International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 11, Issue 12-2024

Review Article

DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.12.001

Histological and Microscopic study in plant tissue culture: A Review

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Abstract

Combining conventional breeding with *in vitro* culture techniques, supported by histological and microscopic analysis, enhances plant traits, conserves endangered species, and aids in producing valuable compounds in economically important plants. These methods offer essential insights into cellular processes, guiding plant biotechnology research.

In multicellular plants, traditional breeding with *in vitro* culture techniques enhances crop traits and conserves rare medicinal plants. Calluses, masses of undifferentiated cells play a key role in tissue culture regeneration. Histological analysis, which examines tissue under a microscope, is crucial for understanding cellular processes during embryogenesis, callus formation, and regeneration.

Microscopic techniques, like scanning and transmission electron microscopy, reveal cellular structures and organelles, advancing plant tissue culture technology and aiding species conservation and compound production. Histological studies also reveal structural changes during tissue culture, optimizing culture conditions. Somatic embryogenesis, through direct or indirect methods, offers unique advantages for propagation and biotechnology. This review encourages further use of histological techniques to improve tissue culture applications for societal benefit.

Keywords: Histology, Plant tissue culture, Microscopic, Callus culture

Introduction

Histological techniques are essential for examining tissue structures related to cell biology, anatomy, and physiology, focusing on groups of cells that arise from cell divisions (Karabiyik & Sen, 2023). Microscopy including electron microscopy has advanced *in vitro* plant culture studies providing detailed views of plant tissues and cellular processes. The SEM and TEM allow high-resolution examination of plant cell internal structures and organelles, enhancing knowledge of biological mechanisms in plant cells. (Oluf *et al.,* 1995). Histological studies reveal structural changes during somatic embryogenesis and other developmental events, aiding research on cellular processes. Plant regeneration in tissue culture involves direct and indirect organogenesis, with histological analyses clarifying regeneration patterns and guiding further research. These insights are crucial for advancing callus regeneration understanding (Kruglova *et al.,* 2023).

Micropropagation is pivotal in preserving endangered genotypes and advancing plant tissue culture. Microscopy, paired with *in vitro* culture technology enables significant progress in plant growth and development studies (Moyo *et al.,* 2015). For instance, in *Rubus adenotrichos* (blackberry) optical and TEM techniques helped analyze callus induction and cell suspensions to improve phenolic compound production (Duran *et al.,* 2016).

Somatic embryogenesis, where a somatic cell forms an embryonic stem cell under specific conditions, is vital for cloning, synthetic seed production, and conservation.This process passes through developmental stages (globular, heart, torpedo, and cotyledonary) before forming plants (Guan *et al.,* 2016).

The present review is an overview of the application and importance of microscopic and histological techniques in plant tissue culture. Table 1 presents the application of the technique for various objective factors by further detailing.

Table 1: Review of Histological and Microscopic study of callus in plant tissue culture:

No.	Name of Plant	PGR	Explant	Type of Study	Observations	References
1	*Akebia trifoliata* (Akebia quinate)	BA	Root	M	Confirmed the development process of SE via the globular, heart, torpedo, and cotyledonal stages.	Zou, *et al.,* 2019
				H	Observed the SE directly initiating from the root of epidermal cells. Vascular connections between the SE and material tissue.	

2	*Taraxacum belorussicum*	IAA TDZ PSK	Cotyledons, hypocotyls, meristems, Roots.	H	Confirmed indirect organogenesis, somatic embryogenesis and the high morphogenetic ability of *T. belorussicum.*	Galuszka, *et al.,* 2019
3	*Swietenia macrophylla* (Mahogany)	Kinetin, BAP, 2,4-D	seed	H	Embryo development from globular-shaped SE to the cotyledonary stage was confirmed.	Arias, *et al.,* 2019
4	*Lepianthes umbellata* (Wild pepper)	IBA	Leaf Shoot bud	H	Showed indirect organogenesis in which embryoids and shoot buds initiated from the callus masses and cytological variations in callus cultures.	Manasa, *et al.,* 2019
5	*Acrocomia aculeata* (Macaw palm)	FAA	Leaf	H	Observed starch accumulation only in the adjacencies, undergoing intense cell division.	Meira, *et al.,*2019
6	*Elaeis guineensis* (Oil palm)	BAP,	Root	H	Observed indirect somatic embryogenesis induced to test different growth regulators from the root explant.	Vilela, *et al.,*2019
				M	Callus formation on. 1mg.L-1 picloram-containing media, exhibited cells with embryogenic characteristics	

7	*Ascochyta rabiei* (Phoma rabies)	NAA, BAP	Seeds	H	Comparative analysis of two cultivars showing two different reactions.	Kadiri, *et al.,*2019
8	*Nigella damascene L.* (Love-in-a-mist)	NAA, Kinetin BAP	Seed	H	Development of embryo from callus tissue on the hormone-free medium.	Chodacka, *et al.,* 2020
9	*Vernonia condensata*	BAP, NAA	Leaf	H	Formation of friable calli on 4.44 µM BAP associated with a larger % of calli with embryogenic masses.	Rodrigues, *et al.,* 2020
10	*Ficus carica* L.cv. Blackjack	BAP, 2-iP	Bud	H	Histological analysis proved that the adaxial surface obtained a better response for callus induction on the leaf.	Han, *et al.,* 2020
				M	SEM analysis confirmed that no structural distortion was caused by cytokinin.	
11	*Echinacea purpurea* (purple Coneflower)	BA NAA	Seed	H	It is found that the somatic embryogenesis of *E. purpurea* occurs at high levels of BA and low levels of NAA with the addition of coconut milk and hydrolysate.	Ardakani, *et al.*, 2020

12	*Polianthes tuberosa* (Tuberosa)	BAP, NAA 2,4-D	Root	H	Histological study of *in vitro* nematode infected tuberose roots. This *in vitro* method enables the production of disease-free plantlets for mass propagation.	Singh, *et al.,* 2020
13	*Plinia peruviana* (Jaboticaba)	2,4-D BAP	Seed Leaf	H	The calluses that were formed from leaf discs showed non-embryogenic characteristics.	Silveira, *et al.,* 2020
14	*Actinidia deliciosa* (Kiwifruit)	TDZ	Seed	H	Showed cells in the bulgy and ball-shaped structures arranged regularly, with visible intercellular spaces.	Czernicka, *et al.,*2021
				M	Globular cells of various sizes were seen on the surface of the organogenic callus with the deposition of phenols. SEM observed the more or less closely attached cells and a membranous layer. Long-term culturing of the callus's shapeless part past and the protuberances.	
15	*Vanda Tricolor* (Orchid)	BAP & NAA	Leaf basal	H	Demonstrated the progression of the pro-embryo into a mature embryo.SE development began with embryogenic callus, followed by the globular phase at 10 days of culture.	Hardjo *et al.,*2021
16	*Asparagus cochinchinesis* (Chinese Asparagus)	BAP, NAA, & Kinetin	Leaf	H	Indicated the existence of crystal idioblasts in young leaves was the onset of leaf differentiation.	Kim, *et al.,*2021

17	*Phaseolus vulgaris L.* (Bean)	BAP, 2,4-D, NAA	Plumule, radicle, cotyledon embryo,	M	It was found that the callus plumule and radicle were fibrous due to loose parenchyma cell arrangement, while the callus from cotyledon and embryo was more rigid. Callus from plumule and radicle showed root and shoot induction after over thirty days of incubation.	Besra, Basak 2021
18	*Yucca Gloriosa Variegata* (Palm Lily)	TZN	Leaf	H	Evident the saponins distribution in intact plants and different developmental stages of culture parts were varied.	Ansam, *et al.,*2021
19	*Oryza sativa* (Rice)	2,4-D	Seed	H	Observed different developmental stages showed two types of cells. The inner cell layer consisted of small, dense cytoplasm isodiametric cells with active metabolism resembling meristematic cells, and large cells with a small nucleus and large vacuole. The center of the calli contained large cells with starch granules and abundant intercellular spaces on embryogenic calli.	Mesbah, *et al.,*2021
20	*Dioscorea nipponica* Makino	BA+NAA	Seed, leaf, root.	H	Confirmed indirect shoot regeneration of *D. nipponica*. Features of the stem were observed in the cross-section of the initial explant, including, the epidermis, collenchyma, sclerenchyma, and vascular bundles scattered throughout the parenchyma cells.	Dang, *et al.,*2022

21	*Biancaea sappan* (Sapanwood)	2,4-D BAP IBA	Seed	H	Revealed round parenchymatous cells stained with safranin, containing polyphenols, and transitioning into shoot primordia over several weeks.	Ebbie, *et al.,*2022
22	*Asteracantha longifolia Nees* (Talamkhana)	NAA BA 2,4-D	Plant twigs, nodes	H	The organogenic potential of compact, green callus was confirmed, with more meristematic regions seen after 45 days on MS medium with NAA 0.5 mg/l and BA 2.0 mg/l. Vascular nodules indicated shoot meristem formation, confirming the effect of growth regulators on plant regeneration.	Kumar & Nandi. 2022
23	*Artemisia maritima* L. (Old women)	BAP NAA	Stem, Leaf	H	Observed somatic embryos developed into globular, heart-shaped, and bipolar stages on BAP and NAA.	Nabi, *et al.,*2022
24	*Coffea arabica* L.	2,4-D TZN BAP	Leaf	H	Confirmed that the callus exhibits a friable and embryogenic texture and initiates the development of various stages of SE formation.	Arimarsetiowati, *et al.,*2022
25	*Sorghum bicolor* L. (Sweet sorghum)	2,4-D NAA Kinetin	Shoot	H	Analysis of sweet sorghum embryogenic calli revealed the development of embryogenic shoots.	Yadala, *et al.,*2022

26	*Digitalis purpurea* L.	BAP 2,4-D	Seed Shoot hypocotyl	M	It is carried out to determine the origin of somatic embryos from hypocotyl calli. The SEM revealed the presence of different stages of SE. (globular and heart-shaped) on callus surfaces.	Bansal, *et al.,* 2022
27	*Anaphyllum wightii Schott*	2, 4-D NAA	Petiole	H	Observed the embryogenic callus revealed various stages of SE indicating an asynchronous type of embryogenesis.	Lekshmi & Swapna 2022
28	*Hancornia speciosa gomes* (Mangabeira)	2,4-D BAP	Leaf, Node	H	Cells in dedifferentiation were observed including maturation of SE.	Santana, *et al.,*2023
29	*Prunus persica* L (Peach)	ZT, 2,4-D+IAA BA+IBA	Anthers, Buds	H	The callus cells varied in size and shape observed with	Yang, *et al.,* 2023
				M	Nuclei containing intact membrane and uniform chromatin.	
30	*Glycine max* (Soybean)	TDZ, AgNO3, IBA	Each part	H	It revealed that leafy shoot-like structures originated from the cortical region of hypocotyl segments confirming a phenomenon of direct shoot regeneration.	Patel, *et al.,* 2023
31	*Guizotica abyssinica L.* (Niger)	IBA, 2,4-D, KN	Seed	M	It confirmed the ploidy level of regenerants as compared to diploid control.	Maurya, *et al.,* 2023

32	*Manihot esculenta crantz* (Cassava)	BA MS+CuSO4 2 Um	ILLs ABs	H	A 2-week-old callus shows the meristematic area where small cells are densely packed and also found that the cells actively divided to appear dense and dark.	Fariroh, *et al.,* 2023
				M	The Kaspro and Kuning cultivars, a distinctive embryogenic spherical shape and a densely packed structure containing dense cells were observed.	
33	*Nigella sativa* L. (Black cumin)	IBA NAA	Seed Hypocotyl cotyledon	H	It showed the ideal developmental stages of SE. (i.e., spherical, heart, torpedo-shaped, and cotyledonary-stage embryos).	Higazy, *et al.,* 2023
34	*Camelina*	IAA BAP	IZEs	H	It revealed the formation of adventitious shoots, which were initiated by locally activated cell division and proliferation in the epidermis and the outer cortex of the hypocotyl.	Rezaeva, *et al.,* 2024
35	*Coffea arabica* L.	2,4-D 2-iP	Leaf	H	Uncovered the progression of different stages and confirmed the critical role of auxin and cytokinins in initiating SE.	Ibrahim, *et al., 2*024
36	*Sorghum bicolor*	2,4-D	Seed	H	Revealed root organogenesis from type 1 and SE from type 2. Differentiation was evident in meristematic cells with intense cytoplasmic staining and distinct vascular elements. Globular SE was observed.	Wu, et al.,2024

(H: Histological, M: Microscopic)

Callus induction

Callus induction is a critical initial step in plant tissue culture and is essential for embryogenesis and shoot regeneration. Histological studies on various plants have shown that specific growth regulators and explant types significantly influence callus formation. In *Nigella damascena*, effective callus induction was achieved across multiple media and explants, with cotyledons producing a higher callus frequency than hypocotyls. Callus formation was greater in media with synergistic combinations of BAP and NAA than with Kinetin and NAA. In *Ficus carica*, an ornamental plant, the best callus response occurred on the adaxial leaf surface in media containing BAP and 2-iP (Chodacka *et al.,* 2020). Similarly, in *Asteracantha longifolia,* the combination of NAA (0.5 mg/l) and BAP (0.25 mg/l) increased callus formation, outperforming other growth regulator treatments (Kumar & Nandi, 2022).

Histological analysis of the *Coffea arabica* AS2K clone showed that calluses exhibited a friable, embryogenic texture that initiated somatic embryogenesis (Arimarsetiowati, *et al.,* 2022). *Polianthes tuberosa* showed callus initiation from leaf edges two weeks post-inoculation on MS media with 2,4-D, BAP, and NAA, achieving high callus production (Singh, *et al.,* 2020).

In *Dioscorea nipponica,* callus formation varied across explants, with seeds and rhizomes showing high frequency, while leaves rarely formed callus. Paraffin section analysis identified three callus types, with Callus III showing the highest cell differentiation and bud formation ability, highlighting indirect organogenesis as a primary morphogenesis route (Dang *et al.,* 2022).

Comparisons of callus formation from different explants

Callus formation refers to an unstructured cluster of cells that originates from plant tissue explants. In *Dioscorea nipponica* callus derived from seeds, stem segments, and rhizomes exhibited diverse differentiation capabilities, and it was observed by microscopic observation that the callus formation underwent three stages: Initial, mitotic, and formation (Induction, proliferation, and differentiation).

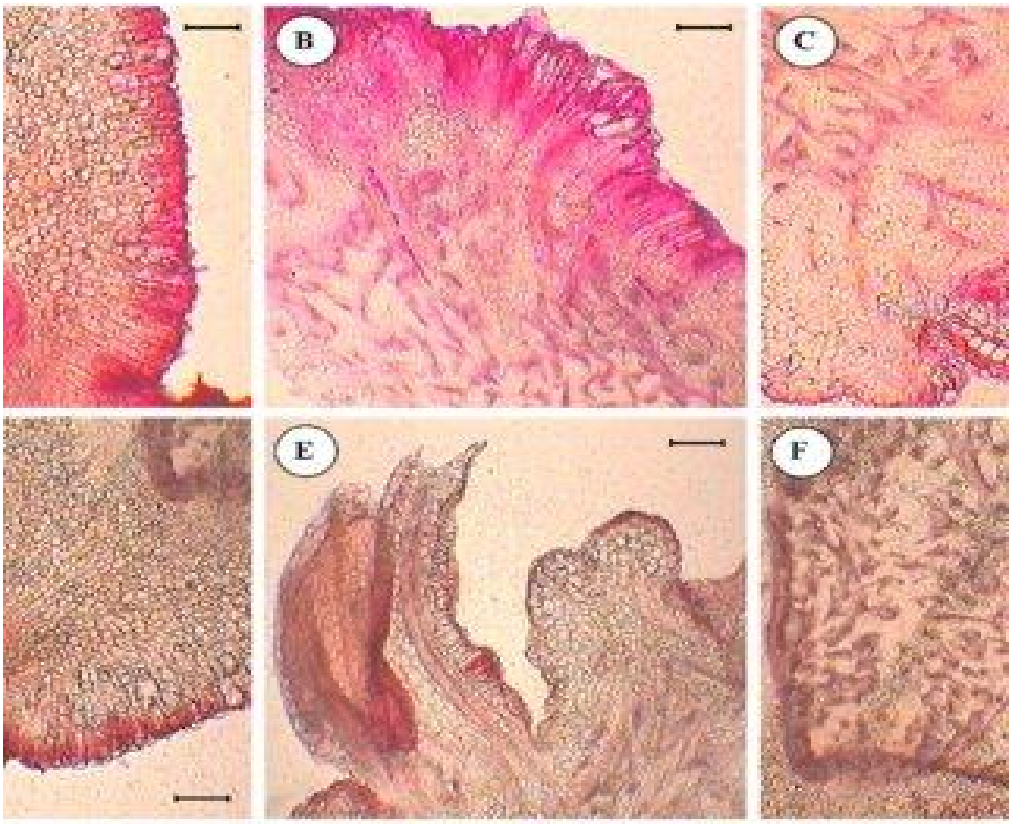
Differentiated callus was observed such as callus type I, which was more vascular tissue nodules of tightly arranged cells, the callus contained numerous tracheids and clusters of gathered meristem cells within its structure. In type II, larger parenchyma cells with small nuclei were evenly distributed and arranged in layers, although fewer vascular tissue nodules were observed within the callus, and in type III, the cells were densely packed with minimal interstitial substance and exhibited well–developed tracheids. Meristematic cells formed clusters both on the surface and within the interior of the callus (Dang, *et al.,* 2022).

Histological Features of Primary Morphogenic Calli under Microscope

Research experiments have demonstrated that *in vitro* callus formation is determined by the intricate interaction between internal and external factors. Current research in this field focuses on gene regulatory networks and epigenetic factors that influence callus formation *in vitro*, with studies often involving specific mutants of *Arabidopsis thaliana*. Histologically, the presence of morphogenetically competent cells within explant tissues is considered a key internal factor in this process. These cells can be referred to as "initial callus cells." Exogenous factors, primarily hormones in the induction medium and stress factors such as wounding, trigger the reprogramming of these cells toward morphogenesis *in vitro* callus formation. The principal issue is this: do the initial cells already have the competence to form calli under planta conditions, or is it the conditions of *in vitro* cultivation that stimulate their reprogramming by gaining competence.

Under ideal *in vitro* conditions, explant cells have the potential to develop into primary morphogenic calli. The morphological characteristics of calli that form on the surfaces of various explants and

are capable of undergoing morphogenesis during subsequent *in vitro* cultivation are quite similar across many plant species. These calli typically appear as compact, nodular structures (Mishra, *et al.,* 2015) (Figure 1(1).



(Courtesy: Tanmayee, Goyal, & Sen. (2015)).

Figure 1. Stages of *in vitro* morphogenesis in calli. Histology of various stages of embryogenesis observed under light microscope (A) Embryonic axis; (B) & (C) Region of tissue differentiation; (D) Region of embryo induction; (E) Region of shoot primordia; (F) Part of the embryonic axis. Scale = 20 µm.

Histological Method for Evaluating in vitro Morphogenesis Pathways in Calli

Morphogenesis is a biological process that shapes tissues or organs by regulating the spatial arrangement of cells during embryonic development. Morphogenesis pathways facilitate the regeneration of fully mature plants when provided with optimal *in vitro* conditions. The regeneration of plants from calli is described as "de novo plant regeneration" (Wan, *et al.,* 2023), "*in vitro* plant regeneration" (Lee, *et al.,* 2022), or "indirect plant regeneration" (Neves, *et al.,* 2021).

Different histological analyses showed unique morphogenesis pathways in calli during the later stages of *in vitro* cultivation.

Organogenesis: (callogenesis type, involving the formation and development of shoots; rhizogenesis type, involving the formation and development of roots; or gemmorhizogenesis type, involving the formation and development of a structure that combines both shoots and roots).

In *Taraxacum belorussicum* the vigor of the morphogenetic response of preferred types of explants of *Taraxacum belorussicum* was expressed as the intermediate number of adventitious shoots produced during *in vitro* culture. The anecdotal presence of somatic embryogenesis was not approved for its proper quantification.

During histological observation, it was observed that hypocotyl and meristem explants showed superior morphogenetic responses compared to cotyledon and root explants after 20 and 45 days of culture on ½ MS medium supplemented with (IAA, TDZ, and PSK). Adventitious shoots of *Taraxacum belorussicum* were detected on the surfaces of all explant types, and their regeneration efficiency was evaluated accordingly.

Somatic Embryogenesis (Involving the generation and progression of somatic embryos.)

Callus cells that have been reprogrammed display pluripotency and totipotency characteristics when provided with suitable *in vitro* conditions. Scientists have focused significant attention on *in vitro* somatic embryogenesis as a technique for plant regeneration in calli. Somatic embryogenesis is a synthetic method in which a plant or embryo is produced from a single somatic cell. This process involves the formation of somatic embryos from plant cells that do not usually participate in embryo development under normal conditions. Somatic embryogenesis can be categorized into two forms: direct somatic embryogenesis and indirect somatic embryogenesis. Direct somatic embryogenesis involves the generation of embryos directly from explant cells, such as those from immature embryos, where embryogenic callus is generated from explants as an intermediary step of callus formation before somatic embryo development takes place. This process is commonly used in plant propagation, genetic transformation, and biotechnology applications (Rose & Song 2019).

In *Akebia trifoliata* microscopic observation showed that multicellular embryogenic protuberances ascended directly from the surface of the maternal root apex. The microscopic analysis confirmed the development process of SE via the globular, heart, torpedo, and cotyledonal stages with two cotyledon-like structures and one root pole that are similar to the zygotic embryos of the plant, and also histological observation observed of SE directly initiating from the root of epidermal cells, and vascular connections between the SE and material tissue (Zou, *et al.,* 2019).

Microscopic and histological examination of *Taraxacum belorussicum* validated its strong morphogenetic capacity, with indications of somatic embryogenesis (SE) detected in hypocotyl and cotyledon explants, the greatest efficiency of somatic embryogenesis, manifested as small, light-green globular structures, was observed on ½ MS medium enriched with TDZ, IAA, and PSK. The organogenesis ¯analysis confirmed the presence of two types of callus tissue the first type comprised loosely distributed cells of various shapes with intercellular spaces and the second type exhibited a compact structure with meristematic centers giving rise to adventitious shoots, and the SE/PSK (phytosulfokine) model/long darkness histological observation of the cotyledon explants confirmed that indirect organogenesis and indirect somatic embryogenesis and revealed structures similar to somatic embryos formed from explant tissue overgrown with endogenous callus, and histological analysis of hypocotyl explant revealed changes in the axial roller and parenchyma of the primary cortex cells and endogenous callus featuring sizable intercellular spaces was also observed within the primary cortex (Galuszka, *et al.,* 2019).

In *Elaeis guineensis,* histological analysis of indirect somatic embryogenesis induced to test different growth regulators from the root and microscopic treatments showed callus formation 1mg.L-1 picloram exhibited cells with embryogenic characteristics that developed somatic embryos (Vilela, *et al.,*2019). *Echinacea purpurea* is a popular plant valued for both its medicinal and ornamental purposes.

Embryogenesis occurs indirectly through the formation of the callus. The calli were categorized into three unique types: undifferentiated, embryogenic, and organogenic. The embryogenic calli were dark green, cohesive, and exhibited a faster growth rate. The media with 3 mg L^{-1} BA+0.1 and 0.2 mg L^{-1} NAA + 1 g. L^{-1} combined activated charcoal, coconut milk, and casein hydrolysate exhibited the highest chlorophyll content and growth characteristics in acclimatized plantlets and histological analysis verified somatic embryogenesis in *Echinacea purpurea* was found to occur at high concentrations of BA and low concentrations of NAA, combined with the inclusion of coconut milk and casein hydrolysate. (Ardakani, *et al*., 2020). Histological and SEM analysis of somatic embryos in *Swietenia macrophylla* revealed that the embryos encased within the callus lacked a visible vascular link to the parent tissue. Globular-shaped somatic embryos were brighter and lighter in color compared to the callus. Globular structures connected to the original callus were observed using SEM. Heart-shaped embryos exhibited a characteristic notch at the apex and another on the surface of the structure. Heart-stage embryos elongated and developed into somatic embryos at the torpedo stage and exhibited a well-defined procambium as they progressed into torpedo-shaped structures (Arias, *et al.,* 2019). In *Lepianthes umbellate* histological investigations revealed that embryoids and shoot buds initiate from the callus masses, i.e. indirect organogenesis (Manasa, *et al.,* 2019). In *Vanda Tricolor* (Orchid) observed demonstrated the progression of the pro-embryo into a mature embryo.SE development began with embryogenic callus, followed by the globular phase at 10 days of culture,

The optimal medium for inducing SEs from embryogenic calli is half-strength MS medium supplemented with 0.01 mg L^{-1} NAA and 0.05 mg L^{-1} BAP (Hardjo *et al.,* 2021). In *Artemisia maritima,* somatic embryos progressed through globular, heart-shaped, and bipolar plantlet stages when cultured with BAP and NAA, as confirmed by SEM and histological analyses (Nabi, *et al.,* 2022). In *Sorghum bicolor* for effective regeneration and transformation methodology for sweet sorghum *in vitro* culture from immature embryo callus was developed. Histological examinations of sweet sorghum embryogenic calli elucidated the progression of embryogenic shoots (Yadala, *et al.,* 2022).

In *Digitalis purpurea* conducted to ascertain the source of SE from hypocotyl calli, SEM observed the presence of different stages of SE globular and heart-shaped on callus surfaces. The globular embryos were distinguished more prominently during the investigation compared to the other embryonic stages (Bansal, *et al.,* 2022). In *Anaphyllum wightii* Schott is an ethnomedicinally significant plant endemic to the southern region of Western Ghat. Stereomicroscopic histological observations of the embryogenic callus showed multiple stages of somatic embryo development, suggesting an asynchronous pattern of embryogenesis. NAA at a concentration of 2 mg L^{-1} demonstrated the highest embryogenic callus induction (S Lakshmi & TS Swapna 2022). In *Hancornia speciosa* histological examination on explants of *Hancornia speciosa* response of nodal and leaf segments in inducing embryogenic calluses and potentially maturing somatic embryos (Santana, *et al.,* 2023). In *Manihot esculenta Crantz* SEM analysis a distinctive embryogenic spherical shape and a densely packed structure containing dense cells were observed, and histological observation on a 2-week-old callus shows the meristematic area where small cells are densely packed and also found that the cells actively divided to appear dense and dark (Fariroh, *et al.,* 2023).

In *Nigella sativa*, histological images of the embryonic calli reveal that the optimal developmental stages of somatic embryos consist of the globular, heart, torpedo-shaped, and cotyledonary stages (Higazy, *et al.,* 2023). Similarly, in *Coffea arabica* histological analysis of the somatic embryogenesis process demonstrated the progression through various stages of development. Injured leaf cells dedifferentiate to develop into an embryogenic callus characterized by enlarged nuclei, dense cytoplasm, and thickened cell walls. Histological

progression occurs through distinct stages, transitioning from the globular phase to oblong, heart-shaped, elongated, torpedo and initial cotyledonary stages, with visible procambium tissue in the oblong phase and cotyledon formation as a slight bulge at the apical edge, Subsequent to the elongation of the procambium during the torpedo phase, two cotyledons emerge at the top of the embryo in the cotyledonary stage (Ibrahim, *et al.,* 2024). In *Sorghum bicolor* histological analysis revealed root organogenesis from white and loose non-embryogenic callus, root organogenesis from parenchyma tissues, and SE from yellow and compact embryogenic callus. Differentiation was evident in meristematic cells with intense cytoplasmic staining and distinct vascular elements. Globular SE was observed under the histological (Wu, *et al*., 2024).

Isodiametric cells

Isodiametric cells are cells that have similar diameters in all directions, or the same dimensional. These are small cells that are similar in size and can be found in callus tissue. They can have a thin primary wall, a thin cytoplasmic layer around the wall, and large vacuoles. Formation of Isodiametric cells was observed in *Swietenia macrophylla.* Explants responded to *Swietenia macrophylla* by forming three types of calluses which varied in color, texture, and friability. The calli exhibited diverse morphological characteristics, appearing as friable and white, compact and cream-colored, or spongy with a brown texture. The friable calli were made up of small, isodiametric cells with a distinct nucleus and dense cytoplasm, whereas dense, isodiametric meristematic cells with large, clearly defined nuclei were also observed (Arias, *et al.,* 2019).

Phytohormone/ Plant Growth Regulators

Plants are vital for the planet, providing food and environmental stability. Phytohormones are key regulators of plant growth, yield, and stress responses. These hormones fall into two main classes: those promoting growth and those managing stress responses. Recent research has emphasized the importance of phytohormones like salicylic acid, abscisic acid, ethylene, and jasmonates in plant processes (Sabagh, *et al.,* 2022). The involvement of cytokinins, gibberellins, auxin, and relatively novel Phytohormones such as brassinosteroids, and strigolactones in plant growth and development has been documented under normal and stress conditions. Their signaling networks make them valuable for optimizing growth and stress management in agriculture. Future studies and research will investigate these hormones and their uses in other domains (Asif, *et al.,* 2022).

Cytological variation

Cytological variation refers to differences observed in the cellular characteristics of cultured plant cells or tissues. This can include variations in cell size, shape, ploidy level, chromosome number, organelle structure, and metabolic activity of cytological variation commonly encountered in tissue culture due to factors such as genetic instability, somaclonal variation, epigenetic changes, and environmental factors during culture. In *Lepianthes umbellata* the cytological variation was observed in callus cultures through histological analyses that showed embryoids from the callus masses specifically, indirect organogenesis (Manasa, *et al.,* 2019).

Starch accumulation

Starch accumulation in plant tissues involves the storage of starch, a complex carbohydrate. In plant tissue culture, starch accumulation can be observed in the form of granules within plastids, particularly amyloplasts, in cells. In *Acrocomia aculeata,* starch accumulation was observed by histological observations, within the cells of cultured plant tissues. Managing factors such as nutrient availability, hormonal levels, physiological stress, developmental stages, light intensity, and culture conditions are important in controlling starch accumulation and optimizing the growth of cultured plant tissues. In *Acrocomia aculeata* there is starch accumulation was shown near centers of intense cell division during somatic embryogenesis, The presence of starch during somatic embryogenesis is commonly

related to embryogenic cell differentiation (Meira, *et al.*, 2019).

Confirmation of no structure distortion

Structural distortion refers to any alteration or deformation in the arrangement and system often resulting in a deviation from its organization. SEM analysis confirmed the organization of cells and tissue in the in vitro apical buds of *Ficus carica* L.cv. Blackjack treated with BAP and 2-4D at various concentrations showed no structural distortion. The presence of latex produced by the plant during sectioning was observed (Han, *et al.,* 2020).

Detection of diseases

Diseases in plants caused by pathogens and environmental conditions bacterial diseases such as fire blight, to viral infections like mosaic viruses. In tuberose (*Polianthes tuberosa*) cultivation suffers greatly from *Meloidogyne incognita* infection. In *in vitro* histological analysis revealed root infection starting within 2 days post-inoculation (DPI), with gall formation by 6 DPI, indicating established root infection. This *in vitro* regeneration method can effectively produce disease-free plantlets for mass propagation (Singh, *et al.,* 2020).

New structure formation

The main parts of plants are the leaves, stems, and roots. A histological study of *Glycine max* demonstrated that leafy, shoot-like structures emerged from the cortical region of hypocotyl segments, providing evidence for the process of direct shoot regeneration (Patel, *et al.,* 2023).

Observation of Crystal Idioblasts

Idioblasts are specialized parenchymatous cells that are often found in callus cultures, usually in a narrow band of tissue near the top surface. Idioblasts can be much larger than the surrounding cells, containing crystals often composed of calcium oxalate. In *Asparagus cochinchinensis* crystal idioblast was found through histological observation in young leaves from the early stages of leaf differentiation (Kim, *et al.,* 2021).

Saponins distribution using histology

Saponins generally consist of a hydrophobic aglycone core. They are widely found in plant-derived natural products and display remarkable structural and functional variation. This complexity is enhanced by the presence of numerous functional groups, followed by the addition of hydrophilic sugar chains. Saponin in *Yucca gloriosa* was observed through histological fluorescent localization using a fluorescence microscope that showed saponin distribution in intact plants and different developmental stages of culture parts were varied (Ansam, *et al.,* 2021).

Conclusion

A detailed description of the application of histological and microscopic studies revealed that they were easy but valuable tools in various aspects of plant tissue culture. Both practices very clearly and minutely prove the histological and cytological changes, structure formation, and accumulation of substances occur during callogenesis, morphogenesis and embryogenesis. These minor observations can differentiate the responses of various plants or various plant species against different phytohormones applied during the process. Microscopic and histological studies during the early stages of plant cultures may give a view of the forecast of the cultures. By using any of the techniques one can reduce the long-time duration, which is otherwise compulsory when he or she is totally depend on morphological observations only. Reducing the time duration will make the process of *in vitro* plant regeneration, mass propagation and *in vitro* metabolite production more economical.

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DOI:10.22192/ijarbs.2024.11.12.001

How to cite this article:
Binita Basumatary, Mamta Gokhale, Hemanga Kumar Das and Rumana Faraz. (2024). Histological and Microscopic study in plant tissue culture: A Review. Int. J. Adv. Res. Biol. Sci. 11(12): 1-18.
DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.12.001