet development was better than individual shoots. •Shoot elongated after 15 to 16 weeks under light conditions and explant smaller than 0.5 cm long did not show shoot formation.	Raju (2006)
Coconut cv. **East Coast Tall** Zygotic embryos of 11 to 12 months old palms	Y3 + 45 g L^{-1} sucrose + 2.5 g L^{-1} AC + 8 g L^{-1} agar	150 μM TDZ T: 25 ± 2°C; RH: 60 to 70% Transferred to regeneration medium after 90 days	-	-	Same as SIM	•Shoot induction rate is 75% and about 66% of explants had regenerated. •A maximum of 2 shoots per embryo was obtained. •The average shoot length was 4 cm after 150 days. •The presence of 2,4-D in combination with TDZ inhibited direct organogenesis.	Chandrakala, et al. (2019)
Oil palm *In vitro* shoots from 4 weeks old seedlings	½ MS + 30 g L^{-1} sucrose + 2.5 g L^{-1} phytagel	9.8 μM 2iP	-	PGR free Individual shoots were excised from cluster	PGR free T: 28 ± 2°C; L/D: 16:8 h; L: 60 ± 5 μmol m^{-2} s^{-1}	•Cytokinin was required to induce direct shoot formation. •2iP was the best in promoting direct shoot formation at a concentration of 9.8 μM (54.2%	Romyanon, et al. (2015)

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Table 1 (*continued*)

Palm/Explant	BM/ Pre-culture	SIM	SMM	SEM	RFM/PRM	Outcome	Reference
derived from zygotic embryos				8 weeks culture before root initiation T: 28 ± 2C; L/D: 16:8 h; L: 60 ± 5 µmol m^{-2} s^{-1}		shoot induction rate with five shoots per explant). •High concentration of cytokinin resulted in the formation of type II shoot which were compact shoot clusters with stunted leaves. •The highest shoot formation was obtained when cultured for 4 weeks (69.5%). •Shoots from seedlings less than 3 weeks old were unsuccessful to induce shoot formation.	
Oil palm *In vitro* shoots (2.5 ± 0.5 cm in length) from 4 weeks old seedlings derived from zygotic embryos	½ MS + 30 g L^{-1} sucrose + 2.5 g L^{-1} phytagel	9.8 µM 2iP T: 28 ± 2C; L/D: 16:8 h; L: 60 ± 5 µmol m^{-2} s^{-1}; RH: 60 ± 5%	-	-	-	•½ MS medium containing 50 g L^{-1} sucrose was the best medium for induction of shoot. •Y3 medium did not induce direct shoot formation. •High sucrose concentration lowered the shoot induction frequency in MS and WPM media. •A cluster of single shoots were produced from MS media and WPM medium produced compact shoot clusters. •Minimum osmotic potential for shoot maturation was −2.0 MPa and sucrose was better than sorbitol in the induction of shoot formation. •A reliable protocol for true-to-type propagation.	Romyanon, et al. (2017)
Date palm Shoot tips (0.5 cm long and 0.3 cm wide) with three to five leaf primordia from 2 years old seedlings	MS + 30 g L^{-1} sucrose + 3 g L^{-1} AC + 8 g L^{-1} phytagar	53.7 µM NAA CP: 8 weeks	Liquid SMM: 0.54 µM NAA + 44.4 µM BAP + 30 g L^{-1} sucrose + No AC Agitation speed: 70 rpm; SC: 8 weeks	-	0.54 µM NAA + No AC CP: 8 to 16 weeks	•5 to 10% explant produced axillary bud outgrowth in medium containing 53.7 µM NAA. •Adventitious root formation in medium containing NAA. •Axillary shoots were formed more frequently from the axillary bud outgrowths in liquid medium. •A single shoot could produce up to 20 shoots after 6 months.	Tisserat (1984)
Date palm **cv. Khnazi** Shoot tips (3 to 5 mm^3) from 3 to 4 years old offshoots	MS + 40 g L^{-1} sucrose + 2 g L^{-1} PVP + 3 g L^{-1} AC + 7 g L^{-1} agar	2.28 µM IAA + 2.15 µM NAA or 9.13 µM IAA or 15.74 µM 2iP or 7.1 µM BAP AC only for first two months T: 28 ± 1C; Dark conditions; CP: 4 months	15.74 µM 2iP + 2.15 µM NAA + 30 g L^{-1} sucrose + No AC L/D: 16:8 h; L: 30 µmol m^{-2} s^{-1}; SC: 1 month	SIM + 30 g L^{-1} sucrose + No AC L/D: 16:8 h; L: 30 µmol m^{-2} s^{-1}; SC: 1 month	5.37 µM NAA + 30 g L^{-1} sucrose + No AC L/D: 16:8 h; L: 30 µmol m^{-2} s^{-1}	•31.25% of explant produced bud generative tissue. •6.25 buds were formed from each explant cultured in medium containing 2.28 µM IAA and 2.15 µM NAA. •31.25% explant produced bud generative tissue in medium containing 15.74 µM 2iP or 7.1 µM BAP. •7.3 buds per explant were produced in the shoot multiplication medium after 7	Al Kaabi (1999); Al Kaabi, et al. (2001)

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Table 1 (*continued*)

Palm/Explant	BM/ Pre-culture	SIM	SMM	SEM	RFM/PRM	Outcome	Reference
						months. •7 buds per explant were produced in medium containing 4.57 µM IAA after 7 months. •Shoot induction was affected by the collection time of explant.	
Date palm (multiple female cultivars, one male clone) Segments of inflorescence of 0.5 cm long with two to three closed flower buds	Greshoff and Doy medium + 40 g L^{-1} sucrose + 2 g L^{-1} PVP + 8 g L^{-1} agar	2.69 µM NAA + 8.88 µM BAP + 4.92 µM 2iP Dark conditions; T: 28C; SC: 4 to 6 weeks	Same as SIM L/D: 16:8 h; L: 180 µmol m^{-2} s^{-1}	-	10.74 µM NAA + 4.92 µM 2iP or 4.44 µM BAP	•Shoots required at least 4 months to form from young female inflorescences while mature flowers took 2 to 3 months with limited capacity. •No growth in mature male inflorescences while young ones showed callus or shoot formation. •Female inflorescences were more superior than male for shoot and callus production. •27% young male, 35% young female and 5% old female produced shoots in shoot induction medium.	Loutfi and Chlyah (1998)
Date palm cv. Zaidi, Hussain, Asil Shoot tips (0.4 to 0.6 cm long) from 3 to 4 years old offshoots	MS + 30 g L^{-1} sucrose + 2 g L^{-1} gelrite + 3 g L^{-1} AC	19.68 µM IBA + 4.44 µM BAP Dark conditions; T: 25 ± 2C	2.27 µM TDZ + 4.92 µM 2iP L/D: 16:8 h; L: 2000 lux; CP: 8 weeks	-	-	•The response of each cultivar on shoot induction and multiplication medium was genotype dependent. •Light conditions transformed explant from whitish to green. •TDZ had important role in shoot multiplication. •10 to 15 shoots were produced in shoot multiplication medium for Asil cultivar.	Hussain, et al. (2001)
Date palm cv. Zaghlool Shoot tips (1 cm long) from offshoots of mature female palm	MS + 30 g L^{-1} sucrose + 1.75 g L^{-1} phytagel	5.37 µM NAA + 9.84 µM 2iP Dark conditions; SC: 6 weeks	2.69 µM NAA + 14.76 µM 2iP	-	-	•8.10 shoot buds were proliferated in the multiplication medium. •High sucrose concentration (40 g L^{-1}) and low concentration of phytagel resulted in vitrification.	Taha, et al. (2001)
Date palm cv. Khanezi Shoot tips (1 cm long) from 3 years old offshoots	MS + 30 g L^{-1} sucrose + 7 g L^{-1} agar	0.93 µM KN + 0.49 µM 2iP + 0.44 µM BAP + 0.57 µM IAA + 0.49 µM NOA + 0.54 µM NAA T: 23 to 25C; L/D: 16:8 h; L: 40 W fluorescent lamp; CP: 8 weeks	-	-	-	•High PGRs concentration caused abnormal growth and did not induce shoot or bud. •Low concentrations of PGRs resulted in high fresh and dry weight.	Al-Khateeb (2006)
Date palm cv. Maktoom Shoot tips with two pairs of leaf primordia from 2 to 3 years old offshoots	MS + 30 g L^{-1} sucrose + 2 g L^{-1} AC + 7 g L^{-1} agar	9.84 µM 2iP + 4.44 µM BAP + 4.95 µM NOA + 5.37 µM NAA T: 27 ± 1C; L/D: 16:8 h; L: 1000 lux; SC: 4 weeks; CP: 16 weeks	Liquid SMM: 19.68 µM 2iP + 8.88 µM BAP + 4.95 µM NOA + 5.37 µM NAA Shaker speed: 40 rpm	1.44 µM GA_3 + 5.37 µM NAA	2.69 µM NAA	•80% growth response with an average of 6.2 buds formed in shoot induction medium. •BAP was better than kinetin and 2iP in bud initiation. •12.6 buds were formed in the liquid agitated multiplication medium.	Khierallah and Bader (2007)

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Table 1 (*continued*)

Palm/Explant	BM/ Pre-culture	SIM	SMM	SEM	RFM/PRM	Outcome	Reference
						•Plantlet length was elongated to 5.3 cm. •90% plantlets rooted in root formation medium.	
Date palm cv. Barhi and Maktoom Shoot tips with two pairs of leaf primordia from 2 to 3 years old offshoots	MS + 2 g L^{-1} AC + 90 μM STS + 0.7 mM glutamine + 7 g L^{-1} agar	9.84 μM 2iP + 4.44 μM BAP + 4.95 μM NOA + 5.37 μM NAA T: 27 ± 1̊C; L/D: 16:8 h; L: 1000 lux ; SC: 4 weeks; CP: 16 weeks	19.68 μM 2iP + 8.88 μM BAP + 4.95 μM NOA + 5.37 μM NAA + 1.4 mM glutamine	-	-	•8.1 Barhi and 9.4 Maktoom buds were produced from shoot induction medium. •12.5 Barhi and 14.7 Maktoom shoots were produced from multiplication medium.	Bader and Khierallah (2009)
Date palm cv. Dhakki Shoot tips with one to two pairs of leaf primordia from 3 to 4 years old offshoot	MS + 0.3 g L^{-1} AC + 2.5 g L^{-1} phytagel	0.75 g L^{-1} citric acid + 0.75 g L^{-1} ascorbic acid + 14.76 μM 2iP + 5.37 μM NAA + 13.32 μM BAP + 40 g L^{-1} sugar SC: 2 weeks; L/D: 16:8 h after 5 weeks	2.32 μM KN + 2.22 μM BAP + 5.37 μM NAA + 30 g L^{-1} sugar T: 25 ± 2̊C; L/D: 16:8 h; SC: 6 weeks	-	2.69 μM NAA + 30 g L^{-1} sugar + 1 mg L^{-1} biotin + 6 g L^{-1} agar	•Shoot tip had higher capacity for shoot regeneration compared to leaf primordia and a section of apical meristem which formed callus. •Citric acid and ascorbic acid had significant effects to prevent browning of explant. •Around 8 shoots were produced in the multiplication medium with average shoot length of 8 cm. •High concentration of NAA (8.06 μM) had higher number of roots but lower survival rate.	Khan and Bi Bi (2012)
Date palm cv. Zaghlool Shoot tips from 2 to 3 years old offshoots	Medium for induction of nodular culture: MS + 9.84 μM 2iP + 5.37 μM NAA + 3 g L^{-1} AC + 7 g L^{-1} agar Dark conditions; T: 25 ± 2̊C; SC: 5 weeks; CP: 3 subcultures	24.6 μM 2iP L/D: 16:8 h; L: 3000 lux; CP: 5 weeks	9.29 μM KN + 24.6 μM 2iP L/D: 16:8 h	-	5.37 μM NAA + no AC + 1.75 g L^{-1} gelrite	•85% explant formed shoots in shoot induction medium with 4.5 shoots per explant. •8.1 shoots bud were formed per explant with about 4 cm in height in the multiplication medium. •Silver nitrate also improved the multiplication rate. •NAA was better than IAA or IBA in the induction of root formation. •Plantlets had similar genetic as the mother plant through RAPD analysis.	Bekheet (2013)
Date palm cv. Ekhlass Shoot tips from 4 years old offshoots	Pre-SIM: PGR-free MS + 40 g L^{-1} sugar + 1.5 g L^{-1} AC + 2 g L^{-1} gelrite Dark conditions; T: 25 ± 1̊C; CP: 2 to 4 weeks	4.92 μM 2iP + 2.47 μM NOA + 4.44 μM BAP + 4.65 μM KN + 40 g L^{-1} sugar + 1.5 g L^{-1} AC + 2 g L^{-1} gelrite Dark conditions; T: 25 ± 1̊C; SC: 4 weeks; CP: 12 weeks	0.93 μM KN + 0.98 μM 2iP + 1.14 μM IAA + 50 mg L^{-1} putrescine + 160 mg L^{-1} adenine hemi−sulphate + 30 g L^{-1} sugar + 2.5 g L^{-1} phytagel L/D: 16:8 h; L: 1500 lux; SC: 1 month; CP: 8 weeks	-	1.07 μM NAA + 100 mg L^{-1} spermidine + 30 g L^{-1} sugar + 6 g L^{-1} phytoagar L/D: 16:8 h; L: 3000 lux; CP: 4 weeks	•33.33% explant produced axillary buds in shoot induction medium. •2.11 buds were formed in each explant after cultured in shoot multiplication medium. •All explants formed roots in the root formation medium with 2.67 roots in average and root length of 5.42 cm.	Hegazy (2013)
Date palm cv. Najda Organogenic stem	Organogenic culture induction medium described by	-	½ MS + 2.47 μM NOA + 2.32 μM KN + 30 g L^{-1} sucrose + 1 g L^{-1} PVP + 6 g L^{-1} bactoagar	PGRs-free ½ MS or MS	-	•23.5 shoots were produced from each explant in the multiplication medium.	Mazri and Meziani (2013)

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Table 1 (*continued*)

Palm/Explant	BM/ Pre-culture	SIM	SMM	SEM	RFM/PRM	Outcome	Reference
derived from shoot tip with 5 buds per explant	Beauchesne, et al. (1986)		L/D: 16:8 h; T: 25 ± 2°C			•MS medium was better than Beauchesne medium for shoot multiplication. •More than 90% shoots in PGR-free media survived while shoots cultured in PGRs supplemented medium showed poor survival even though PGRs improved the shoot and root growth.	
Date palm cv. Hillawi Shoot tips from 3 to 4 years old offshoots	MS + 30 g L^{-1} sugar + 2 g L^{-1} AC + 5 g L^{-1} agar	4.65 µM KN + 5.37 µM NAA + 4.95 µM NOA + 4.92 µM IBA Dark conditions; T: 27 ± 2°C	4.44 µM BAP + 2.27 µM TDZ T: 27 ± 2°C; L/D: 16:8 h; RH: 55 to 60%; SC: 6 weeks; CP: 24 weeks	1.44 µM GA_3 + 0.54 µM NAA	1.07 µM NAA	•4.2 and 18.2 buds per explant were produced after 16 and 24 weeks respectively in multiplication medium. •TDZ had significant effect at 2.27 µM on shoot multiplication and suppression occurred at concentration of 2.27 to 9.08 µM.	Al-Mayahi (2014)
Date palm cv. 16-bis Organogenic clusters derived from shoot tip with 4 buds per cluster	Organogenic culture induction medium described by Beauchesne, et al. (1986)[20]	-	MS + 2.5 µM IBA + 2.5 µM BAP + 20 g L^{-1} sucrose + 1 g L^{-1} PVP + 6 g L^{-1} bactoagar T: 25°C; L/D: 16:8 h; L: 40 µmol m^{-2} s^{-1}; SC: 1 month; CP: 3 months	PGR-free + 40 g L^{-1} sucrose	-	•22.3 shoots were produced from each cluster in the shoot multiplication medium. •PGRs at high concentration caused hyperhydricity and precocious rooting. •High amount of sucrose also caused precocious rooting. •The shoots cultured in shoot elongation medium had an average height of 14.9 cm and the roots had an average length of 4.5 cm. •The plantlets had 92.5% survival rate.	Mazri (2014)
Date palm cv. Barhee Shoot tips from 2 to 3 years old offshoots	MS + 30 g L^{-1} sucrose + 2 g L^{-1} AC + 7 g L^{-1} agar	5.37 µM NAA + 4.44 µM BAP + 4.95 µM NOA + 19.68 µM 2iP Dark conditions for 1 month then under 16: 8 L/D; T: 24 ± 1°C; CP: 6 months	5.37 µM NAA + 4.44 µM BAP + 7.38 µM 2iP T: 24 ± 1°C; L/D: 16:8 h; SC: 4 weeks; CP: 3 months	-	5.37 µM NAA	•MS medium was better than ½ MS medium for shoot induction. •30 g L^{-1} sucrose was more suitable than 60 g L^{-1} sucrose for shoot induction. •6 buds was produced per explant in the shoot induction medium. •Shoot multiplication medium resulted in 6.7 buds per explant with an average shoot length of 8.90 cm.	Jazinizadeh, et al. (2015)
Date palm cv. Boufeggous Organogenic clusters derived from shoot tip with 3 buds per cluster	Organogenic culture induction medium described by Beauchesne, et al. (1986)[20]	-	½ MS + 3 µM IBA + 3 µM BAP + 30 g L^{-1} sucrose + 1 g L^{-1} PVP + 6 g L^{-1} bactoagar T: 25 ± 1°C; L/D: 16:8 h; CP: 3 months	PGR-free T: 25 ± 1°C; L/D: 16:8 h; SC: 1 month; CP: 3 months	Same as SEM	•22.9 shoot buds were produced from explant in shoot multiplication medium with 13.2 cm shoot length and 3.5 cm root length. •BAP and KN were better than 2iP and TDZ in shoot multiplication. •High BAP caused more hyperhydration. •Plantlets previously cultured in	Mazri (2015)

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Table 1 (*continued*)

Palm/Explant	BM/ Pre-culture	SIM	SMM	SEM	RFM/PRM	Outcome	Reference
						semi-solid medium survived better.	
Date palm cv. Mejhoul Organogenic clusters derived from shoot tip with 4 buds per cluster	Organogenic culture induction medium described by Beauchesne, et al. (1986)[20]	-	½ MS + 1.14 μM IAA + 1.86 μM KN + 0.99 μM NOA + 1.97 μM 2iP + 30 g L^{-1} sugar + 1 g L^{-1} PVP + 8 g L^{-1} agar T: 25 ± 1̊C; L/D: 16:8 h; L: 1000 lux; CP: 3 month; SC: 1 month	PGR-free	Same as SEM	•12.8 shoots were produced from each explant in the shoot multiplication medium. •Dark conditions or low light intensity (500 lux) resulted in precocious rooting. •High light intensity of 2000 to 3000 lux decreased shoot growth. •1000 lux was the optimal light intensity for shoot multiplication.	Meziani, et al. (2015)
Date palm cv. Medjool and Mazafati Shoot tips from offshoots	MS + 30 g L^{-1} sucrose + 1.5 g L^{-1} AC + 7 g L^{-1} agar	2.69 μM NAA + 2.47 μM NOA + 4.92 μM 2iP + 4.44 μM BAP Dark conditions for the 1st month with T of 22 ± 1̊C; then L/D: 16: 8 with T: 27 ± 1̊C	-	-	-	•4.51 buds were produced in the shoot induction medium. •There was significant difference in the shoot bud formed in different cultivar.	Rad, et al. (2015)
Date palm cv. Alshakr Shoot tips from 2 to 3 years old offshoots	MS + 30 g L^{-1} sucrose + 3 g L^{-1} AC + 6 g L^{-1} agar	5.37 μM NAA + 9.84 μM 2iP Dark conditions; T: 27 ± 2̊C; SC: 5 weeks	5.37 μM NAA + 2.22 μM BAP + 2.32 μM KN T: 27 ± 1̊C; L/D: 14:8 h; CRB-LED red/blue ratio: 18:2; L: 80 to 100 μmol m^{-2} s^{-1}; SC: 5 weeks	-	-	•CRB-LED was a better light source than white fluorescent light as 73.34% buds formed shoots with an average of 8.1 shoots per explant.	Al-Mayahi (2016)
Date palm cv. Mejhoul Organogenic clusters derived from shoot tip	Organogenic culture induction medium described by Beauchesne, et al. (1986)[20]: ½ MS + 14.84 μM NOA + 5.37 μM NAA + 5.71 μM IAA + 0.49 μM 2iP + 30 g L^{-1} sucrose + 0.2 g L^{-1} L-glutamine + 0.1 g L^{-1} myo-inositol + 2 g L^{-1} PVP + 6 g L^{-1} agar Dark conditions; T: 25̊C; SC: 4 weeks; CP: 9 months	-	0.99 μM NOA + 1.14 μM IAA + 1.86 μM KN + 1.97 μM 2iP + 1 g L^{-1} PVP T: 25̊C; L/D: 16:8 h; L: 13.5 μmol m^{-2} s^{-1}; SC: 1 month; CP: 3 months	-	-	•18.7 buds were formed for each explant when cultured in shoot multiplication medium. •High concentration of NH_4NO_3 resulted in increased frequency of hyperhydration. •90% plantlets survived after 3 months.	Mazri, et al. (2016)
Date palm cv. Mejhoul Shoot tips from 2 to 3 years old offshoots	½ MS + 30 g L^{-1} sucrose + 1.5 g L^{-1} PVP + 6 g L^{-1} agar	14.2 μM IAA + 13.4 μM NAA + 0.5 μM 2iP Dark conditions; T: 25 ± 1̊C; SC: 1 month; CP: 9 months	0.9 μM NOA + 1.1 μM IAA + 1.8 μM KN+ 1.9 μM 2iP + 1.5 g L^{-1} DSAC T: 27 ± 1̊C; L/D: 16:8 h; L: 13.5 μmol m^{-2} s^{-1}; SC: 1 month; CP: 3 months	PGR-free T: 25 ± 1̊C; L/D: 16:8 h; L: 40 μmol m^{-2} s^{-1}; SC: 1 month; CP: 3 months	Same as SEM	•Organogenesis occurred in shoot induction medium at 52.5% while 13.9 buds were produced from explant in multiplication medium. •97.5% plantlets survived after 3 months. •DSAC could be used as an natural agent to prevent browning in date palm. •The average shoot and root length were 13.6 cm and 3.85 cm respectively while the number of	Meziani, et al. (2016); Meziani, et al. (2019)

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Table 1 (*continued*)

Palm/Explant	BM/ Pre-culture	SIM	SMM	SEM	RFM/PRM	Outcome	Reference
						adventitious roots formed was 3.6. About 90% of the plantlets survived (Meziani, et al. 2019).	
Date palm cv. Siwi 2 to 3 cm mature female inflorescences of 40 to 50 cm spathe length	MS + 30 g L^{-1} sucrose + 1.0 g L^{-1} AC + 6 g L^{-1} agar	7.57 µM ABA Dark conditions; T: 27 ± 2C; SC: 8 weeks; CP: 6 months	-	-	-	•40% direct shoot formation in the induction medium. •PBZ could not induce direct shoot organogenesis.	Zayed, et al. (2016)
Date palm cv. Siwy Shoot tips (1 to 1.5 cm long) from 3 to 4 years old offshoots	MS + 40 g L^{-1} sucrose + 0.3 g L^{-1} AC + 6 g L^{-1} agar	5.37 µM NAA + 4.44 µM BAP + 4.65 µM KN + 4.92 µM 2iP Dark conditions; T: 25 ± 2C; SC: 8 weeks; CP: 32 weeks	-	-	-	•36.6% of explant formed shoots in the induction medium with an average of 9.2 shoots per explant.	Ali, et al. (2017)
Date palm cv. Shamiya Immature female inflorescences	Cultured in MS + 30 g L^{-1} sucrose + 7 g L^{-1} agar	(1) 22.62 µM 2,4-D + 14.76 µM 2iP + 1.5 g L^{-1} AC CP: 4 weeks (2) 4.52 µM 2,4-D + 14.76 µM 2iP + 5.37 µM NAA + 11.42 µM IAA + 1 g L^{-1} AC SC: 4 weeks; CP: 8 weeks (3) 2.26 µM 2,4-D + 14.76 µM 2iP + 2.69 µM NAA + 0.5 g L^{-1} AC CP: 4 weeks Dark conditions; T: 26 ± 2C	SDM: 1.16 µM KN + 1.11 µM BAP + 0.54 µM NAA + 0.95 µM ABA + 0.2 g L^{-1} AC Dark conditions; T: 26 ± 2C; CP: 3 weeks SMM: 0.27 µM NAA + 1.14 µM IAA + 2.32 µM KN + 2.22 µM BAP + 0.49 µM 2iP + 0.2 g L^{-1} AC T: 26 ± 2C; L/D: 16:8 h; L: 40 µmol m^{-2} s^{-1}; SC: 4 weeks; CP: 9 to 12 months	4.65 µM KN + 2.89 µM GA$_3$ + 4.92 µM IBA + 0.2 g L^{-1} AC T: 26 ± 2C; L/D: 16:8 h; L: 50 µmol m^{-2} s^{-1}; CP: 3 weeks	5.37 µM NAA + 9.84 µM IBA T: 26 ± 2C; L/D: 16:8 h; L: 80 µmol m^{-2} s^{-1}; SC: 4 weeks; CP: 8 weeks	•Shoot multiplication rate was lowered when concentration of AC was greater than 0.2 g L^{-1}. •Inflorescence of 12 cm spathe length was the most responsive but those between 8 to 25 cm could also be used. •Gradual reduction of 2,4-D resulted in direct shoot organogenesis and both direct and indirect somatic embryogenesis. •Culture period more than 3 to 4 weeks in shoot differentiation medium resulted in root formation.	Gadalla (2017)
Date palm cv. Maktoom Inflorescences (0.5 cm segments) from 8 to 10 cm long spathe length	MS + 40 g L^{-1} sucrose + 170 mg L^{-1} NaH$_2$PO$_4$ + 100 mg L^{-1} glutamine + 40 mg L^{-1} adenine sulfate + 2 g L^{-1} PVP + 3 g L^{-1} phytagel	10.74 µM NAA + 17.76 µM BAP T: 26 ± 2C; Dark conditions; CP: 6 weeks	14.76 µM 2iP + 8.88 µM BAP + 200 mg L^{-1} glutamine + 80 mg L^{-1} adenine sulfate + 7 g L^{-1} agar T: 27 ± 2C; L/D: 16:8 h; L: 40 µmol m^{-2} s^{-1}	5.37 µM NAA + 2.22 µM BAP + 0.14 µM GA$_3$ + 200 mg L^{-1} glutamine + 80 mg L^{-1} adenine sulfate + 7 g L^{-1} agar T: 27 ± 2C; L/D: 16:8 h; L: 40 µmol m^{-2} s^{-1}; CP: 6 weeks Then transferred to PGR-free medium for 6 weeks	5.37 µM NAA + 45 g L^{-1} sucrose + 80 mg L^{-1} adenine sulfate + 7 g L^{-1} agar	•High concentration of auxin was required for shoot bud induction. •6.6 buds were produced in liquid shoot multiplication medium in the presence of 200 mg L^{-1} glutamine. •90% of the shoots formed roots.	Khierallah, et al. (2017)
Date palm cv. Siwy	MS + 30 g L^{-1} sucrose + 170 mg L^{-1} NaH$_2$PO$_4$ + 100 mg L^{-1} myo-inositol + 40 mg L^{-1}	5.37 µM NAA + 4.95 µM NOA + 11.1 µM BAP + 12.3 µM 2iP	(1) 2.46 µM 2iP + 2.22 µM BAP + 50 g L^{-1} sucrose + 0.4 mg L^{-1} thiamine HCl	½ MS + 5.37 µM NAA + 1.44 µM GA$_3$ + 50 g L^{-1} sucrose + 0.4 mg	½ MS + 0.54 µM NAA + 13.62 µM PBZ + 40 g L^{-1} sucrose + 0.4 mg	•Date of collection was an important factor for achieving the best growth and bud regeneration	Sidky (2017)

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Table 1 (*continued*)

Palm/Explant	BM/ Pre-culture	SIM	SMM	SEM	RFM/PRM	Outcome	Reference
Shoot tips from 3 to 5 years old offshoots	adenine sulfate + 2 g L^{-1} PVP + 7 g L^{-1} agar	T: 27 ± 1̊C; Dark conditions; CP: 6 months; SC: 4 weeks	CP: 8 weeks; L: 25 µmol m^{-2} s^{-1} (2) ½ MS + 2.46 µM 2iP + 2.22 µM BAP + 50 g L^{-1} sucrose + 0.4 mg L^{-1} thiamine HCl + 10 mg L^{-1} silver nitrate CP: 12 weeks; L:40 µmol m^{-2} s^{-1}; T: 25 ± 2̊C; L/D: 16:8 h; SC: 4 weeks	L^{-1} thiamine HCl + 0.1 g L^{-1} AC	L^{-1} thiamine HCl + 1 g L^{-1} AC L: 40 µmol m^{-2} s^{-1}; CP: 12 weeks; SC: 6 weeks	with low browning frequency (November to March). •Silver nitrate was proven to be a good inhibitor of ethylene and promoted shoot bud growth.	
Date palm cv. Siwy 2 to 3 cm mature female inflorescences of 40 to 50 cm spathe length	MS + 30 g L^{-1} sucrose + 1 g L^{-1} AC	45.24 µM 2,4-D + 7.57 µM ABA + 14.76 µM 2iP T: 27 ± 2̊C; L/D: 16:8 h; L: 40 µmol m^{-2} s^{-1}; CP: 24 weeks; SC: 6 weeks	-	-	0.54 µM NAA + 0.22 µM BAP T: 27 ± 2̊C; L/D: 16:8 h; L: 20 µmol m^{-2} s^{-1}	•Sucrose added should not exceed 30 g L^{-1}. •Shoot induction occurred at sepals and petals of mature female flowers.	Zayed (2017)
Date palm cv. Mejhoul Shoot tips from 3 years old offshoots	½ MS + 30 g L^{-1} sucrose + 1 g L^{-1} PVP + 6 g L^{-1} agar	(1) 14.2 µM IAA + 13.4 µM NAA + 0.5 µM 2iP CP: 6 months (2) 1.1 µM IAA + 1.1 µM NAA + 0.5 µM 2iP + 2.2 µM BAP + 1.5 g L^{-1} PVP CP: 3 months Dark conditions; T: 25̊C; SC: 1 month	0.9 µM NOA + 1.1 µM IAA + 1.8 µM KN + 1.9 µM 2iP + 1.5 g L^{-1} DSAC T: 25̊C; SC: 1 month	-	-	•53.5% explants formed shoot buds after transferred to second shoot induction medium. •12.7 shoots were formed from each explant after 3 months in shoot multiplication medium.	Mazri, et al. (2018)

Key: 2,4-D: 2,4-Dichlorophenoxyacetic acid, 2iP: N6-(2-Isopentenyl)adenine, ABA: Abscisic acid, AC: Activated charcoal, BAP: 6-Benzylaminopurine, BM: Basal medium, CP: Culture period, CRB-LED: Combinations of red and blue light emitting diode, DSAC: Date stone-based activated carbon, GA_3: Gibberellic acid, HCl: Hydrochloric acid, IAA: Indole-3-acetic acid, IBA: Indole-3-butyric acid, KN: Kinetin, L: Light intensity, L/D: Light/dark photoperiod, MS: Murashige and Skoog (1962) medium, NAA: 1-Naphthaleneacetic acid, NOA: Naphthoxyacetic acid, PBZ: Paclobutrazol, PGR(s): Plant growth regulator(s), PRM: Plant regeneration medium, PVP: Polyvinylpyrrolidone, RFM: Root formation medium, RH: Relative humidity, SC: Subculture cycle, SDM: Shoot differentiation medium, SEM: Shoot elongation medium, SIM: Shoot induction medium, SMM: Shoot multiplication medium, STS: Silver thiosulphate, T: Temperature, TDZ: Thidiazuron, Y3: Eeuwens (1976) medium.

The regeneration of shoots is influenced by multiple factors such as genotype, explant age, basal medium, PGRs, photoperiod and elicitors such as mechanical wounding and pathogen infection. Auxins and cytokinins are two external stimuli that have been found to be of critical significance in regulating plant regeneration (Sun et al., 2020). Although the ratio of auxin and cytokinin is still the principal driving force for organogenesis, other PGRs such as ethylene, abscisic acid, gibberellin, polyamine and brassinosteroid also influence organogenesis by affecting the regulatory network (Geneve, 2016). Auxins and cytokinins are particularly important for direct shoot organogenesis of coconut and other palm species as depicted in Table 1. Cytokinins are often incorporated into the medium to promote growth and the proliferation of shoots (George and Debergh, 2008). They can have a significant effect on promoting shoot organogenesis during the induction of shoots from competent cells that acquire organogenic competence (Sugiyama, 1999). This is because cytokinins can promote bud activation by stimulating cell division in the shoot apical meristem. Therefore, cytokinins break the apical dominance and antagonize the effect of auxin which acts as a primary agent to maintain apical dominance. Auxins are thought to inhibit branching by the regulation of root-derived cytokinin transports to the shoots from the roots. Auxins, therefore, limit cytokinin availability for bud activation. Although cytokinins have been known to promote the formation of shoot buds (whereas auxin has the converse effect), their mode of action is still debatable. This is because shoot branching is affected by the regulation of both auxins and cytokinins in terms of their synthesis, meristem function, auxin transport, and the cell cycle (Müller and Leyser, 2011). The balance of auxins and cytokinins is therefore an important key for success in direct shoot organogenesis. This was supported by Ikeuchi et al. (2016) who mentioned that the developmental fate of regenerating organs was determined by the balance between auxins and cytokinins.

The age of the explant is an important factor that can influence direct shoot organogenesis. According to Shahidul Haque et al. (1997), more mature root tip explants (9 to 18 days) of garlic (*Allium sativum* L.) were found more likely to produce adventitious shoots, through direct shoot regeneration, than the younger explants (3 days). Conversely, young finger millet (*Eleusine coracana* L. Gaertn.) explants were found to possess greater direct shoot regeneration capacity than the very young or older explant (Satish et al., 2015). Similarly, Bhagyalakshmi (1999) demonstrated direct shoot organogenesis results for saffron (*Crocos sativus* L.) were better for explants of intermediate age, as the very young ovaries (with achromatic stigma) or old ovaries of open flowers resulted in lower shoot regeneration as compared to intermediate age ovaries with yellow stigma. Young explants which have less differentiated, metabolically active cells and abundant auxin synthesis sites may enhance the organogenesis process (Famiani et al., 1994). However, the influence of explant age on shoot regeneration is likely to link with the age-dependent DNA (deoxyribonucleic acid) methylation (Sun et al., 2020).

The importance of explant age on plant regeneration through shoot induction has been demonstrated on a species more similar to coconut, oil palm (Romyanon et al., 2015). In this study, the best explant age for direct shoot regeneration was 4-week old seedlings, resulting in a 34% shoot induction rate. The percentage of shoot induction decreased significantly to 17% when 8-week old seedlings were used. Success was found to be affected by the presence of more differentiated structures, with well-defined apical meristem and leaf primordia. The shoot meristem from younger or older seedlings had a lower shoot induction rate and fewer shoots were formed. In general, the regeneration capacity of plants declines with age (Ikeuchi et al., 2016), but as the results across species vary by explant age, it is likely that the best explant age is species dependent and needs to be determined empirically for coconut.

Root organogenesis can be categorized into either direct or indirect root organogenesis. Direct root organogenesis does not involve a callus phase whereas indirect root organogenesis originates from callus (Yu et al., 2017). Plant growth regulators such as auxin and cytokinin have a critical influence on the cell fate during organogenesis (Liu et al., 2014). Thus, the appropriate type and concentration of PGRs can affect root organogenesis (Chen et al., 2014). Auxin is the principle PGR for the formation of adventitious roots and has been found to influence the expression of homeobox genes: *WUSCHEL RELATED HOMEOBOX11* and *WUSCHEL RELATED HOMEOBOX12*, which can mediate the first step of stem cell fate transition from procambium (meristematic tissue) to root founder cell (Liu et al., 2014). Like shoot organogenesis, root organogenesis is likely to be explant-age dependent. Older *Arabidopsis thaliana* L. and tomato (*Solanum lycopersicum* L.) leaves have been found to have a decreased capacity for root formation, likely due to an inadequate free auxin level or lower polar transport (Chen et al., 2014). However, the addition of auxin may be more critical to root organogenesis in coconut as it is commonly supplemented in the root induction medium for date palm (as shown in Table 1).

3.2. Regeneration of palms through direct shoot organogenesis

Even though the reported studies on direct shoot organogenesis of coconut are limited (Chandrakala et al., 2019; Raju, 2006; Vidhana Arachchi and Weerakoon, 1997), the potential for using direct shoot organogenesis as a clonal propagation method for coconut is supported by the documented success of direct shoot regeneration in oil palm and date palm. Unlike date palm, neither oil nor coconut palms propagate vegetatively to produce offshoots under natural conditions (Blake, 1983), as they have only a single shoot and they are non-branching (Tisserat, 1997). Hence, shoot tip explants are of limited availability so other explants such as inflorescence tissues may be preferable as they are in relative abundance. Success using coconut palm inflorescence tissues is likely to be dependent upon the stage of maturity of the tissue. Young inflorescence may be better for direct shoot organogenesis as the floral meristem has not yet canalized, whereas the meristem of mature inflorescence is more likely to form floral structures (George and Debergh, 2008). Based on Table 1, the most common culture medium used for direct shoot organogenesis of coconut was Eeuwens Y3 medium (Eeuwens, 1976), while for oil and date palm, Murashige and Skoog (MS) (Murashige and Skoog, 1962) medium was commonly used. Similarly, the culture medium used for each palm species as mentioned above was regarded as the most suitable medium to initiate cell suspension culture for propagation via somatic embryogenesis (Kong et al., 2020).

In the study of Vidhana Arachchi and Weerakoon (1997), shoots were directly regenerated from immature inflorescence tissues of the Sri Lanka Tall coconut palm. Translucent outgrowths were formed directly from the floral primordia within 6 weeks of culture in Y3 medium supplemented with 200 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 2 g L^{-1} activated charcoal. Shoots developed when subcultured into medium supplemented with 8 μM 6-benzylaminopurine (BAP) and 10 μM 2,4-D, while the root was induced by dipping the shoot in 100 μM indole-3-acetic acid (IAA) solution for 3 days, followed by growth in PGR-free medium. Unfortunately, the direct shoot regeneration rate was low, likely due to non-optimal culture conditions. The type and concentration of PGRs (as mentioned above) may be the reasons for low shoot formation through direct shoot organogenesis as Chandrakala et al. (2019) reported the inhibition of 2,4-D in combination with thidiazuron (TDZ) on direct shoot organogenesis in coconut. Similarly, the combination of TDZ with 2,4-D showed a negative effect on the somatic embryo induction of a terrestrial orchid (*Epipactis veratrifolia* Boissier & Hohenacker) (Moradi et al., 2017).

In another study, vegetative shoots of West Coast Tall coconut emerged from the immature inflorescence of 10 to 12 cm inner spathe length, during the third cycle (16 weeks per cycle) in diffused light conditions. These explants were cultured in a modified ½ MS medium supplemented with 20.71 μM picloram and 4.54 μM TDZ under low light conditions (<100 lux) (Raju, 2006), and the number of vegetative shoots formed per explant ranged from 1 to 14 shoots. Thidiazuron was found to be essential for the transformation of floral meristem into shoots. Similarly, TDZ was crucial for shoot organogenesis in another study conducted on the East

Coast Tall coconut, employing zygotic embryos as explants (Chandrakala et al., 2019). The shoot induction and regeneration rate was the highest when 150 μM TDZ was supplemented. Based the study by Chandrakala et al. (2019), the shoot induction rate is 75% and about 66% of the explants had regenerated, but only a maximum of two shoots per embryo was achieved. The results from this study indicate that TDZ plays an important role in direct shoot organogenesis of coconut.

Cytokinin, 6-(γ,γ-Dimethylallylamino)purine (2iP), has been found to play an important role in direct shoot formation in oil palm (Romyanon et al., 2015; Romyanon et al., 2017), and may prove important also for coconut. Direct shoot organogenesis of oil palm, as shown in Fig. 2, used *in vitro* shoots from 4-weeks old oil palm seedlings derived from zygotic embryos as explants. Results showed that 2iP (9.8 μM) was better than BAP in the induction of multiple shoots cultured in ½ MS medium, with five shoots formed per explant and a shoot induction rate of 54.2% (Romyanon et al., 2015). Based on the study, seedlings less than 3 weeks old were found unsuitable for multiple shoot induction. In addition, it was found that Y3 medium could not induce direct shoot formation in oil palm, while Woody Plant Medium produced compact shoot clusters (Romyanon et al., 2017). In this study, carbon source was also found to play an important role in the shoot induction of oil palm. Sucrose was better than sorbitol for shoot induction, with the highest shoot induction rate recorded in ½ MS medium containing 50 g L^{-1} sucrose. Similarly, sucrose was a more suitable carbon source than maltose for the formation of Persian walnut (*Juglans regia* L.) somatic embryos (Jalali et al., 2017). These results are likely to be applicable to the direct shoot organogenesis of coconut.

Date palm has been shown to produce offshoots and shoot tips have often been used as explants for direct shoot organogenesis (Al-Mayahi, 2016; Al Kaabi, 1999; Bader and Khierallah, 2009; Hegazy, 2013; Mazri et al., 2018), as shown in Fig. 3. Other explants such as inflorescence tissues (Gadalla, 2017), as well as organogenic stems (Mazri and Meziani, 2013), and clusters (Mazri et al., 2016), derived from shoot tips have been used. Shoot induction from inflorescence tissues have also been demonstrated to occur at the sepals and petals of mature female flowers of date palm (Zayed, 2017). Thus, shoot tips and inflorescence tissues are likely to be also suitable explants for direct shoot organogenesis of coconut.

The combination of PGRs greatly influences the shoot induction rate of date palm. Several PGRs, including 1-naphthaleneacetic acid (NAA), BAP, IAA, 2iP, indole-3-butyric acid (IBA), TDZ, kinetin and naphthoxyacetic acid, supplemented into different media such as MS medium (half or full strength) and Greshoff and Doy medium (Gresshoff and Doy, 1974), have been used to induce multiple shoots in date palm (refer to Table 1 for more detail). These PGRs were used in low concentrations as high concentrations of PGRs have been demonstrated to cause abnormal growth, suppressed shoot bud formation (Al-Khateeb, 2006), and induced hyperhydricity and precocious rooting (Mazri, 2014). Naphthoxyacetic acid is an auxin influx inhibitor (Lanková et al., 2010), commonly used in the MS medium for date palm direct shoot organogenesis. Therefore, empirical studies are important to determine the effect of PGRs on direct shoot organogenesis as the response may be similar for coconut.

It is likely that the response of various date palm cultivars to direct shoot organogenesis is genotype-dependent (Hussain et al., 2001), and this may also be relevant for coconut. For instance, from Table 1, cultivar Ekhlass was found to have low shoot bud formation (2 buds per explant) (Hegazy, 2013), while cultivar Najda formed a higher number of shoots, at 24 shoots per explant after culture in shoot multiplication medium (Mazri and Meziani, 2013). Also, the effect of PGRs on the direct shoot organogenesis of date palm is dependent on cultivar. For example, TDZ was found to have a significant effect in shoot multiplication of cultivar Zaidi, Hussain and Asil at a concentration of 2.27 μM (Hussain et al., 2001), but suppression occurred at concentrations from 2.27 to 9.08 μM when shoot tips of cultivar Hillawi were used as explant tissues (Al-Mayahi, 2014). As for inflorescence tissues, high auxin concentration was found to be required for the induction of shoot buds (Khierallah et al., 2017), but a gradual reduction of the 2,4-D concentration during subculture was found to be the determining factor for shoot bud formation (Gadalla, 2017). Therefore, the effect of PGRs on direct shoot organogenesis of coconut is likely to be genotype dependent and the type of PGRs used needs to be determined empirically for coconut.

Other than PGRs, direct shoot organogenesis of date palm was found to be affected by the sucrose concentration, gelling agent, collection time of the explant, light conditions, and silver nitrate concentration. A high sucrose concentration (40 g L^{-1}) and a low concentration of phytagel has been shown to cause vitrification in date palm direct shoot organogenesis (Taha et al., 2001). The collection time of the explant could also affect shoot induction (Al Kaabi, 1999), as well as its growth and regeneration capacity. According to Meziani et al. (2015), nil or low light intensity (500 lux) can lead to precocious rooting, while high light intensity at 2000 to 3000 lux can suppress shoot growth in date palm. The shoot multiplication rate has also been found to be improved with the addition of silver nitrate (Bekheet, 2013). This is likely because silver nitrate is a good inhibitor of ethylene and it promotes shoot bud growth (Sidky, 2017). Hence, these factors are likely to have effects on direct shoot organogenesis of coconut and the response could be similar.

In general, the roots of the coconut, date and oil palms have been found to be able to regenerate through organogenesis in a PGR-free medium cultured under light conditions. However, in some studies, IAA and BAP were used in rooting media of coconut, while NAA (the most commonly used), 2iP, BAP and IBA were used for rooting in date palm (Table 1). The adventitious root formation rate was 90 to 100% for date palm (Hegazy, 2013; Khierallah and Bader, 2007; Khierallah et al., 2017). Consequently, it is likely that the effect of PGRs on adventitious root formation in coconut would differ among varieties. In conclusion, these studies on date palm and oil palm provide some valuable insights into the possible factors affecting direct shoot organogenesis of coconut.

4. Research gaps for direct shoot organogenesis of coconut

Direct shoot organogenesis of coconut is an underexplored area that requires paramount attention as it may be an alternative clonal propagation system to alleviate the increasing demand for elite coconut seedlings. The capability to produce multiple stem buds in coconut has

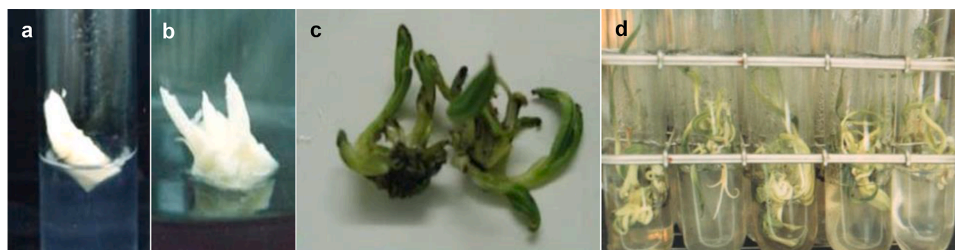

Fig. 3. Direct shoot organogenesis of date palm cv. Zaghloul using (**a**) shoot tips as explants to produce (**b**-**c**) multiple shoots and (**d**) plantlets. The photos are reprinted from Bekheet and El-Sharabasy (2015) with permission.

been so far largely unexplored with a lack of reported studies in this field, as shown in Table 1. Coconut's evolutionary adaptation is a compromised design of a single apical bud which generally restricts growth to a single-stemmed palm (Windsor-Collins et al., 2007). Although, coconut has been observed to produce multiple stems and shoot branching in natural and tissue culture environments (Fisher and Tsai, 1979; Mondal, 2014). This review explored direct shoot organogenesis of closely related species including oil palm and date palm and proposed the possibility of application of these findings for coconut propagation. The research gaps that need further understanding to implement a system for coconut include, but are not limited to, suitable explant types, PGRs (particularly auxin and cytokinin), genotype influence, sucrose concentration and appropriate physical culture conditions (such as light conditions). Based on the literature, the first two mentioned are of utmost importance to initiate and ensure this system is feasible and applicable for coconut. Studies to date have determined that the competent explants for direct shoot organogenesis of coconut are immature inflorescences, zygotic embryos and *in vitro* shoot tips or meristems. Immature coconut inflorescences with inner spathe length of 10 to 12 cm are preferable because they have been demonstrated to be more responsive than other spathe lengths for multiple shoot induction according to Raju (2006). Explants such as zygotic embryos were found to result in lower shoot production (Chandrakala et al., 2019), however, *in vitro* shoots derived from coconut seedlings may be suitable for direct shoot organogenesis as multiple shoots can be formed from *in vitro* shoots of 4-week old oil palm seedlings derived from zygotic embryos (Romyanon et al., 2015). Young explants are likely to be better as they have higher regeneration capacity which declines with age (Ikeuchi et al., 2016). It is vital to note the importance of TDZ alone, or in combination with auxin, as likely to improve success for the establishment of direct shoot organogenesis for coconut. In trials by Chandrakala et al. (2019), the treatment of zygotic embryos with TDZ (150 μM) led to a 75% shoot induction rate. Whilst, the combination of TDZ (4.54 μM) with picloram (8.28 μM) successfully induced a maximum of 14 shoots per explant from immature coconut inflorescence tissues (Raju, 2006). Thus, TDZ has a crucial role in direct shoot organogenesis of coconut and the concentration applied depends on the explant type. The use of Y3 medium is likely to be ideal for this system because it is superior for growing coconut tissue through *in vitro* culture conditions (Kong et al., 2020). As the response of direct shoot organogenesis in date palm has been inferred to be genotype-dependent (Hussain et al., 2001), it is important to compare different tall and dwarf elite varieties of coconut for a similar response. Sucrose at 30 g L^{-1} is possibly the most appropriate concentration for growth as high sucrose concentration can cause vitrification in date palm direct shoot organogenesis (Taha et al., 2001). As for the physical culture conditions, dark conditions may be the best for coconut in early shoot bud formation, followed by exposure to light conditions during the elongation stage. This is because high light intensity, at 2000 to 3000 lux, supressed shoot growth in date palm (Meziani et al., 2015). Thus, these successful findings for similar species and gaps for coconut encourage a wider exploration of this system to test the viability and efficiency of direct shoot organogenesis for clonal propagation of coconut.

5. Conclusion

Coconut palm is an economically and culturally valuable tree occupying 12 million hectares of land worldwide (Batugal et al., 2009), and holding a significant position in supporting the livelihood of millions of farmers. There is an increasing demand for products derived from the coconut fruit (Kong et al., 2019), but decreased coconut production in some regions due to senility, biotic and abiotic stresses (Perera, 2016), is a growing issue. The pressure to produce more coconut fruit, particularly elite varieties, is pushing researchers to seek alternative propagation pathways such as somatic embryogenesis. This review proposes an alternative method, direct shoot organogenesis, as another coconut clonal propagation pathway. It is a process that enables an explant to produce multiple adventitious shoots. Although coconut is considered to be non-branching, the occurrence of occasional shoot branching under natural conditions is a novel trait that implies the possibility of producing multiple *in vitro* adventitious shoots. Even though multiple shoots can be regenerated *in vitro* from either axillary or adventitious origin, the factors affecting shoot regeneration are similar. Direct shoot organogenesis of coconut has been reported, but the small number of shoots regenerated indicates an urgent need for further research to enable this pathway to be established. It is encouraging to discover that date palm has been shown to produce a relatively high number of *in vitro* shoots (>20) per explant, with a high survival rate (90%), so studies of oil and date palm are likely to provide a strong foundation for coconut research in this field. Direct shoot organogenesis research for coconut could uncover not just a viable alternative clonal propagation pathway to SE, but an efficient system with high reliability and reduced risk of somaclonal variation.

Author contribution

EK, JB, MF, BP and SWA designed the framework of the paper. EK composed the manuscript, figures and tables. JB, MF, BP and SWA corrected the grammar and style. All authors read and approved the manuscript.

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