A REVIEW ON SOME FACTORS THAT AFFECT THE PROCESS OF PLANT CALLUS CULTURES

Pham Thi My Tram[(1)]
(1) Thu Dau Mot University
Corresponding author: tramptm@tdmu.edu.vn

DOI: 10.37550/tdmu.EJS/2025.03.672

Article Info

Volume: 7
Issue: 3
Sep: 2025
Received: Jun. 9th, 2025
Accepted: Aug. 30th, 2025
Page No: 734-743

Abstract

The callus is a disorganized mass of cells that grow in plants in response to various biotic and abiotic stimuli. Callus plays an important role in cell cultures in vitro, as a starting material for many subsequent studies such as single-cell culture, protoplast culture, micropropagation, etc. In this review, callus as well as the factors affecting callus formation and proliferation (plant growth regulator, mineral medium, carbon source, type of explant, light condition, callus line) are explored to provide a brief overview of callus culture.

Keywords: callus, cultures, factor, in vitro, plant

1. Introduction

The term "plant tissue culture" is widely used to refer to the cultures of sterile plant cells, tissues, and organs under defined chemical, and physical conditions, which is an important method in basic and applied research (Thorpe, 2007).

Applications of plant tissue culture include micropropagation; the creation of disease-free plants; maintenance of plant sprouts; haploid production from pollen culture; studies on plant morphogenesis; production of valuable compounds; genetic engineering (Smith, 2001).

In vitro plant cell culture media contain the same essential nutrients as *in vivo* culture media, including macronutrients, micronutrients, vitamins, plant growth regulators, sugars, other organics, and some gelling agents (Husain et al., 2012).

In cell cultures, the formation of callus is the first and most important step. The callus is the starting material for further studies such as tissue and cell differentiation, cell line selection, protoplast culture, adventitious root culture, hairy root culture, single-cell culture, and somatic embryo culture. Therefore, callus culture requires optimal nutrients for them to develop fully (Bhatia et al., 2015).

The main objective of writing this review is to introduce tissue culture techniques, callus culture as well as some of the factors affecting callus formation and proliferation.

2. Callus culture

Callus culture can be defined as the proliferation of a disorganized mass of cells from isolated plant cells, tissues, or organs by culturing them on artificial nutrient media under sterile and special conditions (injury, treatment with plant growth regulators (Sharma et al., 2015).

The callus is formed by de-differentiation of the original cells to produce meristem cells and actively proliferate. This causes the explants to become thick, hard, and swollen. Rapid division of cells produces disorganized cell masses on the explant surface (Mastuti et al., 2017). Depending on the plant species, type of explant, and culture conditions, different types of callus can be formed (Figure 1).

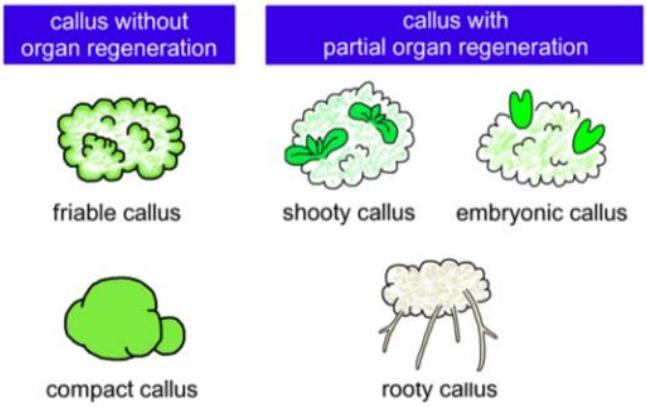

Figure 1. Types of plant callus (Ikeuchi, 2013).

The explants can be placed in dark or light conditions at 25°C. Callus may form after several days or weeks. Some explants are better able to form callus under light conditions, but, conversely, they are also prone to browning due to the overproduction of polyphenols under light. The callus is often the starting material for many common cultures *in vitro*, such as cell suspension culture, cell culture, and plant propagation (Petersen & Alfermann, 1993).

3. Some factors affecting the process of plant callus cultures

The callus is a mass of rapidly proliferating, undifferentiated cells that can be obtained by culturing from explant sources such as seeds, shoots, leaves, meristems, and root tips. Culture medium contains nutrients and plant growth regulators with a standard recipe of chemicals (Ullah et al., 2007).

The capacity of callus formation is highly dependent on physiological, biochemical, genotype, and culture conditions (Luong & Tien, 2006). Therefore, in callus culture, important factors such as mineral medium, plant growth regulator, carbon source, explant origin, and light conditions need to be investigated to select the callus culture medium that is appropriate for each plant species.

3.1. Plant growth regulator

Among these components in the nutrient medium, plant growth regulators are the most important for initiating callus culture (Mastuti et al., 2017). Auxin, cytokinin, and

gibberellin are growth regulators usually used in tissue culture. In particular, auxin plays a role in promoting the initiation of callus. 2,4-dichlorophenoxyacetic acid (2,4-D), which is a synthetic auxin, is most used in callus formation (Sari et al., 2018). Auxins have an indirect effect on cell wall elongation by acidifying the cell wall. Auxins induce the active transport of H+ ions from the cytoplasm to the cell wall. Acidic pH activates enzymes in the wall, breaking the cross-links between cellulose fibers. Loose fibers make cells more elastic. This also activates more K+ ions to be pumped into the cytoplasm, stimulates water uptake, creates a swelling pressure inside the cell, and increases the cell volume. Thus, proper auxin use can help cells become more loose, watery, and friable. However, different cells have different elongation capacities. Older cells are less sensitive to acidic pH and the cell wall is less enlarged than younger cells (Majda & Robert, 2018).

According to the study of callus induction by *Panax vietnamensis* Ha et Grushv., petiole explants were grown on Murashige and Skoog (MS) medium containing 1mg/L 2,4-D. The percentage of callus explants was 100% with friable, watery, and white callus. In medium supplemented with a low concentration of 2,4-D (0.5mg/L), the explants were almost not induced to form callus but to form roots. In the treatment without 2,4-D, the explants turned brown and died (Cuong et al., 2012). In addition, different concentrations of plant growth regulators significantly affected callus induction. Research results on callus formation in *Rosa hybrida* L. showed that high concentrations of 2,4-D (from 3.0mg/L to 5.0mg/L) may cause callus browning, which would seriously affect the regeneration (Liu et al., 2018). The combination of the two auxins has also been reported to be effective in callus induction in many plant species. For example, in *Panax vietnamensis* Ha et Grushv., friable callus was formed at a rate of 100% when the medium was supplemented with 1mg/L 2,4-D, and 1mg/L naphthalene acetic acid (NAA). Callus biomass was 1.6 times higher compared with the treatment without NAA (Cuong et al., 2012). For *Celastrus paniculatus*, leaf explants cultured on MS medium supplemented with 1.5mg/L 2,4-D and 1mg/L NAA produced friable, milky callus (Anusha et al., 2016). However, in selected *Indica* rice (*Oryza sativa* L.), the results noted that callus grown on medium supplemented with 2,4-D (3mg/L) was better than on medium supplemented with 2,4-D (3mg/L), and NAA (2.5-10mg/L). Furthermore, lower callus induction of all varieties was observed when higher auxin concentrations were used (Binte & Wagiran, 2018).

Cytokinins are involved in most phases of the cell cycle. It is a signal transducer that helps cells move from the G1 phase into the S phase by rapidly regulating the expression of the CycD3 fusion gene, a cyclin that plays a key role in the regulation of plant cell division both *in vitro* and *in vivo* (Kieber & Schaller, 2014). In some cases, cytokinins can be added to the culture medium to assist with auxins in the stimulation of callus formation and proliferation by regulating nutrient transport and protein uptake (Kieber & Schaller, 2018). In *Melastoma malabathricum*, Keng et al. (2008) reported that the use of 2,4-D, NAA alone, or in combination, could help create friable callus. However, the callus biomass was very low, not enough as a raw material for the cell suspension culture. Therefore, the authors investigated the effect of the combination of benzylaminopurine (BA) and NAA on callus formation. Leaf samples placed on MS medium containing 1mg/L BA and 6mg/L NAA produced the highest callus volume of all investigated treatments. In contrast, in *Elaeagnus angustifolia* L, callus formed from leaf samples placed on MS medium supplemented with 1mg/L 2,4-D and 0.5mg/L BA (white, friable callus) was better than in samples placed on medium added with NAA combined with BA or thidiazuron (TDZ) combined with BA (Zeng et al., 2009). In another result, the combination of 0.5 and 1mg/L 2,4-D with 0.5mg/L

kinetin (KIN) induced the highest fraction of *Arabidopsis thaliana* hypocotyl to induce callus. This showed that 2,4-D was more effective for initiating callus formation than IAA and IBA. 2,4-D was the most common auxin used for initiating callus growth because it could convert the sample cells to an undifferentiated state and initiate active proliferation. The combination of all auxins with KIN produced friable callus and may have formed root regeneration (Mastuti et al., 2017).

3.2. Mineral medium

Plants, like other living things, need food to grow and develop. 16 elements are necessary for plants. In it, carbon, hydrogen, and oxygen are obtained from the atmosphere and soil water. The remaining 13 essential elements, including nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, zinc, manganese, copper, boron, molybdenum, and chlorine, are supplied from soil minerals and organic matter in the soil or organic or inorganic fertilizers. Each type of plant has a different minimum nutrient requirement (Uchida, 2000). Similarly, cells grown *in vitro* also require similar or higher nutrient requirements. According to many studies, the nutrient concentration of callus was lower than that of intact plants, even though they were cultured on medium added with higher concentrations of nutrients. This may be explained by the fact that plants *in vivo* have roots, through root hairs and mycorrhizal fungi that are easier to absorb nutrients compared to callus. In addition, the nutrient contents in the environment *in vivo* are kept steady while in the medium *in vitro*, the nutrient concentrations are initially very high but then decrease rapidly (Vasic et al., 2001). Thus, mineral components play an important role in the induction, proliferation, and morphogenesis of plant callus. In which, Murashige and Skoog (1962) (MS) medium is most commonly used. Although MS medium is not optimal for many plant species, many tissues will grow on it to some extent and it is often used for initiating cell cultures. Since then, many other media have been developed from the MS medium (Niedz & Evens, 2007).

In *Barringtonia racemosa*, leaf explants were cultured on Woody plant medium (WPM), Gamborg (B5), and MS medium supplemented with 2,4-D from 0.5mg/L to 3mg/L. The study showed that friable callus was induced on WMP and B5 media, but hard callus was induced on MS medium. In which, WPM medium supplemented with 2,4-D 2mg/L was the best medium to induce callus formation (Behbahani et al., 2011).

In *Zea mays* L., the results showed that callus was formed with the highest rate on $N6^{++}$ medium (N6 medium supplemented with 25mM L-proline, 100mg/L casein hydrolysate, and 1mg/L 2,4-D) (Rafiq et al., 2005). This difference suggested that the interaction between genotype and culture is significant. In contrast, in *Oryza sativa* L., the rate of callus formation on MS medium was higher than on N6 and Linsmaier and Skoog (LS) medium for rice varieties (Binte & Wagiran, 2018).

In another study, three media, including MS, ½MS, and B5, were investigated for callus induction from leaf explants of *Nepeta binaloudensis* Jamzad (Lamiaceae). The results showed that the types of culture medium and BA levels had a substantial impact on fresh callus. The highest fresh weight of callus was recorded in ½MS medium. According to the authors, ½MS medium can reduce the production of phenolic and toxic compounds in the environment. No callus formation was observed in the medium without growth regulators. No callus was formed when explants were cultured on control MS and B5 medium (Sagharyan et al., 2020). In *Hyssopus officinalis* callus cultures, the results showed that the mineral composition was the most essential element in the biosynthesis of phenolics and

saponin. NAA and KIN (3mg/L and 2mg/L) have been shown to support the production of these compounds for MS medium, but not B5 medium (Babich et al., 2021).

Therefore, each plant species with different explants will use a different type of suitable medium.

3.3 Carbon source

It is essential to provide a carbon source to the culture medium, as the photosynthetic activity of *in vitro* tissues is often reduced. These compounds are also needed in the environment as agents to ensure proper permeability. In addition, sugars are also signaling molecules that repress or activate plant genes involved in many important metabolic processes such as photosynthesis, glycoxylate metabolism, respiration, starch and sucrose synthesis and breakdown, nitrogen metabolism, regeneration, cell cycle regulation, pigmentation, and senescence. Therefore, sugar greatly affects the physiology, growth, and differentiation of cells. Of these, sucrose is most commonly used in tissue and cell cultures, because it is the major translocated sugar in the phloem of many plants. However, some plants can grow on carbon sources other than sucrose (Alina et al., 2006). Therefore, in the process of *in vitro* culture, people often investigate the carbon source to find the most suitable sugar source for culture.

Martinez et al. (2021) studied the effect of the carbon source type (sucrose and glucose) and its concentration (1.0, 2.3, 3.2, and 5.5% w/v) on callus induction and proliferation of *Taraxacum officinale* (L) Weber Ex FH Wigg. The results showed hypocotyls and roots from sterile seedlings were the best explants for callus formation (100% of friable callus). The explants on medium added with sucrose at 2.3% (w/v), placed in complete darkness, had the effect of inducing callus with low organogenesis and high friability. While, in the callus culture of *Datura stramonium*, it was demonstrated that the addition of 2% lactose was more suitable for callus induction than sucrose, glucose, fructose, galactose, maltose. The authors noted that lactose is only detected in a few plant species. When added to the tissue culture medium, it has been found to induce the activity of β-galactosidase enzyme (Amiri & Kazemitabar, 2011). On the other hand, in *Lippia multiflora* Moldenke, among the investigated sugars (sucrose, glucose, fructose, and maltose at a concentration of 30g/L), the maximum rate of callus formation was observed from leaf samples on MS medium added with 30mg/L of fructose (André et al., 2015).

Besides, in rice tissue culture, some studies have shown that maltose is considered an ideal carbon source for callus induction and regeneration. Maltose has a role in promoting signals to activate carbohydrate metabolism in callus formation. The extracellular hydrolysis of maltose occurs at a slower rate than that of sucrose and it is absorbed slowly. This activity may be the main reason for maltose's ability to control phenolic secretion in the medium. Accumulation of toxic products such as phenolic compounds and nutrient depletion can lead to cell death and deceleration of biomass (Binte & Wagiran, 2018; Repalli et al., 2019). Similarly, in callus culture of *Gossypium hirsutum* L. cv. SVPR-2, 3% maltose was found to be the best carbon source for callus culture, followed by glucose. Maltose showed that it had only a small effect on browning (due to phenolic secretion) of callus both at lower and higher concentrations of 3%. Meanwhile, fructose increased phenolic secretion at all tested concentrations. Therefore, fructose has no significant role in the callus culture of SVPR-2 cotton (Kumar et al., 2015).

3.4. Type of explant

Callus can be created from many different types of organs of a plant. The ability to induce callus formation and proliferation in explants of different origins was different. The younger the cell, the more susceptible it is to the impact, the process of creating callus will take place faster and more efficiently than old cells.

According to Bosila et al. (2001), explants were obtained from different organs of *Digitalis lanata* Ehrh (shoot tips, leaves, hypocotyls, and roots), 2 and 4 weeks old, taken from plants grown *in vitro* and 8, 12, and 20 weeks from plants grown *in vivo*, cultured on sterile MS added with 5.0mg/L of 2,4-D and 0.5mg/L of BA. Among all the types and sample ages studied, callus derived from 2 or 20-week old leaf samples produced the highest biomass and glycoside content.

Similarly, in *Nepeta binaloudensis* Jamzad (Lamiaceae), three different explants (leaves, shoot apical meristems, and stems) were used for callus induction. Among them, the leaf explants showed the highest callus induction. Several studies have reported that leaf specimens are suitable for callus formation in plants. Different explant responses to hormone-supplemented treatments can lead to different physiological and biochemical processes in the explants (Sagharyan et al., 2020). On the report of the study on callus formation in *Actinidia deliciosa,* leaf examples, after 12 days of culture, started to initiate callus. Leaf explant placed with the abaxial surface down induced more callus from the main veins of the leaf to the minor veins and then to the wound part. Because it facilitated the absorption and metabolism of nutrients and the transport of water through capillary force (Nhut et al., 2012).

Based on the results of Liu et al. (2018), young leaves of *Rosa hybrida* L. were found to be the most effective in callus formation and whole plant regeneration, compared with petioles and stems. The age and type of explant strongly influenced callus regeneration. Young leaves are ideal explants because they are healthy, non-sclerosing, rich in nutrients, and contain higher levels of endogenous hormones.

In *Momordica cochinchinensis* (Lour.) Spreng, the results showed that the transverse thin cell layers produced hard, yellow-green callus with higher dry biomass than callus produced from the vertical thin cell layers, suitable for cell suspension cultures. (Nguyen et al., 2017).

3.5. Light condition

Light conditions also greatly affect the process of callus formation. There are types of explants that can form callii in both dark and light conditions. But in many plant species, their explants only form callus under certain conditions. In addition to affecting the formation and growth of callus cultured *in vitro*, light also affects the biosynthesis of secondary compounds in cells by involving the activity of some endogenous enzymes (Tien et al., 2010). The color of callus is caused by pigments, nutrients, and light. The yellow-green or green callus is formed when KIN is added, 2,4-D interacts with KIN (cytokinin), because cytokinin has the main function of forming chlorophyll in the callus and when the sample is illuminated. Thus, the color change in the callus from white to green was due to chlorophyll formation (Sari et al., 2018).

In *Barringtonia racemosa*, callus can be formed in both dark and light conditions (16 h light). However, the lycopene content obtained from callus placed in the light was higher than that from callus placed in the dark. Therefore, to obtain lycopene, the sample should

be placed under lighting conditions (Behbahani et al., 2011). Similarly, in the survey results on callus formation and regeneration of *Nicotiana tabacum* L., Siddique & Islam (2015) reported that under white light, callus formed faster than in the dark with higher biomass and green callus. According to the authors, when exposed to light, cells contain photosynthetic pigments, so they can autotrophy. Therefore, the callus may produce carbohydrates and necessary metabolites for their growth.

However, one or more substances required for cell division may also be reduced in levels by light exposure (Yeoman & Davidson., 1971). In addition, light can block the activity of enzymes involved in cell growth (Tien et al., 2010). When explants are placed in the light, the volume of callus may be higher, but browning by polyphenoloxidase enzymes, and callus necrosis may also be higher than that in the dark (Afshari et al., 2011).

For example, in *Saccharum officinarium* L., the rate of callus formation gradually decreased with increasing light intensity (from dark to 5000 lux). Initial samples were cultured in complete darkness, retaining their original cream color for a long time while those cultured under light turned blue over time. Thus, better callus growth was observed in the explants initially incubated in the dark compared with those incubated under low light intensity (between 1000 and 2000 lux). However, high light conditions (from 3000 to 5000 lux) are not favorable for callus initiation. This may be because chlorophyll synthesis reduces callus formation in leaf sheath explants (Sengar et al., 2011).

3.6. Callus line

The *in vitro* plant cell system is a heterogeneous population in which physiological features of plant cell types are different (Mulabagal & Tsay, 2004). Cell line selection may be based on genotype, cell morphology, and structure, growth indices, or ability to metabolize secondary compounds.

In 2010, Tan et al. selected the callus line of *Centella asiatica* L. urban, as a source of raw materials for cell suspension culture to obtain flavonoids through the results of biomass yield and hydrolysed flavonoid content of four different types, based on their morphological characteristics.

In callus culture of *Kaempferia galanga*, cell line selection was based on growth index across subculture cycles. Fast growing lines are lines with a growth index above 2.0, medium growing lines have a growth index from 1.5 to 2.0, while slow growing lines have a growth index lower than 1.5. Callus derived from the roots, leaf sheaths, and rhizomes of this plant made up 26 cell lines when they were induced and maintained on MS medium added with 1mg/L 2, 4-D, 30g/L sucrose, 7.7g/L agar, and inoculated after 21 days and for six subculture cycles. From these 26 cell lines, only 8 cell lines can be selected as stable lines with fast, medium, and slow growth. Among the tested samples, the leaf sheaths gave the most stable and fastest-growing cell lines. Well-developed cell lines can be used as a source of material for cell suspension cultures of *Kaempferia galanga* (Kuen et al., 2011). Similarly, in *Artemisia annua* L., callus cell lines were obtained from leaf samples of five types, grown at two different locations in Vietnam. Thirty-four callus lines were divided into groups based on the growth index. Callus texture also influenced cell growth of *Artemisia annua* L., in which the friable callus produced faster biomass, and more stable cell, suitable for cell suspension culture (Jin & Keng, 2013).

To select betalain-producing callus lines from different varieties of *Chenopodium quinoa* Willd, callus was stained with a fluorescent dye (DAPI) and observed under fluorescence microscopy. Pigments were also analyzed by HPLC-DAD and ESI-MS/MS to

specifically characterize individual pigments in cell lines (Henarejos-Escudero et al., 2018). Next, in the callus culture of *Sorghum bicolor* L. Moench varieties, good cell lines are selected to establish a cell suspension culture system based on variety, structure, biomass, and cell size when viewed under a microscope. (Ramulifho et al., 2019).

4. Conclusion

Callus can form from different types of explants of different plant species on special culture media. Many factors affect the culturing process, including chemical and physical factors. In which, factors such as explants, lighting conditions, plant growth regulators, carbon sources, and mineral environment are often considered to select the optimal environment for callus growth.

Therefore, for each study on callus formation in plants, it is necessary to carefully investigate the culture conditions to produce the appropriate type of callus for the research purpose.

References

Afshari, R. T., Angoshtari, R., & Kalantari, S. (2011). Effects of light and different plant growth regulators on induction of callus growth in rapeseed (*Brassica napus* L.) genotypes. *Plant Omics Journal, 4*(2), 60-67.

Alina, T., Magdalena, J., & Andrzej, T. (2006). The effect of carbon source on callus induction and regeneration ability in *Pharbitis nil*. *Acta Physiologiae Plantarum*, *28*(6), 619-626.

Amiri, S., & Kazemitabar, S. K. (2011). Enhancement of callus induction and regeneration efficiency from embryo cultures of *Datura stramonium* by adjusting carbon sources and concentrations. *African Journal of Biotechnology*, *10*(50), 10101-10107.

André, S. B., Mongomaké, K., Modeste, K. K., Edmond, K. K., Tchoa, K., Hilaire, K. T., & Justin, K. Y. (2015). Effects of plant growth regulators and carbohydrates on callus induction and proliferation from leaf explant of *Lippia multiflora* Moldenke (Verbenacea). *International Journal of Agriculture and Crop Sciences*, *8*(2), 118.

Anusha, T. S., Joseph, M. V., & Elyas, K. K. (2016). Callus induction and elicitation of total phenolics in callus cell suspension culture of *Celastrus paniculatus*–willd, an endangered medicinal plant in India. *Pharmacognosy Journal*, *8*(5), 471-475.

Babich, O., Sukhikh, S., Pungin, A., Astahova, L., Chupakhin, E., Belova, D., & Ivanova, S. (2021). Evaluation of the conditions for the cultivation of callus cultures of *Hyssopus officinalis* regarding the yield of polyphenolic compounds. *Plants*, *10*(5), 915.

Behbahani, M., Shanehsazzadeh, M., & Hessami, M. J. (2011). Optimization of callus and cell suspension cultures of *Barringtonia racemosa* (Lecythidaceae family) for lycopene production. *Scientia Agricola , 68*(1), 69-76.

Bhatia, S., Sharma, K., Dahiya, R., & Bera, T. (2015). *Modern applications of plant biotechnology in pharmaceutical sciences*. Academic Press.

Binte Mostafiz, S., & Wagiran, A. (2018). Efficient callus induction and regeneration in selected indica rice. *Agronomy*, *8*(5), 77.

Bosila, H. A., Mohamed, S., El-Gamal, S., & Bekhit, M. (2001). Factors affecting callus production and glycosidal content of leaf tissue culture of *Digitalis lanata* Ehrh. In *International Conference on Medicinal and Aromatic Plants (Part II) 597*, 289-301.

Cuong, L. K., hien, H. X., Nam, N. B., Huong, T. T., & Nhut, D. T. (2012). Effects of some factors on friable callus induction and cell suspension culture of *Panax Vietnamensis* Ha et Grushv. *Academia Journal of Biology*, *34*(3se), 265-276.

Henarejos-Escudero, P., Guadarrama-Flores, B., Guerrero-Rubio, M. A., Gómez-Pando, L. R., García-Carmona, F., & Gandía-Herrero, F. (2018). Development of betalain producing callus lines from colored quinoa varieties (*Chenopodium quinoa* Willd). *Journal of agricultural and food chemistry*, *66*, 467-474.

Hussain,A., Qarshi, I. A., Nazir, H., & Ullah, I. (2012). Plant tissue culture: Current ttatus and opportunities. *Recent Advances in Plant in vitro Culture*, 1-28. Intechopen.

Ikeuchi, M., Sugimoto, K., & Iwase, A. (2013). Plant callus: mechanisms of induction and repression. *The plant cell*, *25*(9), 3159-3173.

Jin, C. S. & Keng, C. L. (2013). Factors affecting the selection of callus cell lines and the preparation of the cell suspension culture of *Artemisia annua* L. *Plant Tissue Culture and Biotechnology, 23*(2), 157-163.

Keng, C. L., See, K. S., Hoon, L. P., & Lim, B. P. (2008). Effect of plant growth regulators and subculture frequency on callus culture and the establishment of *Melastoma malabathricum* cell suspension cultures for the production of pigments. *Biotechnology, 7* (4), 678-685.

Kieber, J. J., & Schaller, G. E. (2018). Cytokinin signaling in plant development. *Development*, *145*(4), 149344.

Kuen, T. G., Khaladalla, M. M., Bhatt, A., & Keng, C. L. (2011). Callus induction and cell line establishment from various explants of *Kaempferia galanga*. *Int J Curr Res*, *3*, 1-4.

Kumar, G. P., Subiramani, S., Govindarajan, S., Sadasivam, V., Manickam, V., Mogilicherla, K., & Narayanasamy, J. (2015). Evaluation of different carbon sources for high frequency callus culture with reduced phenolic secretion in cotton (*Gossypium hirsutum* L.) cv. SVPR-2. *Biotechnology Reports, 7*, 72-80.

Le, T. T. T., Bui, V. T., & Nguyen, L. D. (2010). Using biotest to estimate taxol production from cell suspension culture of *Taxus wallichiana* Zucc. in different conditions of culture. *Science and Technology Development Journal*, *13*(3), 67-77.

Liu, J., Feng, H., Ma, Y., Zhang, L., Han, H., & Huang, H. (2018). Effects of different plant hormones on callus induction and plant regeneration of miniature roses (*Rosa hybrida* L.). *Horticult Int J*, *2*(4), 201-206.

Luong, N. D. & L. T. T. Tien. 2006. *Cell technology*. National University, Ho Chi Minh City, Viet Nam. [In Vietnamese].

Majda, M., & Robert, S. (2018). The role of auxin in cell wall expansion. *International journal of molecular sciences*, *19*(4), 951.

Martinez, M. E., Jorquera, L., Poirrier, P., Díaz, K., & Chamy, R. (2021). Effect of the carbon source and plant growth regulators (PGRs) in the induction and maintenance of an in vitro callus culture of *Taraxacum officinale* (L) Weber Ex FH Wigg. *Agronomy*, *11*(6), 1181.

Mastuti, R., Munawarti, A., & Firdiana, E. R. (2017). The combination effect of auxin and cytokinin on in vitro callus formation of *Physalis angulata L.*–A medicinal plant. In *AIP Conference Proceedings* (Vol.1908, No. 1, p. 040007). AIP Publishing LLC.

Mulabagal, V., & Tsay, H. S. (2004). Plant cell cultures-an alternative and efficient source for the production of biologically important secondary metabolites. *International journal of applied science and engineering, 2*(1), 29-48.

Nhut, D. T., Ha, T. T. T., Huong, T. T., Cuong, H. V., & Huy, N. P. (2012). Study the formation of callus and single cell of Kiwi (*Actinidia deliciosa*). *Academia Journal of Biology*, *34*(4), 505-514.

Nguyen, C. T., Bui, M. T., & Pham, D. T. (2017). Influence of 2.4D and thin cell layer types on callus induction of Momordica cochinchinensis (Lour.) Spreng, *Vietnam Journal of Science and Technology*, *16*(5), 22-26.

Niedz, R. P., & Evens, T. J. (2007). Regulating plant tissue growth by mineral nutrition. *In Vitro Cellular & Developmental Biology-Plant*, *43*(4), 370-381.

Petersen, M. & Alfermann, A. W. (1993). Plant cell cultures. In H. J. Rehm, & G. Reed, *Biotechnology: Biological Fundamentals* (pp. 577 - 614).

Rafiq, M., Fatima, T., Husnain, T., Bashir, K., & Riazuddin, S. (2005). Effect of different media on callus formation and regeneration of different genotypes of maize (*Zea mays* L.). *Plant Tissue Cult, 15*, 57-65.

Ramulifho, E., Goche, T., Van As, J., Tsilo, T. J., Chivasa, S., & Ngara, R. (2019). Establishment and characterization of callus and cell suspension cultures of selected *Sorghum bicolor* (L.) Moench varieties: A resource for gene discovery in plant stress biology. *Agronomy, 9*(5), 218.

Repalli, S. K., Geda, C. K., Pradhan, N. S. N., & Rao, G. J. N. (2019). Influence of media type and carbon source on callus induction and regeneration response of different indica rice genotypes. *Adv Crop Sci Tech*, *7*(3), 425.

Sagharyan, M., Ganjeali, A., Cheniany, M., & Kouhi, S. M. M. (2020). Optimization of callus induction with enhancing production of phenolic compounds production and antioxidants activity in callus cultures of *Nepeta binaloudensis* Jamzad (Lamiaceae). *Iranian Journal of Biotechnology*, *18*(4), e2621.

Sari, Y. P., Kusumawati, E., Saleh, C., Kustiawan, W., & Sukartingsih, S. (2018). Effect of sucrose and plant growth regulators on callogenesis and preliminary secondary metabolic of different explant *Myrmecodia tuberosa*. *Nusantara Bioscience*, *10*(3), 183-192.

Sengar, K., Sengar, R. S., & Garg, S. K. (2011). The effect of in-vitro environmental conditions on some sugarcane varieties for micropropagation. *African Journal of Biotechnology*, *10*(75), 17122-17126.

Siddique, A. B., & Islam, S. S. (2015).Effect of light and dark on callus induction and regeneration in tobacco (*Nicotiana tabacum* L.). *Bangladesh Journal of Botany, 44*(4), 643-651.

Smith, R. H. (2001). *Plant Cell Culture*. In: eLS. John Wiley & Sons Ltd, Chichester.

Tan, S., Radzali, M., Arbakariya, A., & Mahmood, M. (2010). Effect of plant growth regulators on callus, cell suspension and cell line selection for flavonoid production from pegaga (*Centella asiatica* L. urban). *American Journal of Biochemistry and Biotechnology, 6*(4), 284-299.

Thorpe, T. A. (2007). History of plant tissue culture. *Molecular Biotechnology , 37*(2), 169-180.

Uchida, R. (2000). Essential nutrients for plant growth: nutrient functions and deficiency symptoms. *Plant nutrient management in Hawaii's soils*, 4, 31-55.

Ullah, H., Ullah, I., Jadoon, S. A., & Rashid, H. (2007). Tissue culture techniques for callus induction in rice. *Sarhad Journal of Agriculture*, *23*(1), 81-86.

Vasic, D., Pajevic, S., Saric, M., Vasiljevic, L., & Skoric, D. (2001). Concentration of mineral elements in callus tissue culture of some sunflower inbred lines. *Journal of plant nutrition*, *24*(12), 1987-1994.

Yeoman, M. M., & Davidson, A. W. (1971). Effect of light on cell division in developing callus cultures. *Annals of Botany, 35*, 1085-1000.

Zeng, F. S., Wang, W. W., Zhan, Y. G. & Xin, Y. (2009). Establishment of the callus and cell suspension culture of *Elaeagnus angustifolia* for the production of condensed tannins. *African Journal of Biotechnology , 8*(19), 5005-5010.