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Cell suspension culture: A potential *in vitro* culture method for clonal propagation of coconut plantlets *via* somatic embryogenesis

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ABSTRACT

Cell suspension culture has the potential to achieve more large-scale clonal propagation of coconut (*Cocos nucifera* L.) *via* somatic embryogenesis, and to overcome current uncertainty regarding the quality and quantity of available planting materials, replicating the success of the method for the clonal mass propagation of other palm species. Unlike oil palm (*Elaeis guineensis* Jacq.) and date palm (*Phoenix dactylifera* L.), a high-quality embryogenic cell suspension culture from coconut tissues and the further regeneration of plantlets have not yet been attained. However, similar to oil palm and date palm, the establishment of coconut embryogenic cell suspension cultures is expected to achieve a high multiplication rate arising from rapid cell production, sustained embryogenic potential, and synchronized somatic embryo production. In addition, coconut genetic potential can be improved at the cellular level through biotechnology applications such as plant transformation, protoplast isolation and genome editing. Based on studies conducted on other palms, there are crucial factors which influence the establishment and growth of cell suspension cultures for palm species that are discussed extensively in this review. There are also constraints within the cell suspension culture system which should be critically reviewed prior to considering their application to coconut. Although there may be limitations, this review proposes that a cell suspension culture step is likely to be crucial to scale-up the clonal propagation of coconut, as it currently appears to be the best method to accomplish large-scale renewal and raised productivity in the coconut industry.

1. Introduction

Many palm species are of economic importance and exhibit a multipurpose character to produce many useful products (Johnson, 1999). It is generally believed that coconut (Gomes and Prado, 2007), oil palm (Sayer et al., 2012), date palm (Al-Shahib and Marshall, 2003), and peach palm (*Bactris gasipaes* Kunth) (Clement et al., 2004) are among the most valued. Their economic importance is ranked third after the Poaceae and Fabacease families (Meerow et al., 2012). Coconut is a member of the Arecaceae (palm) family. It has been historically crucial to the successful colonisation of many tropical lands by human explorers. It has become economically important more widely due to its many uses such as food and drink, building materials, fuel, medicines (Aljohi et al., 2016). In addition, the coconut shell, an ideal feedstock for activated carbon, can be modified into organic compost and is a potential resource for the production of biofuels (Archana et al., 2019). As such, it is accurately regarded throughout its global habitats as the 'tree of life' (Foale, 2003). Similar to coconut, oil palm and date palm are also high profile palms due to their economic significance. However, there are many other palm species that now have developed other uses far beyond their traditional uses (Tregear et al., 2011). For instance, peach palm was the staple food of pre-Columbians due to its nutritional value and is now a domesticated fruit crop in Amazonia. It is grown at high density for its 'heart of palm', which is extensively traded in American and European markets. It can also be used as animal feed, for silage making and oil production (Clement et al., 2004).

The coconut, oil, date and peach palms are all seed-propagated, with no natural vegetative propagation capability. Date and peach palm have a minor ability to undergo asexual propagation by forming offsets (Blake, 1983) or offshoots (Mora-Urpí et al., 1997). Oil palm, which lacks secondary shoot formation for vegetative multiplication in nature, is solely amenable to an *in vitro* clonal propagation method (Morcillo et al., 2007). In addition, propagation by seed in date palm causes such heterogeneity that some offspring develop low quality fruit and incompetence in the field (Naik and Al-Khayri, 2016). Offshoots in date palm which produce genetically identical progeny are more favourable, but their availability is limited (Al-Khayri, 2012). Vegetative propagation of offshoots in peach palm is possible, but the formation of

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offshoots is also limited (one to four per year), and the survival rate is low in the field (Mora-Urpí et al., 1997). As for coconut, productivity of the current global resource is decreasing due to biotic or abiotic stress, the fluctuating market demand for its oil as well as a low efficiency of seedling production for replanting (Nguyen et al., 2015).

Due to the propagation challenges for these economically important palm species, *in vitro* clonal propagation through plant tissue culture has been studied extensively and commercialised for some species.

For instance, tissue culture techniques such as embryo culture, somatic embryogenesis (SE) and cryopreservation of coconut have been investigated over the past 60 years (Adkins et al., 2016; Nguyen et al., 2015). Somatic embryogenesis is a promising *in vitro* approach for the clonal propagation of elite coconut germplasm due to its capacity for large-scale clonal propagation (Bandupriya et al., 2016). Nevertheless, there are still limitations in the existing protocol which require improvement to enable large-scale production. The establishment of a highly productive embryogenic cell suspension culture is a plausible objective (Nguyen et al., 2015), but there has been limited research undertaken on cell suspension culture. Past work includes the study on non-embryogenic coconut cell suspension culture (Basu et al., 1988), preliminary studies to initiate embryogenic cell suspension culture of coconut (Bhavyashree et al., 2016; Nguyen, 2018), and the study on somatic embryo formation in liquid culture (Antonova, 2009). Meanwhile the method has been successfully established for oil palm and date palm (Bhaskaran and Smith, 1992; de Touchet et al., 1991) which raises the expectation that the cell suspension culture technique for the rapid mass propagation of coconut could be achieved.

2. Somatic embryogenesis *via* cell suspension culture

Somatic embryogenesis, solely seen in the plant kingdom, is a process of transition from undifferentiated somatic cells into normal embryogenic cells and eventually into entire plants (Zimmerman, 1993). The process begins with the initiation and proliferation of an embryogenic culture, followed by the maturation of somatic embryos and then regeneration into plants (Fig. 1). Proliferation can be achieved on a semi-solid medium as an embryogenic callus or in a liquid medium as an embryogenic cell suspension culture (Von Arnold, 2008). Hence, it is defined as an *in vitro* system typically initiated by inoculating friable embryogenic callus, as the starting material, into a suitable liquid medium with continuous shaking for its multiplication (Mustafa et al., 2011). Friable embryogenic callus is preferable as it needs less force to achieve cell separation as compared to that required for compact embryogenic callus (Bhatia, 2015). The selection of large quantities of high-quality embryogenic callus is an important step in the development of embryogenic cell suspension cultures. The initiating tissues used should consist of embryogenic calli and transparent proembryos, while meristematic globules, cotyledonary stage embryo and compact calli should be removed (Strosse et al., 2003).

Cell suspension cultures are usually agitated continuously to break up cell aggregates, to maintain uniform cell distribution and to provide good gaseous exchange to sustain cell respiration. New individual cells are formed by continuous break-up of the inoculum and the shedding of new cell clusters as cell division proceeds. In nature, plant cells tend to adhere together, therefore, a typical cell suspension culture is often made up of small clusters of cells rather than single cells. In addition to this, the liquid medium used in cell suspension culture is usually similar to that used for callus culture (Bhatia, 2015). The regeneration, therefore, *via* SE and cell suspension culture of date palm, involves several stages: 1) the induction and proliferation of friable embryogenic callus; 2) the establishment of embryogenic cell suspension culture; 3) the development of somatic embryos in the cell suspension culture; 4) the maturation and germination of those somatic embryos; 5) the elongation of shoots, and induction of roots; 6) the acclimatization of the plantlets formed (Abohatem et al., 2017) (Fig. 2).

Cell suspension culture is considered to be superior to callus culture because it offers high medium-to-tissue contact which then promotes a rapid tissue multiplication rate (Soomro and Memon, 2007). A comparatively homogeneous cell culture is developed due to the rapid and uniform transfer of nutrients, the easier access to medium-added plant growth regulators, and precursors and signal compounds (Mustafa et al., 2011). Agitation in a cell suspension culture medium ensures uniform delivery of compounds without gradients being formed around the plant tissues, in contrast to the solidified culture medium system used for callus culture. Hence, suspension culture is the generally favoured approach, as the effects of exogenous applied plant growth regulators are often concentration dependent, and concentrations are kept uniform in an agitated system. Another advantage of this culture system is that there is a reduction in the negative feedback effect of toxins that are commonly released by the cultured tissues into the medium. The inhibition of metabolites secreted by the cells during further growth and development has been found to be minimized by the rapid dispersion and dilution of these substances in the liquid media. In contrast, the toxins would remain in close proximity to the cultured tissues that produced them in a gelled media system (Ascough and Fennell, 2004).

Furthermore, the cell suspension culture system also provides good availability of nutrients and carbon for growth and has a high capability to produce consistent results (Moscatiello et al., 2013). Besides this, the cell suspension culture system is easy to maintain at a high cell population density in a single vessel, allowing the further proliferation of cells when aliquots are placed into additional culture vessels. Hence, it is also a convenient way for studying cell responses that correspond to cell growth, differentiation and regeneration (Wang et al., 1999). Another important advantage is that they have the capacity to mass produce somatic embryos that are all at the same stage of development (Buffard-Morel et al., 1995). This overcomes one of the drawbacks of SE *via* callus culture which produces a mixture of developmental stages (Deo et al., 2010).

Plant cell suspension culture is also often used to culture plant cells on a large-scale (Ahmad et al., 2013), as it induces the efficient production of plant regenerants in large numbers (Wang et al., 1999), whilst a solid medium is not suitable for large-scale propagation (Fki et al., 2011). For instance, banana hybrid cultivar 'FHIA-18'(*Musa* sp. AAAB) was mass-propagated through SE in liquid culture medium and a high regeneration rate was achieved after only 1 month (Kosky et al., 2002). A similar result was observed in oil palm cell suspension culture, whereby cell proliferation occurred rapidly and there was a four-fold increase in initial weight after just 1 month, with the embryogenic competent cells constantly available for more than 18 months (de Touchet et al., 1991). Hence, the advantage of a large-scale clonal propagation allows for the application of a cell suspension culture system in oil palm, date palm and peach palm.

2.1. General uses of the plant cell suspension culture system

A simple model system of plant cell suspension is a good way to the processes of analyse complex cellular and molecular physiology (Moscatiello et al., 2013; Mustafa et al., 2011). Apart from being a tool for the mass propagation and regeneration of somatic embryos, cell suspension culture is a potential renewable source for the synthesis and production of valuable phytochemicals from medicinal plants (Mulabagal and Tsay, 2004). It is also used as plant material for protoplast isolation and cultivation due to the its ready availability of material, and its high stability (Wakita et al., 1996). In fact, it is also a useful aid in studying mutagenesis and cell line selection of desired characteristics, as a large number of cells are available. Cell suspension culture, which requires only a small laboratory space, also offers uniform conditions for chemical treatments (Jeoung et al., 1998). The cost is reduced when the culture system is automated, as production is increased by controlling and regulating cell growth and metabolic pathways (Mulabagal and Tsay, 2004). There are also applications of cell

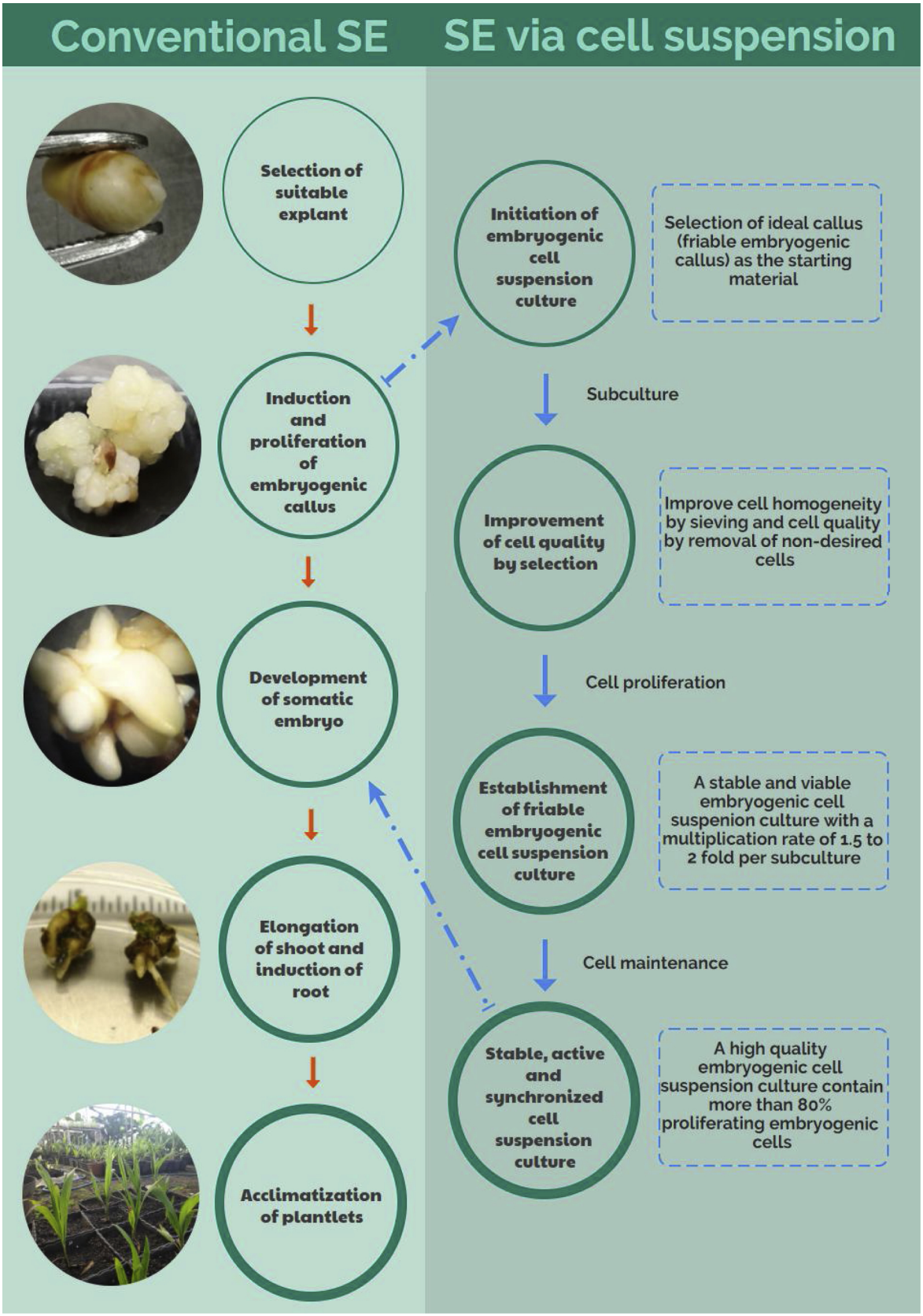

Fig. 1. A flow chart of the conventional clonal propagation of coconut via somatic embryogenesis (left) and the addition of cell suspension culture multiplication step (right) to improve the somatic embryo production rate. (The third and fourth photos are reprinted from Nguyen et al. (2015) and Antonova (2009) with permission. The last photo is a photo courtesy of Sisunandar Sudarma.)

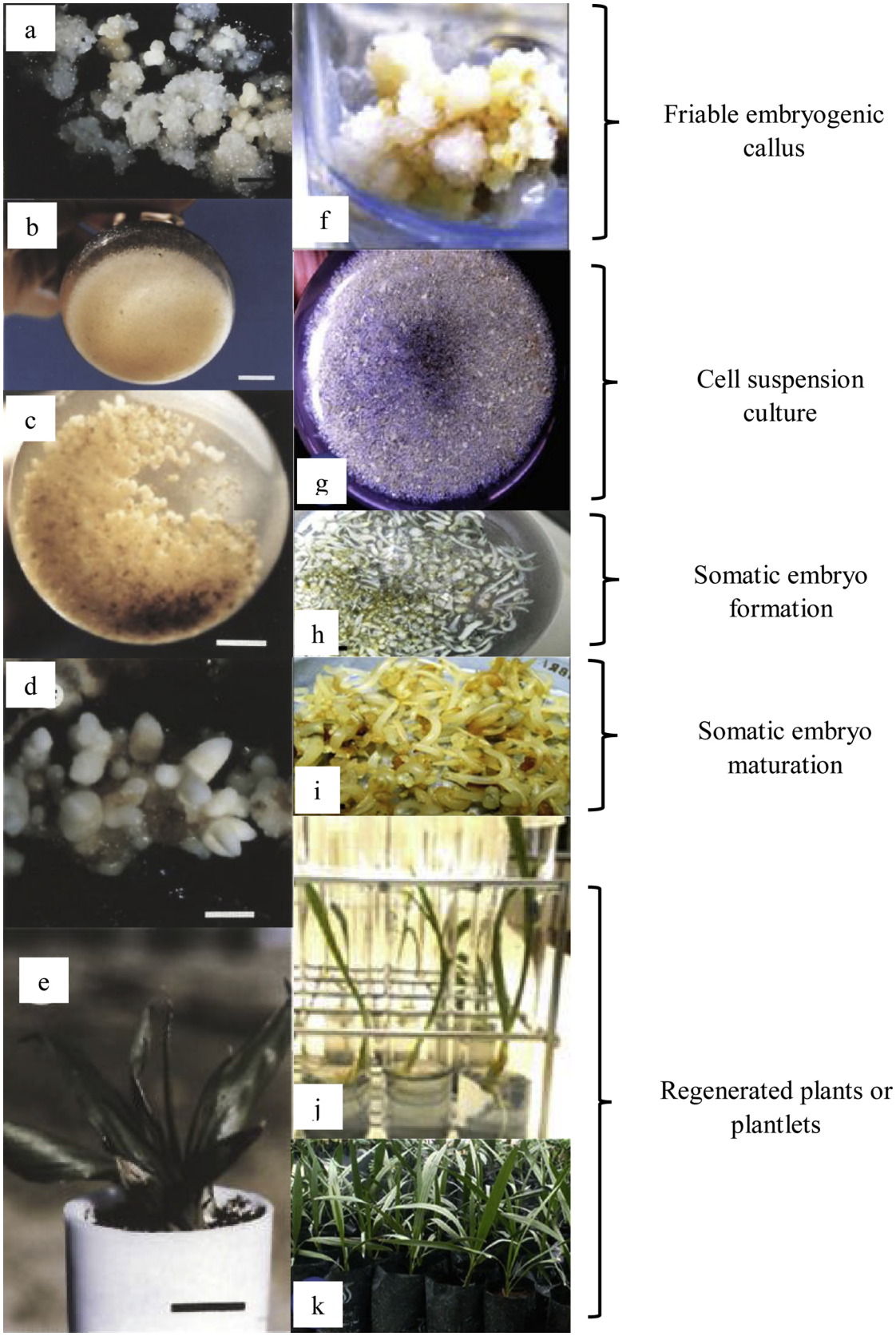

Fig. 2. The stages of establishment and plant regeneration of oil palm (a–e) and date palm (f–k) via somatic embryogenesis through cell suspension culture. (The photos are reprinted from Teixeira et al. (1995) and Abohatem et al. (2017) with permission.).

suspension culture systems for *in vitro* date palm germplasm conservation by cryopreservation (Al-Bahrany and Al-Khayri, 2012), the transformation of maize (*Zea mays* L.) cells resulting in the generation of fertile transgenic plants (Gordon-Kamm et al., 1990), and the genome editing using isolated protoplasts of oil palm (Masani et al., 2014).

3. Factors affecting establishment and growth of embryogenic cell suspension culture

The establishment of a cell suspension culture from highly somatic embryogenic callus tissues, along with the maintenance of their regeneration capacity, and for its preservation for extended periods of time, needs to be achieved prior to the rapid, high-level production of plants can be achieved (Fellers et al., 1995). There are several relevant factors which can be categorized into those that are respectively biological, chemical or physical in nature (Zhong and Yoshida, 1997). Past attempts have been made to form such cultures from coconut (Bhavyashree et al., 2016; Nguyen, 2018) but high-quality cultures and their further regeneration into plants has not yet been achieved. Hence, there is no information currently available on the various culture growth factors involved, but there is information for other palm species (Table 1 and 2) that is expected to be relevant for future coconut work.

3.1. Biological factors

3.1.1. Inoculum density

Initial inoculum density is important for establishing a cell suspension culture for any plant species (Lo et al., 2012). This is because there are interactions that occur just between cells as well as between the cell and the culture medium, and this affects the biological environment of the cultures (Ozeki and Komamine, 1985). The inoculum density should remain above the minimum cell density in order to maintain active cell growth following subculture (Franklin and Dixon, 1994). This is because cell growth does not occur below the minimum cell density nor when a lag phase in growth occurs (Jeong et al., 2009; Yusuf et al., 2013). Although the optimum inoculum density is species-specific, the minimum cell density can be affected by the cell line, culture medium constituents and cell growth rate. In general, a large inoculum size is necessary to create a high cell density and to establish a growing cell suspension culture. However, it is possible to initiate a cell suspension culture with a low inoculum density when conditioned medium is used (Franklin and Dixon, 1994). Cell suspension cultures can be inoculated with either low (0.1 g) or high (1.0–2.0 g) cell inoculum densities per 33–50 mL of medium. However, low inoculum density is often more beneficial. This is because the embryogenic cells may begin to senesce or the non-embryogenic cells (fast growing) may restrict the growth and proliferation of embryogenic cells when the initial inoculum density is high. Whilst, at a low inoculum density, these non-embryogenic cells have only a small effect, they do not survive through further subcultures and have a slow proliferation rate. In most cases, single cells rarely prevail in embryogenic cell suspension cultures as they usually rapidly proliferate into small aggregates of embryogenic tissue. The single cells shed from the aggregates which are most probably non-embryogenic cells will have a low survival capacity at low inoculum density (Finer, 1994).

The effect of inoculum density was studied in the establishment of oil palm cell suspension culture by de Touchet et al. (1991). It was discovered that a low inoculum density of 0.1 g to 0.3 g per 20 mL was the best as the final suspension mass increased four-fold after only 1 month of culture. Similarly, the cell density which determines the plating efficiency was evaluated by Al-Khayri (2012) in date palm cell suspension culture. This researcher discovered the highest plating efficiency (14.6 %) was obtained when the initial cell density was *ca* 10,000 cells per mL. In addition, the number of somatic embryos cultured in liquid media was 3.5-fold greater than could be produced on solid media after 8 weeks of culture. These results demonstrate the importance of inoculum density in establishing the cell suspension culture protocols of these two palm species.

3.1.2. Cell aggregate size

As plant cells tend to aggregate or cluster in suspension culture (Tanaka et al., 1992), the cell aggregate size is believed to be controlled by the degree of cell cohesiveness. This is related to the composition of the cell wall (probably pectic compounds) and the degree of cell wall differentiation (Warren, 1992). Typically, the cell aggregate size can reach up to a few mm in diameter with a cell number of hundreds (Zhao et al., 2003), or even thousands, with cell clump sizes ranging from < 100 μm to over 2 mm (Kolewe, 2011). Therefore, plant cell suspension cultures are heterogeneous with regard to the presence of cell aggregates of various shapes and sizes (Tanaka et al., 1992).

Cell aggregate size is influenced by culture age and medium composition (Wallner and Nevins, 1973). Hence, aggregate size can be controlled by modifying the media constitution as well as by the culture method employed (Bais et al., 2002). In addition, the cell aggregate size has a strong influence on cellular metabolism (Kolewe et al., 2011), including bioprocess performance such as growth and secondary metabolite production (Patil et al., 2013). The cell aggregate size can also affect protein synthesis, substrate and product mass transfer, free amino acids availability, secondary metabolites synthesis and scale-up attainability (Bais et al., 2002). For instance, cell aggregation into large clumps often causes problems in large-scale cultivation of cell suspension culture (Doran, 1993). Therefore, cell aggregate size has been identified as a key feature because it creates microenvironments for the individual cell aggregates with reference to their cell to cell signalling, their nutrient supply and the shear stress applied by the *in vitro* shaking conditions (Kolewe, 2011).

The formation of somatic embryos in date palm embryogenic cell suspension culture was found to be affected by cell aggregate size (Fki et al., 2003). The greatest number of somatic embryos was formed when the aggregates were sieved through a large mesh size of 1000 μm, as compared to a mesh size that was approaching 100 μm. Somatic embryogenesis from embryogenic cell suspension culture of banana cv. 'Da Jiao' (*Musa paradisiacal* ABB L.) was also found to be affected by the cell aggregate size and plating density (Dai et al., 2010). The numbers of somatic embryos obtained were highest in Petri dishes inoculated with cell aggregate sizes of 154–900 μm at a low plating density. Hence, it is proposed that somatic embryos are developed from cell aggregates rather than from single cells.

3.1.3. Growth kinetics and selection of cell lines

Growth kinetics of a culture system need to be understood in order to achieve effective cell culture manipulation. This includes discovering the point at which growth starts, and then the stages that comprise the lag, the exponential or log, linear, deceleration phases and the stationary phase. Then, a growth curve can be plotted based on this information. It is also important to know the performance and metabolic activities that are happening at each growth phase (Al-Khayri, 2012). A sigmoid S-shaped growth curve was obtained for a date palm embryogenic cell suspension culture by measuring its fresh weight over 30 days (Fki et al., 2003), as well as the packed cell volume for 12 weeks (Al-Khayri, 2012). A similar growth curve has been obtained in the cell suspension culture of banana (*Musa acuminata* Colla cv. Mas) (Jalil et al., 2003). The cell suspension culture growth cycle also helps to determine the optimal subculture interval. However, the growth curve is affected by the inoculum size, as smaller inoculum size will result in a longer lag phase, and there may be no cell growth when the inoculum density is below a critical level. A stationary phase is likely to start earlier, if the inoculum density is larger. As a result, the subculture interval is often determined by the aims of the research, such as efficiency in maintenance, optimal cell viability or use in further research for biomass optimization, and production of phytochemicals, ribonucleic acids (RNAs) or proteins (Mustafa et al., 2011).

Table 1
Factors affecting optimization of cell suspension culture system of oil palm, date palm, peach palm and coconut.

Palms	Factor	Outcome	References
Oil palm	• Inoculum density: • 0.05-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4 & 0.4-0.5 g • Plant growth regulator: 2,4-D • Concentrations tested: 25, 50, 80, 100, 150, 200 mg L^{-1}	• Best inoculum density was between 0.1 to 0.3 g with a 4-fold increase in final mass after one month. • Cell suspension cultures obtained in media supplemented with 80 or 100 mg L^{-1} 2,4-D after 2 subculture cycle.	de Touchet et al. (1991)
Oil palm	• Cytokinin types: BAP & KN • Cytokinin concentrations: 0.1, 0.5 & 1.0 mg L^{-1}	• Addition of KN to culture medium shorten the time to establish fine cell suspensions as compared with medium with dicamba alone • Formation of small aggregates with round and dense cytoplasm in medium with addition of 0.1 mg L^{-1} KN	Te-chato et al. (2008)
Oil palm	• Culture medium: MS & Y3 media • Plant growth regulators: NAA, 2,4-D & dicamba • Concentrations tested: 0.1, 0.2, 0.3, 0.4 & 0.5 mg L^{-1} • Carbon sources: 0.2 M sorbitol or 30 g L^{-1} sucrose	• MS medium resulted in higher PCV than Y3 medium • MS medium with addition of 0.4 mg L^{-1} 2,4-D gave the best result in PCV (2.25 mL) with somatic embryos of 2–4 mm in size and produced 20 embryos per vessel • MS +0.5 mg L^{-1} 2,4-D produced 106.6 cell aggregates mL^{-1} • MS + 0.2 M sorbitol gave the best result whereby somatic embryos of 2 mm in size and 11.33 embryos per vessel are produced	Kramut and Te-chato (2010)
Oil palm	• Liquid culture system improvements • MPOB Fast Transfer Technique (MoFaTT) • Two-in-One MPOB Simple Impeller (2-in-1 MoSLIM) • Simple Impeller with Fast Transfer Technique (SLIM-FaTT) • MPOB Modified Vessel (MPOB-MoVess) • MPOB Motorized Vessel (MPOB-MotoVess)	• MoFaTT allows rapid and convenient transfer for liquid media maintenance and embryos maturation in the shaker system • 2-in-1 MoSLIM is an alternative to maintain cultures on a magnetic stirrer instead of a shaker. • SLIM-FaTT ensures efficient medium replenishment in a bottle system (MoSLIM). • MoVess is designed to reduce cost and time for proliferation while MotoVess is improved version of MoVess whereby an automated stand and shaft with impeller are used. • MoVess and MotoVess can be used for scale up production and the performance is similar to the merchandised bioreactors at the same working volume	Tarmizi et al. (2012)
Date palm	• Physical conditions of liquid medium: • Heller support, continuous shaking and shaking for 2, 4, & 6 weeks in liquid medium and subsequent culture in solid medium • Sucrose concentrations: 0, 15, 30 & 60 g L^{-1} • Sucrose starvation period: 0, 1, 2, 3 & 4 weeks	• Best result in liquid culture with physical condition of shaking continuously. Each gram of callus produced 20–40 embryos with size bigger than 5 mm after culture for 3–5 months. • Media with 30 g L^{-1} sucrose significantly produce higher number of embryos. • 2 weeks of sucrose starvation in callus doubled the number of total embryos after 5 months • 40 to 60 embryos per gram were produced from callus after 4 months culture in shaken liquid media with 2 weeks of sucrose starvation and further culture on media with 30 g L^{-1} sucrose. • 10 % of embryos geminated with 90 % survival rate in acclimatization.	Veramendi and Navarro (1996)
Date palm	• Sucrose concentrations: 0, 20, 30, 40 & 50 g L^{-1} • PEG concentrations: 0, 10, 30 & 60 mg L^{-1}	• Medium supplemented with 30 g L^{-1} sucrose was the best for embryo maturation. • Hyperhydration was reduced with addition of PEG to the medium.	Al-Matar et al. (1997)
Date palm	• Cell size • Mesh size (μm): 100, 200 500, 1000	• Somatic embryos originate from cell aggregates whereby number of embryos increase positively with bigger mesh size.	Fki et al. (2003)
Date palm	• Cultivar: Jihel (JHL) & Bousthami Noir (BSTN) • Sucrose concentrations: 15, 30 & 60 g L^{-1}	• Best result in liquid medium containing 30 g L^{-1} of sucrose which resulted in significant increase in embryogenic mass for both cultivar. • The soluble protein and sugar content were enhanced when the sucrose content in medium increase from 15–30 g L^{-1}. • Proteins and phenolics contents are negatively correlated.	Zouine and El Hadrami (2004)
Date palm	• Medium composition: ABA and/or glutamine • ABA concentration: 0.1, 1 & 10 μM • Glutamine concentration: 330, 670, 1340 μM	• Liquid medium containing 10 μM ABA yield 72 embryos in 100 mL of medium after two weeks. • Combination of 10 μM ABA and 670 μM glutamine improved the storage proteins accumulation and the somatic embryos production. • Activated charcoal and phytagel are beneficial for germination of somatic embryos.	Zouine et al. (2005)
Date palm	• Medium type & composition: • Solid or liquid; with & without activated charcoal	• Liquid MS medium produced greatest number of somatic embryos. • Activated charcoal is beneficial in the production of somatic embryos.	Saker et al. (2007)
Date palm	• Medium composition: 2,4-D and Glutamine • Glutamine concentration: 335, 670, 1340 μM • 2,4-D concentration: 0 and 0.1 mg L^{-1} • BAP concentrations in regeneration medium: • 0.05, 0.1 & 0.2 mg L^{-1}	• Addition of 0.1 mg L^{-1} 2,4-D and 670 μM glutamine to the liquid medium improved the proliferation of somatic embryos and the protein and sugar content were also increased. • Addition of 0.05 mg L^{-1} BAP to the medium improved the germination rate while the number of secondary embryos was increased when 0.2 mg L^{-1} BAP was added.	Zouine and El Hadrami (2007)
Date palm	• Plant growth regulator: 2,4-D • Concentrations tested: 0, 0.5, 1.0, 1.5 & 2.0 mg L^{-1}	• Increased concentration of 2,4-D in liquid medium improved the somatic embryo proliferation and performance. • 65 % somatic embryos cultured in solid medium converted into plantlets while only 22 % of somatic embryos germinated and converted into plantlets from cell suspension culture.	Othmani et al. (2009)
Date palm	• Explant type: Shoot tip & primordial leaf • Cultivar: Zaghlool & Samany • NAA concentrations: 0, 1, 3, 5 & 10 mg L^{-1} NAA	• Cell cultures derived from shoot tips had the highest biomass for both cultivars. • Zaghlool cultivar performed better than Samany cultivar. • The addition of 3 mg L^{-1} NAA to the media was the best in terms of cell number.	Taha et al. (2010)

(*continued on next page*)

Table 1 (*continued*)

Palms	Factor	Outcome	References
Date palm	• Plant growth regulator: BAP • Concentrations tested: 0.3, 0.4 & 0.5 mg L^{-1} • Subculture cycle: 7, 15 & 20 days	• Best result obtained in media supplemented with 0.3 mg L^{-1} BAP which produced the greatest number of somatic embryos. • Subculture cycle of 7 days significantly reduce the browning of tissues due to the reduction in phenolic compounds and peroxidase activities, hence, the embryogenic cells proliferation increased.	Abohatem et al. (2011)
Date palm	• Cell density: • 100, 500, 1000, 5000, 10000, 50000 & 100000 cells per mL • Medium type: Solid & liquid media	• Cell density of 10,000 cells per mL has the highest plating efficiency of 14.6 %. • Somatic embryos in liquid media was 3.5-fold more than solid media after 8 weeks.	Al-Khayri (2012); Naik and Al-Khayri (2016)
Date palm	• Culture media system: • Solid, cell suspension & RITA temporary immersion system • Cell suspension culture flask: • Rasotherm flask & Phytacon vessels • Media composition: a) ½ MS +0.5 mg L^{-1} 2,4-D b) ½ MS +1.0 mg L^{-1} 2,4-D c) MS +10 mg L^{-1} NAA +1.5 mg L^{-1} 2iP • Callus source for cell suspension culture: • Callus derived from shoot tips & leaf segments • Embryo formation media composition: Liquid or solid a) MS +0.1 mg L^{-1} NAA +1.5 g L^{-1} AC b) MS +0.1 mg L^{-1} NAA +1.5 g L^{-1} AC +0.05 mg L^{-1} BAP c) PGR-free MS medium	• Cell suspension culture system has the highest biomass of 4 g after 16 weeks. • Suspension culture of callus mass was slightly higher in Rasotherm flask but not significantly different than those in Phytacon vessels. • No significant difference in the callus biomass between different media composition. • Callus derived from shoot tips produced more somatic embryos than those derived from leaf segments. • Liquid medium supplemented with 0.1 mg L^{-1} NAA and 1.5 g L^{-1} AC has the highest number of somatic embryos (160 somatic embryos per 0.5 g callus). • Cell Suspension culture produced somatic embryos of 6–16 times more than solid culture. • Only 6 weeks is required for somatic embryos formation in cell suspension culture instead of 18 weeks in solid culture.	Ibraheem et al. (2013)
Date palm	• Medium type: Solid & liquid media • Plant growth regulators: 2,4-D & BAP • Concentrations of 2,4-D/BAP: 5/1, 10/1, 50/1, 100/1 & 1/0 mg L^{-1} • Regeneration medium: PGRs-free liquid or solid medium	• The best multiplication factor of 9.75 in the biomass was obtained in liquid media supplemented with 1.0 mg L^{-1} 2,4-D after two months culture period. • Homogenous growth was observed in somatic embryos cultured in liquid medium without PGRs. • Signs of hyperhydricity was observed in all somatic embryos.	Boufis et al. (2014)
Date palm	• ABA concentrations: 0, 1, 10, 50 & 100 μM	• Synchronization in the size of somatic embryos were observed in cell suspension medium supplemented with 50–100 μM ABA and the size of majority embryos were greater than 3 mm. • Addition of 1 μM ABA significantly reduced the fresh weight of cell suspension culture and 10 μM ABA inhibit further growth. • Addition of ABA inhibit the production of somatic embryos. • Results refer to cultivar Nabout Saif and may differ in different cultivars.	Alwael et al. (2017)
Date palm	• PEG levels: 0, 5, 10, 15 & 20 % w/v	• MS + 10 % PEG was the best treatment with the increase in the fresh weight, the degree of callus formation and the percentage of normal embryos. • Organic solutes which increased with addition of PEG to the media were concentration dependent.	Helaly et al. (2017)
Peach palm hybrid	• Culture flask closure: • Cotton plugs, rubber and aluminium foil caps	• Cell growth was affected by the gaseous environment in the flask. • About 3-fold of maximum weight to the initial weight after 10 days in cell suspension culture enclosed with cotton plugs and aluminium foil. • Little growth in culture enclosed with rubber cap as browning and death occur progressively while large aggregates and slight aggregation were found in cell suspension culture enclose with cotton plugs and aluminium foil respectively. • The best flask closure was aluminium foil caps which allowed low gaseous exchange and with frequent subculture, cell aggregations and homogeneous rapid growth of cell suspension culture will be obtained.	Marino et al. (1995)
Coconut palm	• pH of medium: 5.0, 6.0, 7.0 & 8.0 • Medium composition: Glutathione, spermidine, methylglyoxal-bis • Glutathione concentrations: 1, 3 & 5 mM • Spermidine concentrations: 0.01, 1 & 100 μM • Methylglyoxal-bis concentrations: 1, 3 & 5 μM	• Cell number was three times more when cultured in medium of pH 7.0 after 48 h. • 5 mM glutathione produced 2-fold increase in cell number while methylglyoxal-bis (guanylhydrazone) inhibit cell growth. • Spermidine at concentration of 1 μM promoted maximum cell growth. • Cell division was positively correlated to glyoxalase-I activity.	Basu et al. (1988)

Key: AC: Activated charcoal, BAP: 6-Benzylaminopurine, 2,4-D: 2,4-Dichlorophenoxyacetic acid, KN: Kinetin, NAA: 1-Naphthaleneacetic acid, ABA: Abscisic acid, IBA: Indole-3-butyric acid, 2iP: N6-(2-Isopentenyl) adenine, PEG: Polyethylene glycol, PVP: Polyvinylpyrrolidone, PGR: Plant growth regulator, MS: Murashige and Skoog (1962) medium, Y3: Eeuwens (1976) medium.

Table 2
Somatic embryogenesis of oil palm, date palm, peach palm and coconut *via* cell suspension culture.

Palms	Initial culture condition	Plant regeneration/Further culture condition	Outcome	References
Oil palm	Establishment • MS +20 g L^{-1} sucrose +30 mg L^{-1} adenine sulphate +1 g L^{-1} AC +1 mg L^{-1} AC +1 mg L^{-1} BAP + 80 or 100 mg L^{-1} 2,4-D • Temperature: 27 ± 1 °C • Light intensity: 50 μmol m^{-2} s^{-1} • Light/dark photoperiod: 12:12 h • Agitation: 90 rpm • Subculture cycle: 4–6 weeks Initial explant: • 500 mg of friable embryogenic calli with white nodules derived from immature leaflet fragments from mature palms	Plant regeneration • Basal medium +30 g L^{-1} sucrose Initial explant: • Direct inoculation of proliferating calli nodules • Synchronous maturing of calli nodules from culture in plant growth regular-free liquid medium	• 4-fold increase of initial weight in a month • No decrease in embryogenic potential over 18 months • Multiplication stage prior to embryo formation. • Reduced multiplication and differentiation of cell clumps • One to three embryos produced from one nodule • First shoot observed after 4–6 weeks • 1.8–18.1% shoot formation	de Touchet et al. (1990), 1991
Oil palm	Establishment • Establish embryogenic cell suspensions based on method of de Touchet et al. (1990), 1991 Initial explant: • Friable embryogenic calli derived from immature leaflet fragments of mature palms	Single embryo encapsulation	• Established embryogenic cell suspension culture for 17 clones • Achieve an embryogenic cell suspensions concentration of 105 cell clusters L^{-1} with 4-fold multiplication capacity each month • First clone palms multiplied from cell embryogenic suspensions planted at La Mé Research Station, Ivory Coast in 1991. • Plantlets developed from encapsulated single embryos	Duval et al. (1995a),
Oil palm	Establishment • Y3 + KM vitamins +500 mg L^{-1} Cysteine +100 mg L^{-1} Inositol +250 mg L^{-1} Ascorbic acid +15 g L^{-1}sucrose +5 g L^{-1} glucose + 10 μM 2,4-D or picloram; pH 6.0 • Temperature: 26–27 °C • Dark condition • Agitation: 140 rpm • Subculture cycle: 2 weeks Initial explant: • Primary globular callus derived from immature zygotic embryos • Friable embryogenic tissue derived from mature zygotic embryos	Plant regeneration • Y3 + Y3 vitamins +100 mg L^{-1} Inositol +20 g L^{-1} sucrose + 15 μM NAA + 2 μM ABA +2 g L^{-1} Gelrite; pH 6.0	• Required 3–5 months to establish cell suspension culture • Only 2 months required to establish cell suspension culture • Optimum inoculum size was 1.5 g of fresh cells in 50 mL of liquid medium • Differentiated embryo observed after 4–6 weeks. • Regeneration frequency: 180 embryos g^{-1} of settled-cell volume or 5400 embryos L^{-1} of cell suspension	Teixeira et al. (1995)
Oil palm	Establishment • Liquid Y3 + inorganic salts +45 g L^{-1} glucose + 2,4-D • Media with or without 0.2 mg L^{-1} 2,4-D • 520 to 860 μm sieved for one month • Temperature: 25 ± 2 °C • Light intensity: 20 μmol m^{-2} s^{-1} • Light/dark photoperiod: 16:8 h • Agitation: 100 rpm • Subculture cycle: 1 week • Initial explant: • 5 g nodular callus of 2 months old	Plant regeneration • Y3 + 20 % coconut water or 0.01 mg L^{-1} NAA or 0.2 mg L^{-1} 2,4-D + 20 % coconut water	• Cell suspension culture was established in 2–3 months. • Fine and friable to nodular suspension cells were obtained in media without the addition of 2,4-D. • 2–3-fold of cells were formed after one month. • Cells below 520 μM were suitable for establishing friable embryogenic cells. • There was no development of plantlets from embryoids. • Shoot, root and haustorium were observed in embryoids cultured in media supplemented with 0.01 mg L^{-1} NAA after 7 days.	Kanchanapoom and Chourykaew (1998)
Oil palm	Establishment • B5 liquid media +30 g L^{-1} sucrose +0.1 mg L^{-1} Kinetin +0.1 mg L^{-1} 2,4-D Initial explant: • Friable embryogenic callus derived from immature leaf fragments of adult palm	–	• Expression of embryogenesis in medium without plant growth regulator • 73.4 % globular embryos present • Synchronized embryo development	Tahardi (1998)

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Table 2 (*continued*)

Palms	Initial culture condition	Plant regeneration/Further culture condition	Outcome	References
Oil palm	Establishment ● Establish embryogenic cell suspensions (de Touchet et al., 1991): Basal medium +100 mg L^{-1} sodium ascorbate +20 g L^{-1} sucrose +30 mg L^{-1}adenine sulphate +1 g L^{-1}AC + 4.44 μM BAP + 450 μM 2,4-D ● Subculture cycle: Monthly Initial explant: ● Friable embryogenic callus derived from immature leaf fragments of adult palm	Plant regeneration ● Embryo development medium: Liquid basal medium +3 g L^{-1} sucrose +0.5 g L^{-1} casein hydrolysate ● 100 μm sieved and plated onto 8 g L^{-1} agar gelled embryo development medium ● Temperature: 27 °C ● Light intensity: 45 μmol m^{-2} s^{-1} Initial explant: Cell clusters	● Expression of embryogenesis in medium without plant growth regulator ● 60–73 % single shoot formation with addition of 1 μM BAP to the embryo development medium but clone dependent ● Short term exposure to BAP result in single shoot formation while long term exposure caused multiple shoot formation	Aberlenc-Bertossi et al. (1999)
Oil palm	Establishment ● Liquid MS medium + 2,4-D + NAA; No AC ● Subculture cycle: Monthly Initial explant: ● Embryogenic callus derived from pinna segments of immature leaves	Plant regeneration ● MS medium + Gelrite + No PGRs Initial explant: Embryoids	● Expression of embryogenesis in medium without plant growth regulator ● 75 % embryoid lines from 26 out of 29 clones result 6–7-fold multiplication rate per subculture after 4th subculture cycle ● Plantlets form after 6 months subculture in solid medium. ● Proliferating lines have embryogenic potential and capable to form shoots after over 24 subculture cycles	Wong et al. (1999)
Oil palm	Establishment ● Establish embryogenic cell suspensions based on Duval et al. (1995a), b ● Liquid medium +100 mg L^{-1} 2,4-D +2 mg L^{-1} AC ● 100 μm sieved ● Subculture cycle: Monthly Initial explant: ● 10–12 mg L^{-1} biomass of friable nodular callus isolated from compact callus	Plant regeneration ● Solid medium Initial explant: ● 0.05 mL packed cell volume of small cell aggregates for each petri dish	● Cell suspension growth rate: 2–3 per subculture cycle ● Achieve an embryogenic cell suspensions concentration of 105 cell clusters/L with 4-fold multiplication each month ● Established embryogenic cell suspension cultures for 20 clones ● Expression of embryogenesis in medium without plant growth regulator ● About 300 embryos per petri dish differentiated ● Increased single shoot formation with addition of 5 μM BAP in maturation medium	Rival (2000)
Oil palm	–	–	● 80–90 % embryogenic palms proliferated in liquid cell suspension culture system ● 60–90 % of embryogenic callus proliferated in cell suspension cultures ● 6–7-fold proliferation rate in every month subculture cycle for liquid culture system while solid culture system only 2-fold every 2 months subculture cycle. ● 90–100% shoot formation in embryos transferred to solid medium	Soh et al. (2003), 2001
Date palm	Establishment ● MS +170 mg L^{-1} $NaH_2PO_4.H_2O$ +40 mg L^{-1} adenine sulphate +0.4 mg L^{-1} thiamine.HCl +30 g L^{-1} sucrose ● Room temperature ● Light intensity: 28 μmol m^{-2} s^{-1} ● Light/dark photoperiod: 16:8 h ● Agitation: 150 rpm ● Subculture cycle: Monthly Initial explant: ● 200 mg of friable callus derived from shoot tips and immature flower buds	Plant regeneration ● MS +170 mg L^{-1} $NaH_2PO_4.H_2O$ +40 mg L^{-1} adenine sulphate +0.4 mg L^{-1} thiamine.HCl +30 g L^{-1} sucrose +8 g L^{-1} Difco agar +3 g L^{-1} AC ● Room temperature ● Light or dark condition ● Light intensity: 28 μmol m^{-2} s^{-1} ● Light/dark photoperiod: 16:8 h ● Culture period: 1 month Initial explant: Somatic embryos	● Hundreds of embryos could be obtained within 3 weeks after first subculture cycle ● 200 mg of embryogenic callus per flask could produce 1000 embryos. ● 40 % of embryos developed into normal plantlets	Bhaskaran and Smith (1992)
Date palm	Establishment ● ½ MS salts + MS vitamins +32 mg L^{-1} Fe-EDTA +1 mg L^{-1} 2,4-D +300 mg L^{-1} AC +30 g L^{-1} sucrose ● 500 μm mesh filter ● Temperature: 28 ± 1 °C ● Light intensity: 28 μmol m^{-2} s^{-1} ● Light/dark photoperiod: 16:8 h ● Agitation: 120 rpm ● Subculture cycle: 7 days Initial explant: ● 500 mg of friable callus derived from leaves and immature inflorescences	Plant regeneration ● MS + MS vitamins +65 mg L^{-1} Fe-EDTA +8 g L^{-1} Difco agar +50 g L^{-1} sucrose ● Temperature: 28 ± 1 °C ● Light intensity: 28 μmol m^{-2} s^{-1} ● Light/dark photoperiod: 16:8 h Initial explant: Proembryogenic masses	● Proembryogenic masses formed after 20 days without subculture. ● 200 ± 10 embryos produced in cell suspension culture in each month from 0.1 g of fresh embryogenic callus. Production rate is 20 times higher than culture in solid medium. ● 10,000 ± 45 embryos production rate in each litre per month. ● Hyperhydration in mature somatic embryos cultured in liquid medium for over 1 month. ● 25 % germination rate in media without PGRs	Fki et al. (2003)

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Table 2 (*continued*)

Palms	Initial culture condition	Plant regeneration/Further culture condition	Outcome	References
Date palm	Establishment ● MS salts +1.5 mg L^{-1} 2,4-D ● Filtered with Whatman No 2 filter paper ● Temperature: 25 ± 2 °C ● Agitation: 120 rpm ● Subculture cycle: 4 days Initial explant: ● Embryogenic callus derived from shoot tips	Plant regeneration ● Initiation: MS + 0–2.5 mg L^{-1} NAA ● Maturation: MS + 0–2.0 mg L^{-1} TDZ ● Germination: MS + 0–1.0 mg L^{-1} BAP or KN Initial explant: ● Initiation: Suspended cells ● Maturation: Globular embryos ● Germination: Matured somatic embryos	● Suspended cells cultured in 1.5 mg L^{-1} NAA give rapid response in somatic embryo development. ● Highest number of somatic embryos matured in media supplemented with 1.0 mg L^{-1} TDZ. ● Addition of BAP at concentration of 0.75 mg L^{-1} is better than KN in germination and conversion of somatic embryos into plantlets.	Aslam et al. (2011)
Date palm	Establishment ● Liquid MS +1 mg L^{-1} 2,4-D ● 1000 μm filter ● Temperature: 27 °C ● Light condition ● Light/dark photoperiod: 12:12 h ● Agitation: 90 rpm ● Subculture cycle: 2 weeks Initial explant: ● 1 g friable embryogenic callus derived from shoot tips	Plant regeneration ● 10 days pre-treatment in PGR free medium ● Further culture in MS + 5 μM BAP ● Addition of 1 mg L^{-1} ANA for rooting Initial explant: Cells from suspension culture	● Viable and homogenous cell suspension culture was obtained. ● Pre-treatment in PGR free medium is good for developing individual somatic embryos ● Globular embryos formed after 3–4 weeks of culture in cell suspension culture.	Selmani et al. (2013)
Date palm	Establishment ● ½ MS salts + ½ DeFossard Vitamins +0.1 mg L^{-1} 2,4-D +0.3 mg L^{-1} BAP +0.15 g L^{-1} AC +30 g L^{-1} sucrose ● 500 μm sieved ● Temperature: 25 ± 2 °C ● Agitation: 100 rpm ● Light/dark photoperiod: 16:8 h ● Light intensity: 28 μmol m^{-2} s^{-1} ● Subculture cycle: 7 days Initial explant: ● 0.5 g of embryogenic callus derived from shoot tips	Plant regeneration ● Maturation medium: ½ MS salts + ½ DeFossard Vitamins +0.15 g L^{-1} AC +30 g L^{-1} sucrose; culture for 2 weeks ● Germination medium: MS salts + DeFossard Vitamins +0.15 g L^{-1} AC +30 g L^{-1} sucrose +7 g L^{-1} agar ● Elongation medium: MS salts + DeFossard Vitamins +0.15 g L^{-1} AC +30 g L^{-1} sucrose +7 g L^{-1} agar +1 mg L^{-1} GA_3; culture for 4 weeks ● Rooting medium: MS salts + DeFossard Vitamins +0.15 g L^{-1} AC +30 g L^{-1} sucrose +7 g L^{-1} agar +0.1 mg L^{-1} NAA Initial explant: Somatic embryos	● Cell growth and somatic embryo formation were stimulated with the addition of 0.15 g L^{-1} AC to the medium. ● Browning was prevented by activated charcoal during the initial months of establishing cell suspension culture. ● Lower concentrations of 2,4-D significantly reduced the presence of abnormal somatic embryos and somaclonal variation.	Abohatem et al. (2017)
Date palm	Establishment and multiplication ● ½ MS +30 g L^{-1} sucrose +0.3 g L^{-1} AC +1 mg L^{-1} 2,4-D ● Temperature: 25 ± 2 °C ● Light intensity: 28 μmol m^{-2} s^{-1} ● Light/dark photoperiod: 16:8 h ● Agitation: 100 rpm ● Subculture cycle: 4 weeks ● Liquid culture period: 8 weeks Initial explant: ● 0.5 g embryogenic callus derived from shoot tips	Further culture ● 2 h partial drying in laminar flow bench and culture in medium ● Solid MS +10 mg L^{-1} 2,4-D +1 mg L^{-1} BAP ● Temperature: 25 °C ● Dark condition Initial explant: 0.4 g embryogenic callus Plant regeneration ● 1000 μM mesh filter ● PGR free liquid medium ● Temperature: 25 ± 2 °C ● Light intensity: 28 μmol m^{-2} s^{-1} ● Light/dark photoperiod: 16:8 h ● Agitation: 100 rpm ● Subculture cycle: 4 weeks	● Browning observed in the first week of culture. ● Somatic embryos and proembryos were produced after 12 weeks of cell suspension culture. ● Robust growth of somatic embryos were 6–7 mm long with one-sided slightly curved.	Boufis et al. (2017)

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Table 2 (*continued*)

Palms	Initial culture condition	Plant regeneration/Further culture condition	Outcome	References
Date palm	Establishment ● ½ MS +0.1 mg L^{-1} 2,4-D +300 mg L^{-1} AC +30 g L^{-1} sucrose ● 500 µm mesh filter ● Temperature: 28 ± 2 °C ● Light intensity: 28 µmol m^{-2} s^{-1} ● Light/dark photoperiod: 16:8 h ● Agitation: 120 rpm Initial explant: ● 500 mg of friable callus derived from juvenile leaves surrounding the apical meristem of offshoots	Plant regeneration ● MS without PGRs ● Root induction in MS +2 mg L^{-1} IBA Initial explant: Somatic embryos	● Embryogenic callus formed from after 12–36 months. ● Proembryogenic masses formed after 20–30 days without subculture. ● Over 10,000 somatic embryos produced in each litre per month. Production rate is 20 times higher than culture in solid medium. ● 83.3 % will survive during acclimatization after a 12 months in vitro hardening stage.	Fki et al. (2017)
Date palm	Establishment ● MS salts +10 mg L^{-1} NAA +1.5 mg L^{-1} 2iP ● Temperature: 25 ± 2 °C ● Agitation: 150 rpm ● Light/dark photoperiod: 16:8 h ● Light intensity: 40 µmol m^{-2} s^{-1} ● Subculture cycle: 2 weeks ● Culture period: 12 weeks Initial explant: ● Embryogenic callus derived from shoot tips	–	● Induction of somatic embryo in plant growth regulator-free medium. ● Maximum biomass production at week 11 from initiation. ● Fresh weight of 62.9 g L^{-1} and dried weight of 7.6 g L^{-1} at week 11. ● Polyphenols production also reach maximum at week 11.	Al-Khayri and Naik (2018); Naik and Al-Khayri (2016), 2017
Peach palm	Establishment ● Liquid MS + 2,4-D + BAP ● Temperature: 21 °C ● Dark condition ● Agitation: 100 rpm ● Subculture cycle: 2 weeks Initial explant: ● 1 cm^3 callus derived from dormant stems	–	● Soft, paste-like callus was produced in Sunhigh genotype while hard and compact callus was obtained in KV862478 genotype after cultured in cell suspension. ● Physical appearance of callus does not affect the establishment of suspension cells. ● Single, clusters of cells and large aggregates were found in the cell suspension cultures of all genotypes. ● 289,900 ± 7150 cells per mL was produced after 4 months culture for Sunhigh genotype and 46,310 ± 2530 cells per mL for KV862478 genotype in media supplemented with 1.0 µM 2,4-D and 5 µM BAP.	Schiavone and Wisniewski (1990)
Peach palm	Establishment ● Liquid MS +2 mg L^{-1} 2,4-D +0.1 mg L^{-1} IBA +30 g L^{-1} sucrose ● Temperature: 24 °C ● Dark condition ● Agitation: 115 rpm ● Subculture cycle: 14–20 days ● Initial explant: ● 3–4 callus of 3 cm in diameter derived from dormant xylem tissue	Further culture after 2 months ● Liquid MS +1 mg L^{-1} 2,4-D +30 g L^{-1} sucrose ● Subculture cycle: 10 days	● Growth kinetics showed short lag phase and reached maximum growth after 9 days, which was about 55 % packed cell volume.	Arora and Wisniewski (1995)
Coconut palm	–	Plant regeneration ● Embryo formation media: Y3 + MW vitamins + 250 µM 2, 4-D + 5 µM 2iP + 5 µM BAP +88 mM sucrose +2.5 g L^{-1} AC with or without 7 g L^{-1} agar ● Temperature: 27 ± 1 °C ● Dark condition ● Agitation: 140 rpm ● Culture period: 6 week Initial explant: ● 0.1 g white nodular embryogenic callus derived from inflorescence tissues	● 2-fold embryogenic masses produced in liquid media with addition of 250 µM 2, 4-D as compared to solid media. ● Cell suspension culture media supplemented with 0.01 µM spermine, 10 % smoke saturated water, 500 µM 2, 4-D, 2.5 g L^{-1} AC and no cytokinins (5 µM 2iP + 5 µM BAP) produced the highest embryogenic masses fresh weight of 952 mg per explant.	Antonova (2009)

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Table 2 (*continued*)

Palms	Initial culture condition	Plant regeneration/Further culture condition	Outcome	References
Coconut palm	Establishment ● Y3 + 4.5 μM 2,4-D + 34.2 μM glutamine + 882.3 μM KH_2PO_4 + 222 μM adenine +0.3 g L^{-1} AC ● Temperature: 28 ± 2 °C ● Dark condition ● Agitation: 120 rpm ● Subculture cycle: 7 days ● Culture period: 42 days Initial explant: ● 0.5 g embryogenic callus derived from shoot meristem	Further culture ● Y3 + 34.2 μM glutamine + 882.3 μM KH_2PO_4 + 222 μM adenine +0.3 g L^{-1} AC ● Subculture cycle: 20 days	● Addition of 4.5 μM 2, 4-D and 34.2 μM glutamine to the medium was the best for initiation of cell suspension culture. ● 9.9 % of packed cell volume was achieved after 200 days of culture and 52 % were viable cells. ● Addition of 100 mg L^{-1} malt extract, 9.3 μM kinetin and 40.9 μM biotin to the medium result in cell aggregation without further progress. ● Hard and densely aggregated cells were produced and did not obtained cell suspension culture of high-quality.	Bhavyashree et al. (2016)

Key: AC: Activated charcoal, BAP: 6-Benzylaminopurine, 2,4-D: 2,4-Dichlorophenoxyacetic acid, KN: Kinetin, ANA or NAA: 1-Naphthaleneacetic acid, TDZ: Thidiazuron, IBA: Indole-3-butyric acid, 2iP: N6-(2-Isopentenyl)adenine, PGR: Plant growth regulator, MS: Murashige and Skoog (1962) medium, MW Vit: Morel and Wetmore (1951) vitamins, Y3: Eeuwens (1976) medium, KM vit: Kao and Michayluk (1975) vitamins, B5: Gamborg et al. (1968) medium.
– Not mentioned.

A stable homogeneous cell suspension culture may require between 6–9 months to be established (Mustafa et al., 2011). Palm species may need even longer periods as the initiation of a stable date palm embryogenic cell suspension culture required 6–12 months (Assani et al., 2011). However, Strosse et al. (2004) commented that the embryogenic cell suspension cultures of banana (*Musa* sp.) may still remain heterogeneous even after 6 months from initiation. Heterogeneity is due to the presence of cells in clumps at various developmental and differentiation phases. For instance, a date palm cell suspension culture comprised different types of cells including single embryogenic cells (15–20 μm in diameter), non-embryogenic long single cells, dividing embryogenic cells, cell aggregates, microcalli and proembryonic clusters. The range of cells present can be determined by the age of the cell suspension culture (Assani et al., 2011). Also, a 6- to 9-month old embryogenic cell suspension culture from banana also consisted of various types of cells such as embryogenic cell aggregates, yellow meristematic globules, whitish somatic proembryos, whitish isolated dense cells, and highly vacuolated cells (Strosse et al., 2003). However, a more homogenous culture is preferred for achieving reproducible results (Mustafa et al., 2011). According to Assani et al. (2011), the use of a 500 μm sieving step to produce fine (more homogeneous) cell suspension is necessary to achieve success in date palm. Therefore, it may be concluded that the selection of a fine homogeneous embryogenic cell suspension culture is essential for long-term establishment, and is likely to be of equal importance for coconut.

3.2. Chemical factors

3.2.1. Culture media

An artificial media is essential for growth of *in vitro* plant tissues and organs. It can be made up of components including macro- and micronutrients, a carbohydrate source, plant growth regulators, vitamins, a solidifying agent, free amino acids and other nitrogen supplements, undefined additions and buffers. Murashige and Skoog (1962) (MS) nutrients are the most commonly used in plant tissue culture (George and De Klerk, 2008). However, the right medium to use for *in vitro* culture work is dependent upon the genotype and the goal of the experiment (Mustafa et al., 2011). For instance, MS medium is the most suitable medium for embryogenic cell suspension culture of oil palm (Kramut and Te-chato, 2010), while Y3 (Eeuwens, 1976) medium is superior for growth of an *in vitro* coconut tissue culture. This is because the Y3 medium formulation has higher levels of potassium and iodine in comparison to most other nutrient medium formulations. This provides an *in vitro* culture condition for coconut tissue that is similar to its natural germination habitat on the coastal strand, where the soil has often had in contact with potassium and iodine-rich sea water. Besides that, the addition of organic sources of reduced nitrogen have been found to be indispensable for the competent *in vitro* growth of coconut tissues (Eeuwens, 1976). Growth was also prompted when acid hydrolysed casein, or mixtures of certain L-amino acids were used as the only source of nitrogen (Eeuwens, 1978).

Plant growth regulators (PGRs) have a substantial effect in the management of vegetative and reproductive growth of plants. They can produce a profound reaction at low concentrations while controlling the growth and development of plants by affecting cell division, expansion and differentiation. The effect depends on the concentration of the PGRs, the presence of target cells or tissues and their developmental stage, the concentration of other PGRs present, the target plant's health, nutrition and water status, as well as physical environmental conditions (Coggins and Lovatt, 2014). Auxins and cytokinins are the two most important PGRs used in tissue culture to aid growth regulation and morphogenesis (Machakova et al., 2008). Therefore, research on the media composition of oil palm and date palm cell suspension culture has been focused on these PGRs. For instance, the addition of 2,4-dichlorophenoxyacetic acid (2,4-D) had a great effect on success with oil palm (de Touchet et al., 1991; Kramut and Te-chato, 2010) while

similar results were observed in date palm (Boufis et al., 2014; Othmani et al., 2009). In addition, media supplemented with the cytokinin kinetin was found to shorten the time to establish a fine cell suspension culture of oil palm (Te-chato et al., 2008). As for date palm, Abohatem et al. (2011) reported the addition of another cytokinin 6-benzylaminopurine (BAP; 0.3 mg L^{-1}) to media as a way to obtain a high production of somatic embryos. Zouine et al. (2005) discovered the production of date palm somatic embryos and the accumulation of storage proteins were improved by the addition of abscisic acid, plus glutamine and 2,4-D in combination in the embryogenic cell suspension culture media. As for coconut, 2,4-D is commonly used for initiation, but the concentrations used differ from other palms, ranging from 4.5 to 6 μM (Bhavyashree et al., 2016; Nguyen, 2018). The optimum concentration of PGRs for each coconut genotype may vary and should be determined through further experimentation.

Apart from PGRs, the effects of other additives such as polyethylene glycol (PEG), spermidine, glutathione and methylglyoxal-bis (guanylhydrazone) have been studied in palms. Polyethylene glycol was found to reduce the effect of hyperhydration in date palm by Al-Matar et al. (1997), whereas Helaly et al. (2017) discovered that 10 % PEG could improve the fresh weight, formation of embryogenic callus and normal embryos. The addition of 5 mM glutathione to the non-embryogenic cell suspension culture of coconut, derived from somatic leaf cells, was found to increase cell number two-fold while methylglyoxal-bis (guanylhydrazone) inhibited cell growth. As for spermidine, it promoted maximum cell growth of coconut at a concentration of 1 μM (Basu et al., 1988). Activated charcoal (AC) is another agent added to the cell suspension media of oil palm (de Touchet et al., 1991), and date palm (Fki et al., 2003). Activated charcoal is often used to remove toxic or growth-inhibiting substances produced in autoclaved media or by the explanted tissues, due to its adsorptive properties (Ebert and Taylor, 1990). Activated charcoal was found to be beneficial in the production of somatic embryos from the embryogenic cell suspension culture of date palm (Saker et al., 2007). However, AC was shown to adsorb 99.5 % of 100 μM 2,4-D in the liquid medium within 5 days (Ebert and Taylor, 1990). Ebert and Taylor (1990) also found that the age of the solid medium containing 100 μM 2,4-D and 10 μM BAP affected the fresh weight of callus obtained from coconut inflorescence tissue, whereby the highest fresh weight was obtained when cultured in a 9-day-old medium as compared to a 1-day-old or 5-day-old medium. The author suggested that AC adsorbed the toxic compounds present in the media (most likely excessive amounts of 2,4-D), hence preventing damage to the inflorescence tissues.

3.2.2. Carbon source

In vitro cultures often require carbohydrates as a carbon source, to provide energy as well as to function as an osmotic agent. Carbohydrate is the main dissolved substance responsible for most of the osmotic potential of a culture medium. The uptake of carbohydrates into plant cells and tissues is partly due to passive permeation and partly due to active transport. Certain development and growth processes are closely related to the tissue carbohydrate status whereby genes are induced, repressed or affected minimally with the changing status of tissue carbohydrate (Thorpe et al., 2008). In general, cell cultures are heterotrophic because carbon has to be incorporated in the form of carbohydrates but some are photoautotrophic, which assimilate carbon dioxide to satisfy their energy requirements (Endress, 1994). Therefore, a carbon source is essential in the normal process of culturing plant cells, tissues and organs. Pure sucrose is the universally used carbon source in plant tissue cultures, although refined white domestic sugar is also sufficiently pure to use (Thorpe et al., 2008). For example, sucrose is the most efficient energy source in inducing protocorm-like body formation in cell suspension cultures of *Phalaenopsis* orchids (Tokuhara and Mii, 2003). The synthesis of chlorophyll and non-photosynthetic pigments seems to be controlled by sucrose (Tognetti et al., 2013). However, the addition of sucrose specifically inhibited the formation of chlorophyll and photosynthesis processes, resulting in low autotrophic growth (Thorpe et al., 2008). Other carbohydrates that have been used for the cultivation of plant cells are glucose, glycerol, pentoses and uronic acid (Endress, 1994).

In general, for the growth of callus or suspension cells, the optimal sucrose concentration is 20–40 g L^{-1}. However, this optimal concentration for callus growth may not be the best for morphogenesis and plantlet development (Thorpe et al., 2008). Therefore, the type and concentration of carbon source in the establishment and development of cultures are usually experimentally determined (de Paiva Neto and Otoni, 2003). For instance, Kramut and Te-chato (2010) found MS medium supplemented with 0.2 M sorbitol gave the best result in terms of size and number of somatic embryos produced from cell suspension culture of oil palm. In date palm, the optimal sucrose concentration was found to be 30 g L^{-1} (Al-Matar et al., 1997; Veramendi and Navarro, 1996; Zouine and El Hadrami, 2004). However, starvation of sucrose for 2 weeks prior to culture in 30 g L^{-1} sucrose was found to be beneficial to somatic embryo development from a cell suspension culture of date palm (Veramendi and Navarro, 1996). Similarly for the initiation of coconut cell suspension culture, 30 g L^{-1} of sucrose was trialled in the liquid culture medium (Nguyen, 2018). A viable cell suspension culture was initiated, but no further work was carried out due to limited availability of friable embryogenic callus used for initiation.

3.2.3. pH of media

The concentration of hydrogen ions present in the culture medium determines the pH of that medium. The culture medium should have a pH that does not disrupt the development of the plant tissues (Thorpe et al., 2008). Therefore, the pH of a typical culture medium is usually adjusted to pH 5.5–6.0 prior to autoclaving, as it usually drops after autoclaving, by 0.6–1.3 units (Smith, 2013). However, the pH of a culture medium is not always constant and changes when ions and compounds are taken up by the plant tissue. Media containing nitrate and ammonium ions usually decline slowly in pH, but sometimes pH may also increase over time (Thorpe et al., 2008). Medium pH is affected by the carbon source, inorganic salts, AC, gelling agent and the medium storage conditions. For instance, the post-autoclave pH of a medium without a carbon source, or with the addition of sucrose, was the highest, while a medium with maltose, glucose or fructose as the carbon source had a lower post-autoclave pH. The difference was found to be related to the buffering capacity of the medium (Owen et al., 1991).

The pH of media can control the availability of certain salts, as well as influence the nutrient and exogenous PGR uptake. It can also affect the chemical reactions - particularly those induced by enzymes, as well as the efficiency of the gelling agent. In the development of a cell suspension culture from coconut leaf cells (non-embryogenic), cell division was found to be affected by the pH of the culture media. The cell number increased three-fold when cultured in a medium of pH 7.0, as compared to the culture growing at pH 5.0 (Basu et al., 1988). The unfavourable effect of low pH is thought to be closely related to the non-availability of ions and the lack of uptake of nutrients rather than on cell death (Thorpe et al., 2008).

On the other hand, PGRs such as auxin, induce the efflux of hydrogen ions through the plant cell wall to encourage cell growth and this process is accompanied by the influx of potassium ions (Thorpe et al., 2008), which has a major role in the regulation of cell pH and its osmotic status (George and De Klerk, 2008). The medium will become more acidic and then the pH of the cell sap will rise when auxin is added to the culture. However, the addition of AC to the medium containing auxin may influence cell growth. This is because the adsorption of 2,4-D by AC was found to be accelerated at low pH (Dehghani et al., 2014; Ebert and Taylor, 1990). Therefore, pH of the culture medium is an important variable to manage in establishing embryogenic cell suspension cultures of coconut.

3.3. Physical factors

3.3.1. Agitation

A fast multiplication rate in *in vitro* cultures requires adequate gaseous exchange. The decline often seen in the growth rate is associated with a limited oxygen supply (George and Davies, 2008). Agitation is used to supply the cells with oxygen for proliferation (Fukui et al., 1999). Agitation also promotes homogeneity in terms of nutrient supply and cell biomass (Georgiev et al., 2009), as it facilitates cell dispersion (Razdan, 2003). Hence, the speed of agitation has direct effects on cell growth and viability due to aeration and agitation (Singh and Chaturvedi, 2012). The frequency of agitation also affects the size of cell aggregates produced and the amount of oxygen supplied (Endress, 1994). A shaker speed of 30–150 rpm is optimal for most tissues in suspension culture (Razdan, 2003). Whilst, for cell suspension culture, the speed of agitation is usually between 90–125 rpm. However, the optimal shaker speed depends on the type of shaker as plant cells may experience shear stress at high speeds which negatively affects cell viability (Mustafa et al., 2011).

The shaker speed varies by species for instance, the cell suspension culture of Chinese ginger (*Boesenbergia rotunda* L.) has been shown to grow healthily and actively multiply at shaker speeds of 80 and 100 rpm (Yusuf et al., 2013). On the other hand, the culture of toothache plant (*Spilanthes acmella* Murr.) cells at lower shaker speed of 60–90 rpm resulted in cell death due to aggregation and clumping. At higher shaker speeds of 150–180 rpm, the cell viability and biomass obtained were poor and the best cell growth was obtained at a shaker speed of 120 rpm (Singh and Chaturvedi, 2012). The shaker speed used for cell suspension culture of different palm species can be different, ranging from 90 to 150 rpm (Table 1 and 2). Similarly, for the oil palm, in a bioreactor, the agitation speed also played an important role in cell proliferation. Bioreactor agitation speeds of between 120–300 rpm were found to be suitable for the culture of oil palm cells (Choi et al., 2008). Hence, the variation in the shaker speed used for the coconut palm should be considered as the speed of agitation can favourably influence cell growth and viability.

3.3.2. Temperature

Temperature is the principal physical factor that impacts upon the rate of plant growth and development. The temperature used varies, as each species has an optimum, and a minimum and maximum where growth does not occur (Hatfield and Prueger, 2015). Sometimes, the optimum temperature can be different among closely related genotypes. In the natural environment, temperature fluctuates, *in vitro* cultures are often maintained at a constant day and night temperature in a growth chamber (George and Davies, 2008). The standard optimum temperature for the growth of plant cell suspension cultures was found to be 26 ± 3 °C (Endress, 1994). Tropical and subtropical species are likely to grow best *in vitro* at a mean temperature of 27.7 °C, in a range 24–32 °C (George and Davies, 2008). Most cell suspension cultures of oil, date and coconut palms are cultured in a temperature ranging from 23 to 28 °C, while peach palm requires a lower temperature of 21 °C (Table 1 and 2).

Temperature also has a strong effect on plant ontology and metabolic activity (Ramakrishna and Ravishankar, 2011). Therefore, the establishment and growth of a culture and the development and morphogenesis of plantlets, are all dependent on temperature (George and Davies, 2008). For instance, in the cell suspension culture of *B. rotunda*, cell growth was slow at low temperature while at higher temperature, the growth was vigorous, but less healthy (Yusuf et al., 2013). Also, the induction of secondary embryogenesis is influenced by temperature. A higher temperature of 30 °C was found to be beneficial for the induction of secondary somatic embryos from cell suspension culture of the Japanese raisin tree (*Hovenia dulcis* Thunb.) while a lower temperature of 20 °C was more suitable for the further development of the embryos produced, as well as their transformation into plantlets, and the success of transplantation (Yang et al., 2013). On the other hand, low temperature was found to be better for the improvement of somatic embryo germination for date palm (Shareef et al., 2016), as well as in the application of slow growth maintenance in embryogenic cell suspension cultures of banana (*Musa* spp.) (Kulkarni and Ganapathi, 2009). According to Ebert and Taylor (1990), temperature could also affect the adsorption of 2,4-D by AC as adsorption was accelerated when a high temperature range, from 20–30 °C was applied. Hence, it is crucial to determine the most suitable temperature for culture, as it affects the concentration of 2,4-D available in the medium when AC is present. In turn, this is likely to affect coconut work, as both 2,4-D and activated charcoal are common additives in coconut culture media.

3.3.3. Light

Most plants depend on light for energy and consequently survival (Hangarter, 1997). *In vitro* plant growth and morphogenesis are affected by the photo-environment in three ways *viz* flux density (intensity), wavelength and photoperiod. Both photosynthesis and photomorphogenesis are affected by these features of the light environment. Light is important for photosynthesis in tissue culture systems if the culture is photoautotrophic. However, light can sometimes have inhibitory effects on cell division and callus growth and development. (George and Davies, 2008). Hence, the effects of light on photoautotrophic, photomixotrophic and heterotrophic cultures are different (Endress, 1994). For instance, red and green light, which were similar to dark culture conditions were suitable for SE of carrot (*Daucus carota* L.) cells from suspension culture. On the other hand, white and blue light were found to inhibit growth and the formation of carrot somatic embryos (Michler and Lineberger, 1987). Similarly, callus of royal lily (*Lilium regale* Wilson) underwent differentiation when cultured in dark condition, and light was only required prior to plantlet formation (Pelkonen and Kauppi, 1999). Light condition was also found to cause browning, somatic embryo necrosis and low plantlet conversion rate for *Phalaenopsis* orchids (Gow et al., 2008). It is also likely to have a significant impact on the production, maturation and germination of blue agave (*Agave tequilana* Weber var. Azul) somatic embryos (Rodríguez-Sahagún et al., 2011). Light is essential in the establishment of cell suspension culture of some palms (Table 1 and 2). For instance, oil palm and date palm cells were commonly cultured in light conditions with various photoperiods (light:dark) such as 12:12, 16:8 and 14:10 h at different intensity, whereas the dark conditions are used for coconut palm and peach palm cells. The dark condition is often used in establishing cultures, as it can reduce visible blackening in tissues and prevent the formation of growth inhibitors. In general, callus formation is often initiated in the dark as it lacks chlorophyll. Subsequently, light is required for regeneration through organogenesis. In some species, light may be essential for callus initiation, but not required for culture maintenance (George and Davies, 2008). Therefore, the effect of illumination can vary among different species as well as at different growth stages. Hence, light is another important factor to be considered for establishing the optimal coconut embryogenic cell suspension culture environment.

4. Cell suspension culture of economically important palms

Cell suspension culture has been used in several palm species for propagation *via* SE and subsequent plantlet regeneration. Research on palms has been focused extensively on oil palm and date palm and there are hardly any studies reported on coconut or peach palm (Table 1 and 2). Oil palm, date palm and peach palm have been involved since the 1990s, with the first reports made by de Touchet et al. (1990); Bhaskaran and Smith (1992), and Schiavone and Wisniewski (1990), respectively. Reports on the establishment of a basic embryogenic cell suspension culture for coconut are more recent (Bhavyashree et al., 2016). In this study, cell aggregation occurred and there was no further progress of SE. Failure in obtaining a high-quality cell suspension

culture was reasoned to be the "hard" nature of coconut embryogenic calli produced.

Murashige and Skoog (1962) medium was used with oil palm in the protocol developed by de Touchet et al. (1991), and has been widely applied in other studies of oil palm. It can be initiated in a shorter time (within 2 months) when friable embryogenic tissue is used as the starting material (Teixeira et al., 1995). A culture with a four-fold multiplication rate is generally achieved each month for oil palm but there have been studies which achieved at a higher rate of six to seven-fold (Soh et al., 2003, 2001; Wong et al., 1999), and a lower rate of only two to three-fold (Kanchanapoom and Chourykaew, 1998) for oil palm cells every month. In contrast, a solid culture system has been found to have a much lower multiplication rate of only two-fold every 2 months for oil palm (Soh et al., 2003, 2001). Teixeira et al. (1995) achieved a high regeneration frequency of 180 embryos in 1 g of settled-cell volume, or 5,400 embryos in 1 L of oil palm cell suspension culture solution, with an optimum inoculum size of 1.5 g of fresh cells in 50 mL of liquid medium. Yet, the highest regeneration frequency reported is 300 oil palm embryos per Petri dish from 0.05 mL of packed cell volume of small cell aggregates (Rival, 2000), and 90–100% shoot formation in the embryos transferred to solid medium (Soh et al., 2001). In some cases, proliferating cell lines have been found to have embryogenic potential and capability to form shoots, after more than 24 subculture cycles (Wong et al., 1999).

In most cases, friable embryogenic callus derived from shoot tips is used to establish date palm cell suspension culture. According to the date palm protocol developed by Fki et al. (2003), pro-embryogenic masses started to form after 20 days without subculture. The embryo production rate was 20 times higher than those in the solid culture system. Maximum biomass production was achieved at week 11 after initiation, and achieved a fresh weight of 62.9 g L^{-1} and a dry weight of 7.6 g L^{-1} (Naik and Al-Khayri, 2017). The highest regeneration frequency documented has been 200 date palm embryos from 0.1 g of fresh embryogenic callus each month and 10,000 embryos from 1 L of cell suspension culture each month (Fki et al., 2003) which is similar to the result of Fki et al. (2017). However, the germination rate was only 25 % in plant growth regulator-free media (Fki et al., 2003). Despite that, Bhaskaran and Smith (1992) have achieved 40 % normal plantlets developed from the embryos derived from a date palm cell suspension culture. Plantlet regeneration stages still need to be improved to achieve a higher percentage of plantlet production. As a result, a study on developing an improved date plant regeneration medium for initiation, maturation and germination was conducted by Aslam et al. (2011). Based on the results, the response was better in a medium supplemented with 1.5 mg L^{-1} NAA (1-Naphthaleneacetic acid), 1.0 mg L^{-1} TDZ (Thidiazuron) and 0.75 mg L^{-1} BAP for the initiation, maturation and germination stages respectively. Media supplemented with BAP was found to be better than kinetin in terms of germination and somatic embryo conversion into plantlets.

It has been found that 2,4-D has an important role in peach palm and the one study on coconut palm. High cell production was achieved by Schiavone and Wisniewski (1990) in peach palm whereby 289,900 ± 7,150 and 46,310 ± 2,530 cells per mL was achieved for Sunhigh and KV862478 genotypes respectively after 4 months culture in media added with 1.0 μM 2,4-D and 5 μM BAP. For coconut, 9.9 % of packed cell volume was achieved by Bhavyashree et al. (2016) after 200 days and 52 % were viable cells. The addition of 4.5 μM 2,4-D and 34.2 μM glutamine to the media were found to be ideal for the initiation of coconut cell suspension culture. However, a high-quality cell suspension culture was not obtained due to the formation of hard and densely aggregated cells. Despite that, Nguyen (2018) suggested that a viable cell suspension culture can be produced from friable secondary embryogenic callus. Hence, it is crucial to obtain friable embryogenic callus for coconut. As for plant regeneration, Y3 (Eeuwens, 1976) liquid media supplemented with spermine, smoke-saturated water (SSW), high auxin and activated charcoal, without the addition of cytokinin, were shown to improve the production of coconut embryogenic masses derived from inflorescence tissues (Antonova, 2009). Although the conversion from somatic embryos to plantlets has not been attained yet, it is plausible to consider this method for the mass propagation of coconut from suspended cells to somatic embryos and then further develop them into plantlets.

5. Limitations of cell suspension culture

In spite of the significance of cell suspension culture system in plant propagation, it is still not a routine technique for economically important species. One of the major problems is hyperhydricity (Preil, 2005). This is defined as the formation of abnormal cells and tissues with brittle and water-soaked appearance (George and De Klerk, 2008). This is a morphological and physiological disorder in plants that is recognized by a glassy appearance. Hyperhydricity is affected by the microenvironment and ethylene concentration in the culture vessel (Hazarika, 2006). Hence, hyperhydricity may be reduced by modifying solutes and gel concentrations, or water evaporation, from tissue when in contact with air. Carbon sources such as galactose and fructose can be effective in reducing hyperhydricity. Also, osmoregulators such as sorbitol, mannitol and maltose, and inhibitors for biosynthesis of gibberellin including ancymidol and paclobutrazol may prevent hyperhydricity (Thorpe et al., 2008). In addition, a medium containing PEG was found to reduce hyperhydration in date palm suspension cultures (Al-Matar et al., 1997).

Oxygen deprivation is another limitation as the oxygen concentration is usually inadequate to fulfil the respiratory needs of immersed cells and tissues. Hence, the oxygen concentration in the medium should be raised, or direct contact should be allowed between cells and tissues with the air (Thorpe et al., 2008) which is closely related to the agitation. Some other limitations shown in banana are low embryogenic response, a prolonged period in establishing embryogenic cell suspension, contamination incidents, and the risk of somaclonal variation (Strosse et al., 2003). Larkin and Scowcroft (1981) concluded that plant cell culture itself can induce genetic variability or somaclonal variation. Somaclonal variation involves deoxyribonucleic acid (DNA) and phenotypic variations caused by chromosome or epigenetic changes. It can be observed as unusual cytological activities, sequence changes, recurrent phenotypic mutations, and activation and silencing of genes (Kaeppler et al., 2000). Although somaclonal variation can lead to plant improvement as a source of beneficial variants with superior agronomic attributes (Bairu et al., 2011), the variation is most likely due to DNA methylation (epigenetic mechanism) as it appears to be more recurrent (Miguel and Marum, 2011).

Rival et al. (2013) studied the variation in DNA methylation of oil palm embryogenic cell suspension cultures that had undergone long-term proliferation. The results indicated that DNA hypermethylation was induced by *in vitro* proliferation in a time-dependent mode. However, most of the cell lines (except one of the five cell lines) retained their embryogenic capacity to produce somatic embryos after 12 months and regenerate into plantlets. Similarly, somaclonal variations in coffee (*Coffea arabica* L.) embryogenic suspension showed an exponential increase as the age of the suspension culture increased. The results showed only 1.3 % somaclonal variants in a 3 months old coffee cell suspension culture but 25 % variants in 12 months old suspension culture, although these results differed among genotypes. The somaclonal variants produced showed less vigorous growth and low fruit productivity (Etienne and Bertrand, 2003). Therefore, newly initiated cells are recommended for plant regeneration because the genetic stability and regeneration capability is reduced as the duration of the culture period increases (Mathur and Koncz, 1998).

6. Conclusion

Although coconut SE has established a pathway to improve the

conventional breeding production rate of one seedling to many seedlings per fruit, the process can be further improved to achieve a much higher multiplication rate, and to do this in a shorter period of time. A cell multiplication step utilizing cell suspension culture is anticipated to be able to significantly improve the production rate of plantlets. This possibility is indicated by the successful work undertaken on oil and date palm, which have shown a high and rapid cell multiplication rate. Studies have reported 5400 and 10,000 embryos produced from each litre of oil palm (Teixeira et al., 1995) and date palm (Fki et al., 2003) in cell suspension culture medium respectively. Hence, this technique offers the possibility of increasing the large-scale multiplication rate of coconut. This is urgently needed by the industry, as high-quality seed coconuts are in short supply due to increasing demand for coconut products and the rapidly decreasing productivity of old plantations worldwide. For future research, it is important to optimize the cell suspension culture system by determining the critical factors (biological, chemical and physical) which should be studied experimentally. This would enable the production, in as short a time-frame as possible, of embryogenic cell suspension cultures with a high multiplication rate. As hyperhydricity and somaclonal variation are common problems for other species including palms, these should be carefully considered. The significance of cell suspension culture for coconut is evident as it provides the basic requirements for scaled-up bioreactor propagation, as an initiation point for plant transformation, protoplast isolation and genome editing. Hence, success in the establishment of coconut embryogenic cell suspension culture will likely be the key to other important biotechnology applications. Plant cell suspension culture development is indeed a worthwhile venture, critical to the future of the global coconut industries.

Author contribution statement

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

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The authors declare no conflict of interest in this paper.

References

Aberlenc-Bertossi, F., Noirot, M., Duval, Y., 1999. BA enhances the germination of oil palm somatic embryos derived from embryogenic suspension cultures. Plant Cell Tiss. Org. Cult. 56, 53–57. https://doi.org/10.1023/A:1006241215717.

Abohatem, M., Zouine, J., El Hadrami, I., 2011. Low concentrations of BAP and high rate of subcultures improve the establishment and multiplication of somatic embryos in date palm suspension cultures by limiting oxidative browning associated with high levels of total phenols and peroxidase activities. Sci. Hortic. 130, 344–348. https://doi.org/10.1016/j.scienta.2011.06.045.

Abohatem, M.A., Bakil, Y., Baaziz, M., 2017. Plant regeneration from somatic embryogenic suspension cultures of date palm. In: Al-Khayri, J.M., Jain, S.M., Johnson, D.V. (Eds.), Date Palm Biotechnology Protocols: Tissue Culture Applications. Humana Press, New York, pp. 203–214. https://doi.org/10.1007/978-1-4939-7156-5_17.

Adkins, S., Nguyen, Q.T., Foale, M., 2016. Improving the availability of valuable coconut germplasm using tissue culture techniques. Cord. 32, 27–35.

Ahmad, S., Garg, M., Tamboli, E.T., Abdin, M.Z., Ansari, S.H., 2013. *In vitro* production of alkaloids: factors, approaches, challenges and prospects. Pharmacogn. Rev. 7, 27–33.

Al-Bahrany, A.M., Al-Khayri, J.M., 2012. Optimizing *in vitro* cryopreservation of date palm (*Phoenix dactylifera* L.). Biotechnology 11, 59–66. https://doi.org/10.3923/biotech.2012.59.66.

Aljohi, H.A., Liu, W., Lin, Q., Zhao, Y., Zeng, J., Alamer, A., Alanazi, I.O., Alawad, A.O., Al-Sadi, A.M., Hu, S., Yu, J., 2016. Complete sequence and analysis of coconut palm (*Cocos nucifera*) mitochondrial genome. PLoS One 11, e0163990. https://doi.org/10.1371/journal.pone.0163990.

Al-Khayri, J.M., 2012. Determination of the date palm cell suspension growth curve, optimum plating efficiency, and influence of liquid medium on somatic embryogenesis. Emir. J. Food Agric. 24, 444–455.

Al-Khayri, J.M., Naik, P.M., 2018. Biomass accumulation and polyphenols quantification in cell suspension culture of date palm (*Phoenix dactylifera* L.). In Vitro Cell. Dev. Biol. Plant. 54, S117–S118.

Al-Matar, S.M., Abo El-Nil, M.M., Al-Khayri, J., Klingaman, G., 1997. Effect of sucrose and PEG concentration on somatic maturation and hyperhydration in date palm suspension cultures. HortScience 32, 462.

Al-Shahib, W., Marshall, R.J., 2003. The fruit of the date palm: its possible use as the best food for the future? Int. J. Food Sci. Nutr. 54, 247–259 https://doi.org/637480120091982.

Alwael, H.A., Naik, P.M., Al-Khayri, J.M., 2017. Synchronization of somatic embryogenesis in date palm suspension culture using abscisic acid. In: Al-Khayri, J.M., Jain, S.M., Johnson, D. (Eds.), Date Palm Biotechnology Protocols: Tissue Culture Applications. Humana Press, New York, pp. 215–226. https://doi.org/10.1007/978-1-4939-7156-5.

Antonova, I.D., 2009. Somatic Embryogenesis for Micropropagation of Coconut (*Cocos nucifera*). Ph.D. Dissertation. School of Land, Crop and Food Sciences, The University of Queensland.

Archana, A., Pradhap, Vijay, Singh, M., Chozhavendhan, S., Gnanavel, G., Jeevitha, S., Muthu Kumara Pandian, A., 2019. Coconut shell as a promising resource for future biofuel production. In: Praveen Kumar, R., Bharathiraja, B., Kataki, R., Moholkar, V.S. (Eds.), Biomass Valorization to Bioenergy. Springer, Singapore, Singapore, pp. 31–43. https://doi.org/10.1007/978-981-15-0410-5_3.

Arora, R., Wisniewski, M.E., 1995. Ultrastructural and protein changes in cell suspension cultures of peach associated with low temperature-induced cold acclimation and abscisic acid treatment. Plant Cell Tiss. Org. Cult. 40, 17–24. https://doi.org/10.1007/BF00041113.

Ascough, G.D., Fennell, C.W., 2004. The regulation of plant growth and development in liquid culture. S. Afr. J. Bot. 70, 181–190. https://doi.org/10.1016/S0254-6299(15)30234-9.

Aslam, J., Khan, S.A., Cheruth, A.J., Mujib, A., Sharma, M.P., Srivastava, P.S., 2011. Somatic embryogenesis, scanning electron microscopy, histology and biochemical analysis at different developing stages of embryogenesis in six date palm (*Phoenix dactylifera* L.) cultivars. Saudi J. Biol. Sci. 18, 369–380. https://doi.org/10.1016/j.sjbs.2011.06.002.

Assani, A., Chabane, D., Shittu, H., Bouguedoura, N., 2011. Date palm cell and protoplast culture. In: Jain, S.M., Al-Khayri, J.M., Johnson, D.V. (Eds.), Date Palm Biotechnology. Springer, Netherlands, Dordrecht, pp. 605–629. https://doi.org/10.1007/978-94-007-1318-5_29.

Bairu, M.W., Aremu, A.O., Van Staden, J., 2011. Somaclonal variation in plants: causes and detection methods. Plant Growth Regul. 63, 147–173. https://doi.org/10.1007/s10725-010-9554-x.

Bais, H.P., Walker, T.S., McGrew, J.J., Vivanco, J.M., 2002. Factors affecting growth of cell suspension cultures of *Hypericum perforatum* L. (St. John's Wort) and production of hypericin. In Vitro Cell. In Vitro Cell. Dev. Biol., Plant 38, 58–65.

Bandupriya, H.D.D., Femando, S.C., Vidhanaarachchil, V.R.M., 2016. Micropropagation and androgenesis in coconut: an assessment of Sri Lankan implication. Cocos 22, 31–47. https://doi.org/10.4038/cocos.v22i1.5810.

Basu, A., Sethi, U., Guha-Mukherjee, S., 1988. Induction of cell division in leaf cells of coconut palm by alteration of pH and its correlation with glyoxalase-I activity. J. Exp. Bot. 39, 1735–1742. https://doi.org/10.1093/jxb/39.12.1735.

Bhaskaran, S., Smith, R.H., 1992. Somatic embryogenesis from shoot tip and immature inflorescence of *Phoenix dactylifera* cv. Barhee. Plant Cell Rep. 12, 22–25. https://doi.org/10.1007/BF00232416.

Bhatia, S., 2015. Plant tissue culture. In: Bhatia, S., Sharma, K., Dahiya, R., Bera, T. (Eds.), Modern Applications of Plant Biotechnology in Pharmaceutical Sciences, first ed. Academic Press, Boston, pp. 31–107. https://doi.org/10.1016/B978-0-12-802221-4.00002-9.

Bhavyashree, U., Jayaraj, K.L., Muralikrishna, K.S., Sajini, K.K., Rajesh, M.K., Anitha, K., 2016. Initiation of coconut cell suspension culture from shoot meristem derived embryogenic calli: a preliminary study. J. Phytol. 8, 13–16. https://doi.org/10.19071/jp.2016.v8.2979.

Blake, J., 1983. Tissue culture propagation of coconut, date and oil palm. In: Dodds, J.H. (Ed.), Tissue Culture of Trees. Springer, US, Boston, MA, pp. 29–50. https://doi.org/10.1007/978-1-4684-6691-1_4.

Boufis, N., Khelifi-Slaoui, M., Djillali, Z., Zaoui, D., Morsli, A., Bernards, M.A., Makhzum, A., Khelifi, L., 2014. Effects of growth regulators and types of culture media on somatic embryogenesis in date palm (*Phoenix dactylifera* L. cv. Degla Beida). Sci. Hortic. 172, 135–142. https://doi.org/10.1016/j.scienta.2014.04.001.

Boufis, N., Titouh, K., Khelifi, L., 2017. Desiccation-enhanced maturation and germination of date palm somatic embryos derived from cell suspension culture. In: Al-Khayri, J.M., Jain, S.M., Johnson, D.V. (Eds.), Date Palm Biotechnology Protocols: Tissue Culture Applications. Humana Press, New York, pp. 107–117. https://doi.org/10.1007/978-1-4939-7156-5_10.

Buffard-Morel, J., Verdeil, J.L., Dussert, S., Magnaval, C., Huet, C., Grosdemange, F., 1995. Initiation of somatic embryogenesis in coconut (*Cocos nucifera* L.). In: Oropeza, C., Howard, F.W., Ashburner, G.R. (Eds.), Lethal Yellowing: Research and Practical Aspects. Springer, Netherlands, Dordrecht, pp. 217–223. https://doi.org/10.1007/978-94-011-0433-3_18.

Choi, D.-S., Andrade, M.H.C., Willis, L.B., Cho, C., Schoenheit, J., Boccazzi, P., Sambanthamurthi, R., Sinskey, A.J., Rha, C., 2008. Effect of agitation and aeration on yield optimization of oil palm suspension culture. J. Oil Palm Res. 1, 23–34.

Clement, C.R., Weber, J.C., van Leeuwen, J., Astorga Domian, C., Cole, D.M., Arévalo Lopez, L.A., Argüello, H., 2004. Why extensive research and development did not

promote use of peach palm fruit in Latin America. Agroforest. Syst. 61, 195–206. https://doi.org/10.1023/B:AGFO.0000028999.84655.17.
Coggins Jr., C.W., Lovatt, C.J., 2014. Plant growth regulators. In: Ferguson, L., Grafton-Cardwell, E.E. (Eds.), Citrus Production Manual, first ed. University of Carlifornia Agriculture and Natural Resources, California, pp. 215–226.
Dai, X.-M., Xiao, W., Huang, X., Zhao, J.-T., Chen, Y.-F., Huang, X.-L., 2010. Plant regeneration from embryogenic cell suspensions and protoplasts of dessert banana cv. 'Da Jiao' (*Musa paradisiacal* ABB Linn.) via somatic embryogenesis. In Vitro Cell. Dev. Biol. Plant 46, 403–410. https://doi.org/10.1007/s11627-010-9314-7.
de Paiva Neto, V.B., Otoni, W.C., 2003. Carbon sources and their osmotic potential in plant tissue culture: does it matter? Sci. Hortic. 97, 193–202. https://doi.org/10.1016/S0304-4238(02)00231-5.
de Touchet, B., Duval, Y., Pannetier, C., 1990. Oil Palm (*Elaeis guineensis* Jacq.) Regeneration From Embryogenic Suspension Culture. VIIth International Congress on Plant Tissue and Cell Culture, Amsterdam p. 1.
de Touchet, B., Duval, Y., Pannetier, C., 1991. Plant regeneration from embryogenic suspension cultures of oil palm (*Elaeis guineensis* Jacq.). Plant Cell Rep. 10, 529–532. https://doi.org/10.1007/BF00234588.
Dehghani, M., Nasseri, S., Karamimanesh, M., 2014. Removal of 2,4-Dichlorophenolyxacetic acid (2,4-D) herbicide in the aqueous phase using modified granular activated carbon. J. Environ. Health Sci. Eng. 12https://doi.org/10.1186/2052-336X-12-28. 28-28.
Deo, P.C., Tyagi, A.P., Taylor, M., Harding, R., Becker, D., 2010. Factors affecting somatic embryogenesis and transformation in modern plant breeding. SPJNS 28, 27–40. https://doi.org/10.1071/SP10002.
Doran, P.M., 1993. Design of reactors for plant cells and organs. Bioprocess Design and Control. Springer Berlin, Heidelberg, Berlin, pp. 115–168. https://doi.org/10.1007/BFb0007198.
Duval, Y., Aberlenc, F., de Touchet, B., 1995a. Use of embryogenic suspensions for oil palm micropropagation. In: Rao, V., Henson, I.E., Rajanaidu, N. (Eds.), International Symposium on Recent Developments in Oil Palm Tissue Culture and Biotechnology. PORIM, Kuala Lumpur, pp. 38–47.
Duval, Y., Engelmann, F., Durand-Gasselin, T., 1995b. Somatic embryogenesis in oil palm (*Elaeis guineensis* Jacq.). In: Bajaj, Y.P.S. (Ed.), Somatic Embryogenesis and Synthetic Seed I. Springer-Verlag, Berlin Heidelberg, Berlin, pp. 335–352. https://doi.org/10.1007/978-3-662-03091-2_22.
Ebert, A., Taylor, H.F., 1990. Assessment of the changes of 2,4-dichlorophenoxyacetic acid concentrations in plant tissue culture media in the presence of activated charcoal. Plant Cell Tiss. Org. Cult. 20, 165–172. https://doi.org/10.1007/BF00041877.
Eeuwens, C.J., 1976. Mineral requirements for growth and callus initiation of tissue explants excised from mature coconut palms (*Cocos nucifera*) and cultured *in vitro*. Physiol. Plant. 36, 23–28. https://doi.org/10.1111/j.1399-3054.1976.tb05022.x.
Eeuwens, C.J., 1978. Effects of organic nutrients and hormones on growth and development of tissue explants from coconut (*Cocos nucifera*) and date (*Phoenix dactylifera*) palms cultured *in vitro*. Physiol. Plant. 42, 173–178. https://doi.org/10.1111/j.1399-3054.1978.tb02543.x.
Endress, R., 1994. Plant Cell Biotechnology. Springer, Berlin Heidelberg, Berlin. https://doi.org/10.1007/978-3-662-02996-1.
Etienne, H., Bertrand, B., 2003. Somaclonal variation in *Coffea arabica*: effects of genotype and embryogenic cell suspension age on frequency and phenotype of variants. Tree Physiol. 23, 419–426. https://doi.org/10.1093/treephys/23.6.419.
Fellers, J.P., Guenzi, A.C., Taliaferro, C.M., 1995. Factors affecting the establishment and maintenance of embryogenic callus and suspension cultures of wheat (*Triticum aestivum* L.). Plant Cell Rep. 15, 232–237. https://doi.org/10.1007/BF00193726.
Finer, J.J., 1994. Plant regeneration via embryogenic suspension cultures. In: Dixon, R.A., Gonzales, R.A. (Eds.), Plant Cell Culture: A Practical Approach, second ed. Oxford University Press Inc., New York, pp. 99–126.
Fki, L., Masmoudi, R., Drira, N., Rival, A., 2003. An optimised protocol for plant regeneration from embryogenic suspension cultures of date palm, *Phoenix dactylifera* L., cv. Deglet Nour. Plant Cell Rep. 21, 517–524. https://doi.org/10.1007/s00299-002-0558-5.
Fki, L., Masmoudi, R., Kriaâ, W., Mahjoub, A., Sghaier, B., Mzid, R., Mliki, A., Rival, A., Drira, N., 2011. Date palm micropropagation via somatic embryogenesis. In: Jain, S.M., Al-Khayri, J.M., Johnson, D.V. (Eds.), Date Palm Biotechnology. Springer Netherlands, Dordrecht, pp. 47–68. https://doi.org/10.1007/978-94-007-1318-5_4.
Fki, L., Kriaa, W., Nasri, A., Baklouti, E., Chkir, O., Masmoudi, R.B., Rival, A., Drira, N., 2017. Indirect somatic embryogenesis of date palm using juvenile leaf explants and low 2,4-D concentration. In: Al-Khayri, J.M., Jain, S.M., Johnson, D.V. (Eds.), Date Palm Biotechnology Protocols: Tissue Culture Applications. Springer New York, New York, pp. 99–106. https://doi.org/10.1007/978-1-4939-7156-5_9.
Foale, M., 2003. The Coconut Odyssey Begins, in: The Coconut Odyssey: the Bounteous Possibilities of the Tree of Life. Australian Centre for International Agricultural Research, Canberra, pp. 15–31.
Franklin, C.I., Dixon, R.A., 1994. Initiation and maintenance of callus and cell suspension. In: Dixon, R.A., Gonzales, R.A. (Eds.), Plant Cell Culture: A Practical Approach, second ed. Oxford University Press Inc., New York, pp. 1–25.
Fukui, H., Hasan, A.F.M.F., Ishii, Y., Tanaka, M., 1999. An envelope-shaped film culture vessel for shikonin production by *Lithospermum erythrorhizon* hairy root cultures. Plant Biotechnol. 16, 171–174. https://doi.org/10.5511/plantbiotechnology.16.171.
Gamborg, O.L., Miller, R.A., Ojima, K., 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50, 151–158. https://doi.org/10.1016/0014-4827(68)90403-5.
George, E.F., Davies, W., 2008. Effects of the physical environment. In: George, E.F., Hall, M.A., De Klerk, G.-J. (Eds.), Eds.), Plant Propagation by Tissue Culture: The Background, third ed. Springer, Dordrecht, pp. 423–464.
George, E.F., De Klerk, G.-J., 2008. The components of plant tissue culture media I: macro- and micro-nutrients. In: George, E.F., Hall, M.A., De Klerk, G.-J. (Eds.), Plant Propagation by Tissue Culture: The Background, third ed. Springer, Dordrecht, pp. 65–113.
Georgiev, M.I., Weber, J., Maciuk, A., 2009. Bioprocessing of plant cell cultures for mass production of targeted compounds. Appl. Microbiol. Biotechnol. 83, 809–823. https://doi.org/10.1007/s00253-009-2049-x.
Gomes, F.P., Prado, C.H.B.A., 2007. Ecophysiology of coconut palm under water stress. Braz. J. Plant Physiol. 19, 377–391. https://doi.org/10.1590/S1677-04202007000400008.
Gordon-Kamm, W.J., Spencer, T.M., Mangano, M.L., Adams, T.R., Daines, R.J., Start, W.G., O'Brien, J.V., Chambers, S.A., Adams, W.R., Willetts, N.G., Rice, T.B., Mackey, C.J., Krueger, R.W., Kausch, A.P., Lemaux, P.G., 1990. Transformation of maize cells and regeneration of fertile transgenic plants. Plant Cell 2, 603–618. https://doi.org/10.1105/tpc.2.7.603.
Gow, W., Chen, J., Chang, W., 2008. Effects of genotype, light regime, explant position and orientation on direct somatic embryogenesis from leaf explants of *Phalaenopsis orchids*. Acta Physiol. Plant. 31, 363. https://doi.org/10.1007/s11738-008-0243-6.
Hangarter, R.P., 1997. Gravity, light and plant form. Plant Cell Environ. 20, 796–800. https://doi.org/10.1046/j.1365-3040.1997.d01-124.x.
Hatfield, J.L., Prueger, J.H., 2015. Temperature extremes: effect on plant growth and development. Weather Clim. Extrem. 10, 4–10.
Hazarika, B.N., 2006. Morpho-physiological disorders in *in vitro* culture of plants. Sci. Hortic. 108, 105–120. https://doi.org/10.1016/j.scienta.2006.01.038.
Helaly, M.N., El-Hosieny, H.A.R., El-Sarkassy, N.M., Fuller, M.P., 2017. Growth, lipid peroxidation, organic solutes, and anti-oxidative enzyme content in drought-stressed date palm embryogenic callus suspension induced by polyethylene glycol. In Vitro Cell. Dev. Biol. Plant 53, 133–141. https://doi.org/10.1007/s11627-017-9815-8.
Ibraheem, Y., Pinker, I., Böhme, M., 2013. A comparative study between solid and liquid cultures relative to callus growth and somatic embryo formation in date palm (*Phoenix dactylifera* L.) cv. Zaghlool. Emir. J. Food Agric. 25, 883–898. https://doi.org/10.9755/ejfa.v25i11.16661.
Jalil, M., Khalid, N., Othman, R.Y., 2003. Plant regeneration from embryogenic suspension cultures of *Musa acuminata* cv. Mas (AA). Plant Cell Tiss. Org. Cult. 75, 209–214. https://doi.org/10.1023/A:1025814922547.
Jeong, C.S., Murthy, H.N., Hahn, E.J., Lee, H.L., Paek, K.Y., 2009. Inoculum size and auxin concentration influence the growth of adventitious roots and accumulation of ginsenosides in suspension cultures of ginseng (*Panax ginseng* C.A. Meyer). Acta Physiol. Plant. 31, 219–222. https://doi.org/10.1007/s11738-008-0206-y.
Jeoung, J.-M., Fritz, J.O., Liang, G.H., 1998. Callus induction, plant regeneration and mutagenesis of Eastern Gamagrass. Cytologia 63, 381–386.
Johnson, D.V., 1999. The economic importance of palms to people in tropical areas. In: Ruano, M.C. (Ed.), Proceedings of the Second International Symposium on Ornamental Palms & Other Monocots from the Tropics. International Society for Horticultural Science (ISHS), Leuven, Belgium. pp. 267–276. https://doi.org/10.17660/ActaHortic.1999.486.40.
Kaeppler, S.M., Kaeppler, H.F., Rhee, Y., 2000. Epigenetic aspects of somaclonal variation in plants. In: Matzke, M.A., Matzke, A.J.M. (Eds.), Plant Gene Silencing. Springer Netherlands, Dordrecht, pp. 59–68. https://doi.org/10.1007/978-94-011-4183-3_4.
Kanchanapoom, K., Chourykaew, B., 1998. Somatic embryogenesis from cell suspension cultures of oil palm (*Elaeis guineensis* Jacq.). J. Sci. Soc. Thailand 24, 241–250.
Kao, K.N., Michayluk, M.R., 1975. Nutritional requirements for growth of *Vicia hajastana* cells and protoplasts at a very low population density in liquid media. Planta 126, 105–110. https://doi.org/10.1007/BF00380613.
Kolewe, M.E., 2011. Development of Plant Cell Culture Processes to Produce Natural Product Pharmaceuticals: Characterization, Analysis, and Modeling of Plant Cell Aggregation. Ph.D. Dissertation. Department of Chemical Engineering, University of Massachusetts Amherst.
Kolewe, M.E., Henson, M.A., Roberts, S.C., 2011. Analysis of aggregate size as a process variable affecting paclitaxel accumulation in *Taxus* suspension cultures. Biotechnol. Prog. 27, 1365–1372. https://doi.org/10.1002/btpr.655.
Kosky, R.G., de Feria Silva, M., Pérez, L.P., Gilliard, T., Martínez, F.B., Vega, M.R., Milian, M.C., Mendoza, E.Q., 2002. Somatic embryogenesis of the banana hybrid cultivar FHIA-18 (AAAB) in liquid medium and scaled-up in a bioreactor. Plant Cell Tiss. Org. Cult. 68, 21–26. https://doi.org/10.1023/A:1012905825307.
Kramut, P., Te-chato, S., 2010. Effect of culture media, plant growth regulators and carbon sources on establishment of somatic embryo in suspension culture of oil palm. J. Agric. Technol. 6, 159–170.
Kulkarni, V.M., Ganapathi, T.R., 2009. A simple procedure for slow growth maintenance of banana (*Musa* spp.) embryogenic cell suspension cultures at low temperature. Curr. Sci. 96, 1372–1377.
Larkin, P.J., Scowcroft, W.R., 1981. Somaclonal variation — a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60, 197–214. https://doi.org/10.1007/BF02342540.
Lo, K., Nadali, B.J., Chan, L.K., 2012. Investigation on the effect of subculture frequency and inoculum size on the artemisinin content in a cell suspension culture of' *Artemisia annua*' L. Aust. J. Crop Sci. 6, 801–807.
Machakova, I., Zazimalova, E., George, E.F., 2008. Plant growth regulators I: introduction; Auxins, their analogues and inhibitors. In: George, E.F., Hall, M.A., Klerk, G.-J.D. (Eds.), Plant Propagation by Tissue Culture: The Background, third ed. Springer Netherlands, Dordrecht, pp. 175–204. https://doi.org/10.1007/978-1-4020-5005-3_5.
Marino, G., Berardi, G., Ancherani, M., 1995. The effect of the type of closure on the gas composition of the headspace and the growth of GF 677 peach × almond rootstock cell suspension cultures. In Vitro Cell. Dev. Biol. Plant 31, 207–210. https://doi.org/10.1007/BF02632023.
Masani, M.Y.A., Noll, G.A., Parveez, G.K.A., Sambanthamurthi, R., Prüfer, D., 2014.

Efficient transformation of oil palm protoplasts by PEG-mediated transfection and DNA microinjection. PLoS One 9, e96831. https://doi.org/10.1371/journal.pone.0096831.
Mathur, J., Koncz, C., 1998. Establishment and maintenance of cell suspension cultures. In: Martinez-Zapater, J.M., Salinas, J. (Eds.), Arabidopsis Protocols. Humana Press, Totowa, pp. 27–30. https://doi.org/10.1385/0-89603-391-0:27.
Meerow, A.W., Krueger, R.R., Singh, R., Low, E.-T.L., Ithnin, M., Ooi, L.C.L., 2012. Coconut, date, and oil palm genomics. In: Schnell, R.J., Priyadarshan, P.M. (Eds.), Genomics of Tree Crops. Springer New York, New York, pp. 299–351. https://doi.org/10.1007/978-1-4614-0920-5_10.
Michler, C., Lineberger, R., 1987. Effects of light on somatic embryo development and abscisic levels in carrot suspension cultures. Plant Cell Tiss. Org. Cult. 11, 189–207. https://doi.org/10.1007/BF00040425.
Miguel, C., Marum, L., 2011. An epigenetic view of plant cells cultured *in vitro*: somaclonal variation and beyond. J. Exp. Bot. 62, 3713–3725. https://doi.org/10.1093/jxb/err155.
Mora-Urpí, J., Weber, J.C., Clement, C.R., 1997. Peach Palm, *Bactris gasipaes* Kunth. International Plant Genetic Resources Institute Rome, Italy.
Morcillo, F., Gallard, A., Pillot, M., Jouannic, S., Aberlenc-Bertossi, F., Collin, M., Verdeil, J.L., Tregear, J.W., 2007. EgAP2-1, an AINTEGUMENTA-like (AIL) gene expressed in meristematic and proliferating tissues of embryos in oil palm. Planta 226, 1353–1362. https://doi.org/10.1007/s00425-007-0574-3.
Morel, G., Wetmore, R.H., 1951. Fern callus tissue culture. Am. J. Bot. 38, 141–143. https://doi.org/10.1002/j.1537-2197.1951.tb14804.x.
Moscatiello, R., Baldan, B., Navazio, L., 2013. Plant cell suspension cultures. In: Maathuis, F.J.M. (Ed.), Plant Mineral Nutrients: Methods and Protocols. Humana Press, New York, pp. 77–93. https://doi.org/10.1007/978-1-62703-152-3_5.
Mulabagal, V., Tsay, H.-S., 2004. Plant cell cultures - an alternative and efficient source for the production of biologically important secondary metabolites. Int. J. Appl. Sci. Eng. 2, 29–48.
Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15, 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.
Mustafa, N.R., de Winter, W., van Iren, F., Verpoorte, R., 2011. Initiation, growth and cryopreservation of plant cell suspension cultures. Nat. Protoc. 6, 715–742.
Naik, P.M., Al-Khayri, J.M., 2016. Somatic embryogenesis of date palm (*Phoenix dactylifera* L.) through cell suspension culture. In: Jain, S.M. (Ed.), Protocols for *In Vitro* Cultures and Secondary Metabolite Analysis of Aromatic and Medicinal Plants, second ed. Humana Press, New York, pp. 357–366. https://doi.org/10.1007/978-1-4939-3332-7_25.
Naik, P.M., Al-Khayri, J.M., 2017. Extraction and estimation of secondary metabolites from date palm cell suspension cultures. In: Al-Khayri, J.M., Jain, S.M., Johnson, D.V. (Eds.), Date Palm Biotechnology Protocols: Tissue Culture Applications. Humana Press, New York, pp. 319–332. https://doi.org/10.1007/978-1-4939-7156-5_26.
Nguyen, Q.T., 2018. Clonal Propagation of Coconut (*Cocos nucifera* L.) for Elite Seedling Production and Germplasm Exchange. Ph.D. Dissertation. School of Agriculture and Food Sciences, The University of Queensland.
Nguyen, Q.T., Bandupriya, H.D.D., López-Villalobos, A., Sisunandar, S., Foale, M., Adkins, S.W., 2015. Tissue culture and associated biotechnological interventions for the improvement of coconut (*Cocos nucifera* L.): a review. Planta 242, 1059–1076. https://doi.org/10.1007/s00425-015-2362-9.
Othmani, A., Bayoudh, C., Drira, N., Trifi, M., 2009. *In vitro* cloning of date palm *Phoenix dactylifera* L., cv. Deglet Bey by using embryogenic suspension and temporary immersion bioreactor (TIB). Biotechnol. Biotechnol. Equip. 23, 1181–1188. https://doi.org/10.1080/13102818.2009.10817635.
Owen, H.R., Wengerd, D., Miller, A.R., 1991. Culture medium pH is influenced by basal medium, carbohydrate source, gelling agent, activated charcoal, and medium storage method. Plant Cell Rep. 10, 583–586. https://doi.org/10.1007/BF00232516.
Ozeki, Y., Komamine, A., 1985. Effects of inoculum density, zeatin and sucrose on anthocyanin accumulation in a carrot suspension culture. Plant Cell Tiss. Org. Cult. 5, 45–53. https://doi.org/10.1007/BF00033568.
Patil, R.A., Kolewe, M.E., Roberts, S.C., 2013. Cellular aggregation is a key parameter associated with long term variability in paclitaxel accumulation in *Taxus* suspension cultures. Plant Cell Tiss. Org. Cult. 112, 303–310. https://doi.org/10.1007/s11240-012-0237-3.
Pelkonen, V.P., Kauppi, A., 1999. The effect of light and auxins on the regeneration of lily (*Lilium regale* Wil.) cells by somatic embryogenesis and organogenesis. Int. J. Plant Sci. 160, 483–490. https://doi.org/10.1086/314138.
Preil, W., 2005. General introduction: a personal reflection on the use of liquid media for *in vitro* culture. In: Hvoslef-Eide, A.K., Preil, W. (Eds.), Liquid Culture Systems for *In Vitro* Plant Propagation. Springer Netherlands, Dordrecht, pp. 1–18. https://doi.org/10.1007/1-4020-3200-5_1.
Ramakrishna, A., Ravishankar, G.A., 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav. 6, 1720–1731. https://doi.org/10.4161/psb.6.11.17613.
Razdan, M.K., 2003. Introduction to Plant Tissue Culture, second ed. Science Publishers, Enfield.
Rival, A., 2000. Somatic embryogenesis in oil palm. In: Jain, S.M., Gupta, P.K., Newton, R.J. (Eds.), Somatic Embryogenesis in Woody Plants. Springer Netherlands, Dordrecht, pp. 249–290. https://doi.org/10.1007/978-94-017-3030-3_9.
Rival, A., Ilbert, P., Labeyrie, A., Torres, E., Doulbeau, S., Personne, A., Dussert, S., Beulé, T., Durand-Gasselin, T., Tregear, J.W., Jaligot, E., 2013. Variations in genomic DNA methylation during the long-term *in vitro* proliferation of oil palm embryogenic suspension cultures. Plant Cell Rep. 32, 359–368. https://doi.org/10.1007/s00299-012-1369-y.
Rodríguez-Sahagún, A., Acevedo-Hernández, G., Rodríguez-Domínguez, J.M., Rodríguez-Garay, B., Cervantes-Martínez, J., Castellanos-Hernández, O.A., 2011. Effect of light quality and culture medium on somatic embryogenesis of *Agave tequilana* Weber var. Azul. Plant Cell Tiss. Org. Cult. 104, 271–275. https://doi.org/10.1007/s11240-010-9815-4.
Saker, M.M., Allam, M.A., Goma, A.H., Abd El-Zaher, M.H., 2007. Development of suspension culture system for *in vitro* propagation of date palm. J. Genet. Eng. Biotechnol. 5, 51–56.
Sayer, J., Ghazoul, J., Nelson, P., Klintuni Boedhihartono, A., 2012. Oil palm expansion transforms tropical landscapes and livelihoods. Glob. Food Sec. 1, 114–119. https://doi.org/10.1016/j.gfs.2012.10.003.
Schiavone, F.M., Wisniewski, M.E., 1990. Callus and cell suspension cultures from dormant stems of peach. HortScience 25, 483.
Selmani, C., Chabane, D., Bouguedoura, N., 2013. Production of somatic embryos of date palm (*Phoenix dactylifera* L.) from cell suspension. Acta Hortic. 994, 331–337.
Shareef, H.J., Al-Mayahi, A.M.W., Alhamd, A.D., 2016. Effect of desiccation and cold hardening on germination of somatic embryos in date palm (*Phoenix dactylifera* L.) Berhi cultivar *in vitro*. Adv. Appl. Sci. Res. 7, 58–64.
Singh, M., Chaturvedi, R., 2012. Evaluation of nutrient uptake and physical parameters on cell biomass growth and production of spilanthol in suspension cultures of *Spilanthes acmella* Murr. Bioprocess Biosyst. Eng. 35, 943–951. https://doi.org/10.1007/s00449-012-0679-3.
Smith, R.H., 2013. Media components and preparation. In: Smith, R.H. (Ed.), Plant Tissue Culture: Techniques and Experiments, third ed. Academic Press, San Diego, pp. 31–43. https://doi.org/10.1016/B978-0-12-415920-4.00003-7.
Soh, A.C., Wong, G., Tan, C.C., Chew, P.S., Hor, T.Y., Chong, S.P., Gopal, K., 2001. Recent Advances Towards Commercial Production of Elite Oil Palm Clones. PIPOC International Palm Oil Congress. Malaysian Palm Oil Board, Kuala Lumpur, pp. 33–44.
Soh, A.C., Wong, G., Hor, T.Y., Tan, C.C., Chew, P.S., 2003. Oil palm genetic improvement. In: Janick, J. (Ed.), Plant Breeding Reviews. John Wiley & Sons, Inc., New Jersey, pp. 165–220.
Soomro, R., Memon, R.A., 2007. Establishment of callus and suspension culture in *Jatropha curcas*. Pakistan J. Bot. 39, 2431–2441.
Strosse, H., Domergue, R., Panis, B., Escalant, J.-V., Côte, F., 2003. Banana and plantain embryogenic cell suspensions. In: Vézina, A., Picq, C. (Eds.), INIBAP Technical Guidelines 8. The International Network for the Improvement of Banana and Plantain, Montpellier, pp. 6–20.
Strosse, H., den Houwe, I.V., Panis, B., 2004. Banana cell and tissue culture. In: Jain, S.M., Swennen, R. (Eds.), Banana Improvement: Cellular, Molecular Biology, and Induced Mutations. Science Publishers, Enfield, pp. 1–12.
Taha, H.S., Abdel-Kawy, A.M., Fathalla, M., Abd-El-Kareem, M., El-Shabrawi, H.M., 2010. Implementation of DMSO for enhancement and production of phenolic and peroxides compounds in suspension cultures of Egyptian date palm (Zaghlool and Samany) cultivars. J. Biotechnol. Biochem. 1, 1–10.
Tahardi, J.S., 1998. Somatic embryogenesis from cell suspension culture of oil palm. In: Tahardi, J.S., Darmono, T.W., Siswanto, Santoso, D., Nataatmadja, R. (Eds.), Fourth BTIG Workshop on Oil Palm Improvement Through Biotechnology. Biotechnology Research Unit for Estate Crops, Bogor, pp. 27–32.
Tanaka, H., Aoyagi, H., Jitsufuchi, T., 1992. Turbidimetric measurement of cell biomass of plant cell suspensions. J. Ferment. Bioeng. 73, 130–134. https://doi.org/10.1016/0922-338X(92)90527-2.
Tarmizi, A.H., Zaiton, R., Rosli, M.Y., 2012. Improvements in oil palm liquid culture systems. Trans. Malaysian Soc. Plant Physiol. 20, 118–121.
Te-chato, S., Hilae, A., In-Peuy, K., 2008. Effects of cytokinin types and concentrations on growth and development of cell suspension culture of oil palm. J. Agric. Technol. 4, 157–163.
Teixeira, J.B., Söndahl, M.R., Nakamura, T., Kirby, E.G., 1995. Establishment of oil palm cell suspensions and plant regeneration. Plant Cell Tiss. Org. Cult. 40, 105–111. https://doi.org/10.1007/BF00037662.
Thorpe, T., Stasolla, C., Yeung, E.C., De Klerk, G.-J., Roberts, A., George, E.F., 2008. The components of plant tissue culture media II: organic additions, osmotic and pH effects, and support systems. In: George, E.F., Hall, M.A., De Klerk, G.-J. (Eds.), Plant Propagation by Tissue Culture: The Background, third ed. Springer, Dordrecht, pp. 115–173.
Tognetti, J.A., Pontis, H.G., Martínez-Noël, G.M.A., 2013. Sucrose signaling in plants: a world yet to be explored. Plant Signal. Behav. 8https://doi.org/10.4161/psb.23316. e23316-e23316.
Tokuhara, K., Mii, M., 2003. Highly-efficient somatic embryogenesis from cell suspension cultures of *Phalaenopsis* orchids by adjusting carbohydrate sources. In Vitro Cell. Dev. Biol. Plant 39, 635–639. https://doi.org/10.1079/IVP2003466.
Tregear, J.W., Rival, A., Pintaud, J.-C., 2011. A family portrait: unravelling the complexities of palms. Ann. Bot. 108, 1387–1389. https://doi.org/10.1093/aob/mcr269.
Veramendi, J., Navarro, L., 1996. Influence of physical conditions of nutrient medium and sucrose on somatic embryogenesis of date palm. Plant Cell Tiss. Org. Cult. 45, 159–164. https://doi.org/10.1007/BF00048760.
Von Arnold, S., 2008. Somatic embryogenesis. In: George, E.F., Hall, M.A., De Klerk, G.-J. (Eds.), Plant Propagation by Tissue Culture: The Background, third ed. Springer, Dordrecht, pp. 335–354.
Wakita, Y., Sasamoto, H., Yokota, S., Yoshizawa, N., 1996. Callus proliferation from protoplasts isolated from cell suspension cultures of *Alnus firma* Sieb. Plant Tissue Cult. Lett. 13, 279–284.
Wallner, S.J., Nevins, D.J., 1973. Formation and dissociation of cell aggregates in suspension cultures of Paul's Scarlet Rose. Am. J. Bot. 60, 255–261. https://doi.org/10.2307/2441216.
Wang, Y., Jeknić, Z., Ernst, R.C., Chen, T.H.H., 1999. Efficient plant regeneration from suspension-cultured cells of Tall Bearded Iris. HortScience. 34, 730–735.

Warren, G.S., 1992. The cell biology of plant culture systems. In: Fowler, M.W., Warren, G.S. (Eds.), Plant Biotechnology: Comprehensive Biotechnology Second Supplement, first ed. Pergamon Press, Oxford, pp. 1–18.

Wong, G., Chong, S.P., Tan, C.C., Soh, A.C., 1999. . Liquid suspension culture: a potential technique for mass production of oil palm clones. Proceedings of the 1999 PORIM International Palm Oil Congress, Kuala Lumpur 3–11.

Yang, J., Wu, S., Li, C., 2013. High efficiency secondary somatic embryogenesis in *Hovenia dulcis* Thunb. through solid and liquid cultures. Sci. World J. 2013https://doi.org/10.1155/2013/718754. 718754-718754.

Yusuf, N.A., Annuar, M.S.M., Khalid, N., 2013. Physical stress for overproduction of biomass and flavonoids in cell suspension cultures of *Boesenbergia rotunda*. Acta Physiol. Plant. 35, 1713–1719. https://doi.org/10.1007/s11738-012-1178-5.

Zhao, D., Huang, Y., Jin, Z., Qu, W., Lu, D., 2003. Effect of aggregate size in cell cultures of *Saussurea medusa* on cell growth and jaceosidin production. Plant Cell Rep. 21, 1129–1133. https://doi.org/10.1007/s00299-003-0631-8.

Zhong, J.J., Yoshida, T., 1997. Cell and tissue cultures of *Perilla*. In: Yu, H.-C., Kosuna, K., Haga, M. (Eds.), *Perilla*-The Genus *Perilla*. Taylor & Francis, London, pp. 19–36.

Zimmerman, J.L., 1993. Somatic embryogenesis: a model for early development in higher plants. Plant Cell 5, 1411–1423. https://doi.org/10.2307/3869792.

Zouine, J., El Hadrami, I., 2004. Somatic embryogenesis in *Phoenix dactylifera* L: effect of exogeneous supply of sucrose on proteins, sugars, phenolics and peroxidases activities during the embryogenic cell suspension culture. Biotechnology 3, 114–118. https://doi.org/10.3923/biotech.2004.114.118.

Zouine, J., El Hadrami, I., 2007. Effect of 2,4-D, glutamine and BAP on embryogenic suspension culture of date palm (*Phoenix dactylifera* L.). Sci. Hortic. 112, 221–226. https://doi.org/10.1016/j.scienta.2006.12.041.

Zouine, J., El Bellaj, M., Meddich, A., Verdeil, J.-L., El Hadrami, I., 2005. Proliferation and germination of somatic embryos from embryogenic suspension cultures in *Phoenix dactylifera*. Plant Cell Tiss. Org. Cult. 82, 83–92. https://doi.org/10.1007/s11240-004-6914-0.