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# High Frequency Plant Regeneration System from Transverse Thin Cell Layer Section of *In vitro* Derived 'Nadia' Ginger Microrhizome

Dikash Singh THINGBAIJAM, Sunitibala Devi HUIDROM*

*Medicinal Plants & Horticultural Resources Division, Institute of Bioresources and Sustainable Development, Takyelpat Institutional Area, Imphal-795 001, Manipur, India; sunitibala.ibsd@nic.in (*corresponding author)*

**Abstract**

An efficient and reproducible procedure is outlined for rapid *in vitro* multiplication of *Zingiber officinale* var. 'Nadia' through high frequency shoot proliferation from transverse thin cell layer (tTCL) sections of *in vitro* derived microrhizome. *In vitro* derived microrhizome of size 500 µm in thickness was used as initial explants for induction of somatic embryos. Among the different phytohormones tested, tTCL explants shows maximum calli proliferation in medium containing 2 mg/L 2,4-Dichlorophenoxyacetic acid (88.30±0.11%). Reduced concentration of 2,4-Dichlorophenoxyacetic acid was supplemented with different cytokinins for regeneration of callus. Among the different medium tested, optimum redifferentiation of somatic embryos were observed in medium containing 0.2 mg/L 2,4-Dichlorophenoxyacetic acid and 6.0 mg/L BAP (141.08±0.25). Clump of regenerated plantlets were further subculture and transfer into microrhizome inducing medium containing high sucrose concentration (8%). Plantlets with well developed microrhizome were successfully acclimatized and eventually transferred to the field. The application of studying embryo section for regeneration of plants might be useful alternative to ginger improvement programme. Histological analysis showed formation of somatic embryos and regenerated adventitious shoot.

***Keywords:*** ginger, thin cell layer, callus, embryogenesis, regeneration, histology

## Introduction

*Zingiber officinale* Rosc. is one of the most important spice condiment crop grown in India. It is herbaceous rhizomatous perennial plant belonging to the family Zingiberaceae, under the natural order scitaminae. It is a tropical plant believed to have originated in South East Asia probably India or China (Bailey, 1949; Parry, 1969). Ginger is commonly used as spice and in medicine. Apart from having tangy flavour, it has appreciable quantities of proteins (2.3%), carbohydrates (12%), fats (1%), minerals (1.2%), fibre (2.5%) and moisture (81%) of fresh rhizome (Swaminathan, 1974). It also contains appreciable amount of vitamin A and small amount of vitamin B. Hence, this crop finds a place in naturotherapy and herbal medicine prescription since Vedic period. However, breeding of ginger has not been advanced so far and is limited to only clonal selection. This is because ginger displays high sterility (Adaniya and Shoda, 1998a) as a result of chromosome aberrations such as translocations and inversions (Ramachandran, 1969; Ramachandran, 1982; Adaniya and Shoda, 1998b). Hence, other breeding methods such as mutation and polyploid breeding are required to obtain genetically improved strains.

The establishment of favourable *in vitro* cultural conditions is indispensable for the efficient induction of polyploidy plants. For ginger, *in vitro* propagation systems have been reported by various workers, who investigated *in vitro* systems using different explants types (Hosoki and Sagawa, 1977; De Lange *et al.,* 1987; Bhagyalakshmi, 1988; Inden *et al.,* 1988; Noguchi and Yamakawa, 1988; Ikeda and Tanabe, 1989; Sharma and Singh, 1995). In addition, somatic embryogenesis is frequently regarded as the best system for propagation of superior genotypes (Kim, 2000) mostly because both root and shoot meristems are present simultaneously. It act as an efficient tool for rapid propagation, genetic transformation, somatic hybridization, and somaclonal variation (Gray *et al.*, 1991; Kuksova *et al.,* 1997; Martinelli and Gribaudo, 2001). It also provides cytological and genetic stability of regenerated plants (Bennici *et al.,* 2004). Although somatic embryogenesis may occur spontaneously in some plant species, it is usually induced in tissue culture medium potentially from almost any part of the plant body (Kantharajah and Golegaonkar, 2003; Siong *et al.,* 2011; Ahmed *et al.,* 2011). However, Somatic embryogenesis has been accomplished in some species for producing millions of seeds. The form of somatic seeds through somatic

embryogenesis, is more efficient than organogenesis. Besides, somatic embryogenesis is more preferable in plant genetic improvement through *in vitro* culture and genetic transformation as well, because single cell derived-plant is easier to be controlled as somatic embryo derived-plant. In general, somatic embryogenesis and meristem derived plants are true-to-type and genetically identical to the mother and certain differences might appear according to the plant species characteristics. Moreover, prior to successful agri-biotechnological research on crops, reliable callus induction and efficient *in vitro* regeneration system is urgently required (Abdellatef and Khalafallah, 2008).

In the investigation reported here, we examined a series of experiments to investigate the influence of transverse thin cell layer culture on callus formation and embryo regeneration in *Zingiber officinale* 'Nadia'. An efficient procedure to induce somatic embryogenesis from tTCL (transverse thin cell layer) explants cultures and histological studies were carried out to determine the ontogenic stage in which somatic embryogenesis occurs in ginger.

**Materials and methods**

Aseptic shoots raised from sterilized rhizome buds of *Zingiber officinale* 'Nadia' culture on Murashige and Skoog's (1962) medium supplemented with 1 mg/L α-napthaleneacetic acid (NAA) were used as the initial explants source. For the induction of microrhizome, well developed 4-5 weeks old *in vitro* derived plantlets were transfer into MS medium containing 8% sucrose, 1 mg/L NAA, and 2 mg/L 6-benzyl-amino-purine (BAP). pH of the medium was adjusted to 5.8 prior to adding agar and medium was autoclaved at 15 lbs psi pressure for 20 minutes at 121°C. All the cultures were then kept in a growth chamber under 16 hours light photoperiod at 25±2°C under white fluorescent tubes (photosynthetic photon flux of 25 $\mu mol^{-2}s^{-1}$).

*In vitro* derived plantlets with microrhizome were removed asceptically and microrhiomes were transversely sliced into pieces of about 500 μm in thickness, and the slices from the microhizomes were used as tTCL explants for the induction of callus *in vitro*. Transverse thin cell layer explants were inoculated on MS medium containing 3% (w/v) sucrose with different levels of α-napthaleneacetic acid (NAA), Thidiazuron (TDZ), 2,4-Dichlorophenoxyacetic acid (2,4-D), and kinetin (Kn) (Sigma, USA). 20 tTCL were inoculated for each treatment. The media were solidified with 0.8% (w/v) agar (Sigma, USA). The pH was adjusted to 5.8 with 1 mol $l^{-1}$ NaOH or HCl prior to autoclaving at 121°C for 15 min. The explants were cultured in upright orientations (with the basal, i.e., proximal end cut surface touching on the medium). The cultures were under illumination (35 lmol $m^{-2}$ $s^{-1}$) with a 16/8 h of light/dark cycle at 25±2°C in the culture chamber. The explants were photographed using a stereozoom microscope (Carl Zeiss STEMI 2000C, United States).

Each of the tTCL explants was cultured in 50 mL culture tube and the number of explants response from each of the microrhizome section were recorded after every 5 weeks of culture. The percentage of the explants with callus was calculated as per following formulae:

$$\text{Percentage of explants with callus}\,(\%) = \frac{\text{number of explants with callus} \times 100}{\text{total number of explants inoculated}}$$

Percentages of embryogenic tTCL explants and number of embryos per responsive tTCL were counted after 3 months of incubation. Callus formation and the embryogenic response of tTCL explants were expressed as percentages on a culture tube basis. Callus cultures were maintained by subculturing every 4th weeks on fresh medium. Significant differences in morphogenetic responses of tTCL explants among all the culture treatments were tested using Tukey's multiple range tests. Significance was determined at the 0.05 probability level. All statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to compare means.

For regeneration of shoots and adventitious buds formation, induced calli were removed asceptically and transferred to modified MS medium containing 3% (w/v) sucrose with different concentration and combination of auxin and cytokinins. Cluster of adventitious shoots were then transfer into liquid microrhzome inducing medium containing different concentration of auxin and cytokinin at high sucrose concentration (8%). Average number of adventitious buds per responsive explants was calculated as per following formulae:

$$\frac{\text{Average no. of adventitious buds}}{\text{responsive explants}} = \frac{\text{sum of the number of adventitous buds in each explant}}{\text{number of explants with a shoot}}$$

45 days after the induction of microrhizome the plantlets with 3-4 expanded leaves per shoot and well-developed roots were removed from the culture bottle. The plantlets were then washed gently with running tap water to minimize the chance of fungal attack by high sucrose content. The plantlets were then transferred to small plastic bags containing sterilized soil. All the plastic bags were maintained under shade house condition (Institute of Bioresources and Sustainable Development, Imphal) at 80% relative humidity and about 16h photoperiod

***Histological Observations***

For histological studies, zygotic embryos, callus and embryoids at various stages of development were fixed in FAA II solution of 90 ml 70% ethyl alcohol, 5 ml glacial acetic acid and 5 ml formalin solution. These tissues were dehydrated through an ethanol tertiary butanol series for 48 h and embedded in Paraplast. Specimens were sectioned at 10 to 14 μm and stained with safranin. All sections were mounted with Permount and were viewed under bright-field illumination with a stereozoom microscope (Carl Zeiss STEMI 2000C).

**Results and discussion**

Transverse thin cell layer microrhizome cultured on the medium devoid of plant growth hormones showed negative cells response giving brown colouration and ultimately died after 15 days of incubation. However

callus could be effectively induced from tTCL microrhizome of size 5000-6000 µm (Fig 1a) inoculated on most of the basal media supplement with different plant growth regulators. Different phytohormones *viz*., 2,4-D, NAA, TDZ and Kn were used to test their effect on embryogenic callus induction (Tab. 1). It was observed that among the different plant growth regulators, 2,4-D played an important role in induction of ginger embryogenic callus. Callus induction was enhanced as the concentration of 2,4-D in the medium increased from 1.0-2.0 mg/L but inhibited at above 2.0 mg/L. On the medium with 2mg/L 2,4-D tTCL explants showed optimum embryogenic callus induction and average of 88.30±0.11(%) explants showed callus proliferation (Tab. 1; Fig 1b). Many authors also reported

Tab. 1. The effect of different plant growth regulators on the morphogenic responses from the TCS explants of *Zingiber officinale**

| Plant growth regulators (µM) | Explants producing callus (%) |
|---|---|
| Control | |
| 0.0 | 0.0 |
| 2,4-D | |
| 0.5 | 40.66±0.14[a] |
| 1.0 | 74.38±0.20[c] |
| 2.0 | 88.30±0.11[b] |
| 4.0 | 55.31±0.12[d] |
| TDZ | |
| 0.5 | 50.83±0.25[h] |
| 1.0 | 61.55±0.15[g] |
| 2.0 | 46.30±0.10[f] |
| 4.0 | 45.17±0.07[e] |
| Kn | |
| 1.0 | 42.41±0.08[k] |
| 2.0 | 35.20±.08[l] |
| 4.0 | 17.19±.06[m] |

*Observations were made with explants of 0.5 mm thickness in semisolid medium. Each value is the mean of 20 observations taken from two experiments each with three replicates. Mean separation within the different treatments of a particular phytohormone is by Tukey's multiple range test, p=0.05.

that high concentration of 2,4-D treatment could be regarded as a trigger for producing somatic embryogenesis (Halperin, 1964; Dudits *et al*., 1993) which may inhibited certain specific stages of embryogenesis in this condition. This is supported by findings of Yinghua and Zhang (2005) showing successful establishment and plant regeneration of somatic embryogenic in cell suspension cultures of *Zingiber officinale* Rosc. Masuda *et al.* (1995) also reported that higher 2,4-D concentration (100 mM) resulted in the formation of somatic embryos due to the induction of alfalfa cells proceeding from the G1 phase to the S phase in the cell cycle. Similarly, Kitamiya *et al.* (2000) also reported that high concentration of 2,4-D treatment could serve as a trigger in inducing cell division in the epidermal cells and promoting their further differentiation to somatic embryos. Even though, 2,4-D was most effective for the induction of ginger somatic embryogenesis, the percentage explants producing callus reduced with increase in the concentration of 2,4-D above 2mg/L. The efficiency of NAA, TDZ and Kn on callus formation from tTCL explant were not responsive than that of 2,4-D (Tab. 1). The frequency of responsive tTCL explants increased as the concentration of TDZ ranged from 0.5 to 1.0 mg/L and decreased from 1.0mg/L to 4.0 mg/L. On the medium containing 1.0 mg/L TDZ, 61.55±0.15 of the explants were proliferated to produce callus. The optimal concentration of Kn ranged from 1.0 mg/L to 2.0 mg/L producing average of 35.20±0.08 to 42.41±0.06% callus per tTCL explants (Tab. 1). Moreover, among the plant growth regulators tested, NAA showed negative response and does not show any sign of callus proliferation. Use of tTCL culture system in the present study for *in vitro* propagation of ginger clearly demonstrated the morphogenic potential of the tTCL ginger explants. It was found out that tTCL system exibits high regenerative capacity, which has been attributed by Lakshmanan *et al.* (1995) to two significant features: (i) availability of nutrients and growth promoting substances at the site of regeneration and (ii) elimination of correlative control imposed by other tissues and organs.

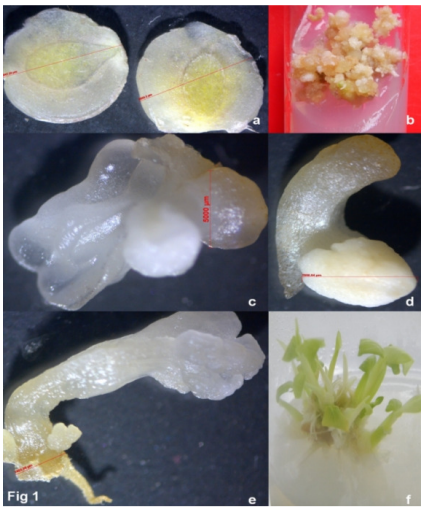


Fig. 1. a) Transverse thin cell layer sections (tTCL) of *in vitro* derived microrhizome. b) Induction of callus on the medium supplemented with 2mg/L 2,4-D. c) Observation of embryo maturation and redifferentiation at MS medium containing 0.2 mg/L 2,4-D and 6 mg/L BAP. d) Appearance of small protuberances from the redifferenting calli of size 1000-6000 µm after 3 weeks of culture. e) Complete plant regeneration along with root after three weeks of culture. f) Formation of adventitious buds into a clump after the first shoot initiation

Although addition of 2,4-D has been found to elicit rapid cell proliferation and callus formation, reduction or removal of 2,4-D from the medium is essential for plant regeneration from calli (Marsolais and Kasha, 1985; Liang *et al.,* 1987; Ball et al., 1993). New gene product synthesized upon the removal of 2,4-D were required for the transition from globular stage to the heart-shaped stage (Zimmerman, 1993). It is known that BAP is important for the maturation and redifferentiation of somatic embryos, and it also promoted the proliferation of shoots and leaves. In the present experiment, attempts were made to redifferentiate tTCL induced embryogenic callus by reducing the concentration of 2,4-D in the medium supplemented with various concentrations of cytokinins such as BAP and Kn. The induced calli from the above experiments were transfer onto modified MS medium containing various concentrations and

combinations of 2,4-D, BAP and Kn. After 4 weeks of incubation, the embryos were promoted to become mature and started dedifferentiation. Lower concentration of 2,4-D in combination of different hormones showed different responses. Among the different hormones tested, BAP was found to be most effective for redifferentiation of shoots than Kn. The preeminent medium for embryo maturation and redifferentiation was observed in MS containing 0.2 mg/L 2,4-D and 6 mg/L BAP (Fig 1c). After 3 weeks, small protuberances gradually emerged from the redifferenting calli of size 1000-6000 µm and then the first shoot subsequently originated from this protruding region (Fig 1d). Although root initiation started from these calli after two weeks of subculturing but complete plants were seen in the differentiating callus after three weeks with single leaf (Fig 1e). In most of the cases redifferentiation of somatic embryos were taken place from the embryonic calli of size ranges from 1000-6000 µm (Fig 1c to e). After the first shoot initiation, the adventitious buds arose from the basal region of the first shoot and ultimately the regenerated shoot and adventitious buds grew into a clump (Fig 1f). A similar morphogenetic process of shoot induction occurred on most of the medium tested but the induction efficiencies were different. The optimum concentration for shoot regeneration was found to be most effective in 0.2 mg/L 2,4-D supplemented with 2.0-6.0 mg/L BAP (82.43±0.15 to 141.08±0.25) (Tab. 2) (Fig 2a). This shows that reduction of 2,4-D concentration from the medium is crucial for plant regeneration in ginger. After the complete maturation of plantlets (5 weeks), cluster of adventitious shoots were transfer into microrhizome inducing liquid medium prepared in 250 ml phytajar (Fig 2b). Since, induction of microrhizome depended on the manipulation of plant growth hormones, culture environment and sucrose concentration, adventitious shoots cluster were transfer into a liquid MS medium containing 8% sucrose + 1 mg/L NAA + 2mg/L BAP (data not shown). Well developed microrhizomes along with roots could be seen after 6 weeks of culture (Fig 2c). This indicates that *in vitro* formation of microrhizome can be facilitated by increasing sucrose concentration which may be due to the presence of high carbon energy. Similar reports of microrhizome induction were already reported by many workers (Thorpe 1982; Ross and Davies 1992; Sunitibala *et al.,* 2001). Regenerated shoots along with microrhizome and roots were successfully acclimatized under shade house condition (IBSD, Imphal) (Fig 2d) and eventually transferred to the experimental garden.

Tab. 2. The effect of media with different phytohormones on the regenerated rate of *Zingiber officinale**

| Plant growth regulators type (µM) | | | No. of shoots generated |
|---|---|---|---|
| 2,4-D | BAP | Kn | (≤ 0.5 cm) |
| 0.2 | 1.0 | | 75.32±0.13$^{q}$ |
| 0.2 | 2.0 | | 82.43±0.15$^{r}$ |
| 0.2 | 4.0 | | 96.30±0.15$^{s}$ |
| 0.2 | 6.0 | | 141.08±0.25$^{t}$ |
| 0.2 | | 1.0 | 27.32±0.13$^{i}$ |
| 0.2 | | 2.0 | 32.60±0.16$^{k}$ |
| 0.2 | | 4.0 | 44.31±0.13$^{m}$ |
| 0.2 | | 6.0 | 26.35±0.14$^{h}$ |
| 0.4 | 1.0 | | 28.59±0.20$^{j}$ |
| 0.4 | 2.0 | | 54.37±0.14$^{m}$ |
| 0.4 | 4.0 | | 72.20±0.12$^{p}$ |
| 0.4 | 6.0 | | 56.22±0.11$^{o}$ |
| 0.4 | | 1.0 | 15.37±0.15$^{c}$ |
| 0.4 | | 2.0 | 17.34±0.13$^{d}$ |
| 0.4 | | 4.0 | 24.30±0.12$^{g}$ |
| 0.4 | | 6.0 | 19.22±0.13$^{e}$ |
| 1.0 | 1.0 | | 27.36±0.14$^{i}$ |
| 1.0 | 2.0 | | 26.17±0.10$^{h}$ |
| 1.0 | 4.0 | | 34.40±0.14$^{l}$ |
| 1.0 | 6.0 | | 21.54±0.23$^{f}$ |
| 1.0 | | 1.0 | 17.13±0.10$^{d}$ |
| 1.0 | | 2.0 | 12.32±0.13$^{b}$ |
| 1.0 | | 4.0 | 10.40±0.16$^{a}$ |
| 1.0 | | 6.0 | 11.52±0.17$^{b}$ |

*Shoots were counted per callus at the size of 1.5 $cm^2$. p=0.05 with three replication.

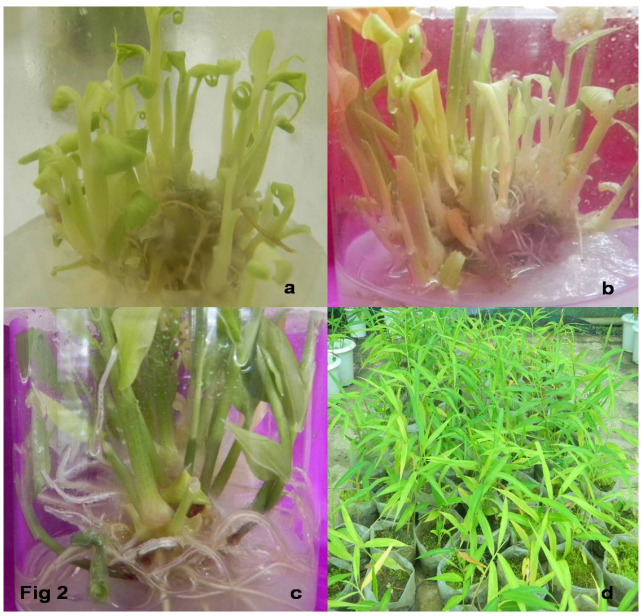


Fig. 2. a) Optimum shoot regeneration at MS medium containing 0.2 mg/L 2,4-D supplemented with 2.0-6.0 mg/L BAP. b) Transfer of adventitious shoots cluster into liquid MS medium prepared in 250 ml phytajar containing 8% sucrose concentration. c) Induction of microrhizomes along with roots after 6 weeks of culture. d) Successful establishment of in vitro derived plantlets under shade house condition (IBSD, Imphal)

### *Histological study of embryogenic calli*

The ginger embryo developed from tTCL was white, ovate and measured about 3000-4000 µm in length (Fig 3a). Histological examination shows that callus originated from the subepidermis layer of the tTCL explants showing central meristematic activity (Fig 3b). The cells in such centers were smaller than cells in the outer part of the callus, and they stained more intensely and contained distinctly prominent nuclei (Fig 3c). The callus cells outside of the meristematic centers are non-

embryogenic containing small starch grain, and are highly vacuolated (Fig 3d). Later the meristematic centers appeared to differentiate into embryoids. Later after 1 week of culture, histological observations on embryogenic calli shows two types of cells. The outer cell layer consisted of 8-12 rows of small and isodiametric cells with prominent nucleus and a dense cytoplasm (Fig 3e). In the initial stages, both periclinal and anticlinal division take place which may be due to an active cells metabolism. This shows that cells present in external part of the embryonic calli resembles meristematic cells. Cell layers below the embryogenic calli consist of larger non-embryogenic cells with larger vacuoles and small nucleus. A cluster of proembryogenic cells were observed in 2 weeks of incubation in the peripheral region (Fig 3f). Different stages of calli differentiation were observed immediately below this region. Shortly after 1 week, well-defined protodermis were observed indicating the formation of proembryo. Proembryos undergoes a series of division which ultimately give rise to the globular stage with well define epidermis cells and nucleus (Fig 3g). This stage of embryo lack vascular connection and are connected to mother tissue by suspensor like structure. The next stage of the embryo regeneration i.e., heart shape were observed after 1-2 weeks of culture (Fig 3h). Cells still remained with prominent nucleus and dense cytoplasm.

After 1-2 weeks, torpedo shaped somatic embryo were regenerated and later shows polarization with apical and redical meristems in opposite poles (Fig 3i). The embryo at this stage consists of well differentiated bipolar structures and consists of shoot and root apices, which were connected by the vascular system (Fig 3j). The root pole is usually blunt and flattened while the shoot apex is surrounded by the cotyledon. In this stage, the vascular connection between the callus and the embryos were completely lost. This existence of typical bipolar orientation and also the absence of vascular connection of these structures with the callus allowed the distinction of the process of embryogenesis from that of organogenesis. At more advanced stage, the presence of shoot apices became evident, and after 3 weeks, complete and distinct single shoot were developed simultaneously along with root (Fig 3j-k). However, root maturation shows much faster than shoot. A developed root shows hairy and shows all the characteristic of mature root (Fig 3j). Later after 2 weeks of culture, longitudinal section of completely regenerated plants shows the presence of distinct leaf primodia surrounding the single first originated leaf (Fig 3k).

The main morphological characteristic of somatic embryos is the bipolarity (the presence of opposite shoot and root poles) and the absence of connection with the explant vascular tissue (Falco *et al.* 1996; Gatica *et al.,* 2007). The present study demonstrates without ambiguity that somatic embryogenesis arises after the transfer of calli into the shoot inducing medium and this development corresponds to the development morphology of zygotic embryos of ginger. Somatic embryos of this single cell origin are widely encountered by many workers in various plant species *viz.,* date palm, *Ranunculus*, carrot, guinea grass, *Trifolium*, borage, cork oak and *Vanda* orchid (Konar *et al.,* 1972; McWilliam *et al.,* 1974; Tisserat and DeMason, 1980; CY Lu and Vasil, 1985; Cui *et al.,* 1988; Quinn *et al.,* 1989; Maataoui *et al.,* 1990; Kanchanapoom *et al.,* 1991). The somatic origin of the embryoids obtained from single cells may be highly differentiated with numerous starch grains. When somatic embryos have unicellular origin, coordinated cell divisions are observed and the embryos are connected to the maternal tissue by a suspensor-like body. Such cells contain numerous starch grains in the cytoplasm as a source of energy. Thomas *et al*. (1972) considered starch to be an indicator of the tissue development towards somatic embryogenesis. Starch accumulation present in the callus and bipolar embryoids of the ginger indicating that starch accompanies the formation of somatic embryos (Fig 3j). Indirect somatic embryogenesis observed in the present study is characterised by the induction of somatic embryos directly from embryogenic calli developed from tTCL microrhizome section. During this process re-determination of differentiated cells take place which is clearly distinguishes between embryogenic and

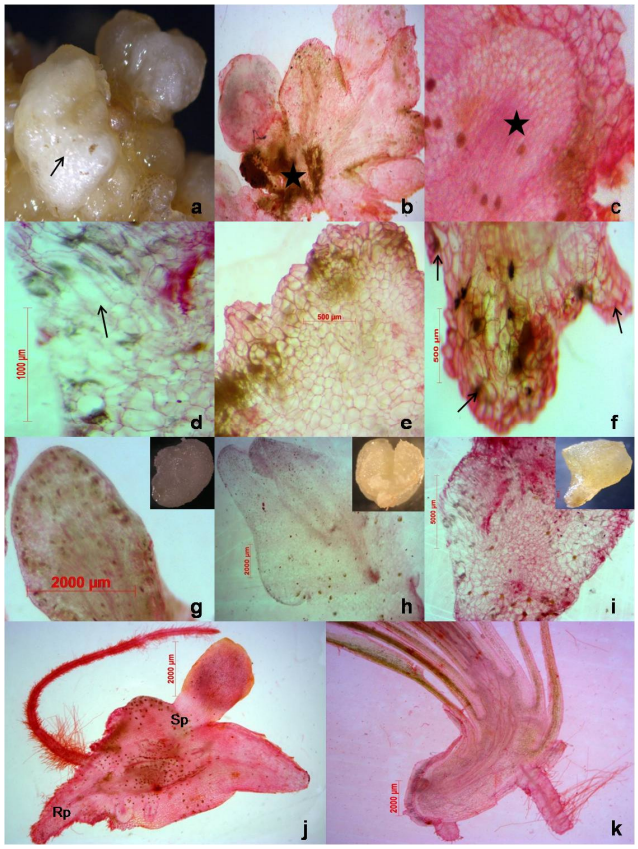


Fig. 3. a) Ginger embryo (arrow) developed from transverse thin cell layer culture.b) Induction of calli from subepidermis layer of explants (★). c) Embryogenic cells (★) at the centre of calli. d) Non-embryogenic cells with large vacuole, small granules, and intercellular space (arrow). e) Embryogenic cells showing isodiametric cells. f) Cluster of pro-embryogenic cells in the peripherial part of the calli (arrow). g) Globular somatic embryo. h) Heart-shaped somatic embryo. i) Torpedo-shaped somatic embryo. j) Mature embryo showing distinct root pole (Rp) and shoot pole (Sp) with simultaneous origin of root and shoot. k) Regenerated plant showing complete root and shoot formation

competent cells (Quiroz-Figueroa *et al.,* 2002). This shows that the auxin 2,4-D plays an important role in the dedifferentiation and cell division in ginger somatic embryogenesis. Similar result was observed by Meneses *et al*. (2005) in rice. Moreover, our histological observations showed that somatic embryos originated from the inner cells of the embryogenic calli, agreeing with previous observations in sugarcane (Jane-Ho and Vasil, 1983), Guinea Grass (CY Lu and Vasil, 1985) and oil palm (Schwendiman, 1988). Since most somatic cells are not naturally embryogenic, an induction phase is required for the cells to acquire embryogenic competence (Namasivayam, 2007).

Present study suggests the use of tTCL in plant regeneration and reports here may be an efficiency method for large scale multiplication and commercialization of ginger germplasm. This shows that somatic embryogenesis is an ideal method of regeneration and histological knowledge concerning ontogeny of ginger embryoids can provide important information for improving the somatic embryogenesis process for this crop. Thus, histological analysis is essential to confirm the origin of embryoids.

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**Conflict of interest**

The authors would like to state that they have no potential conflict of interest regarding submission and publication of this manuscript.

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