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Direct Somatic Embryogenesis in Rice (*Oryza sativa* L.) : Structural and Developmental Patterns

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Abstract
In this study, we used light microscopy to observe the structural and developmental pattern during direct somatic embryogenesis in rice (Oryza sativa L). Initiation of somatic embryogenesis revealed the origin of somatic embryo. It was unicellular origin from the epidermis of scutellum zygotic embryo. The polarity of somatic embryo established when the elongated embryo flattened at the apical end. On the onset of scutellum development, the scutellar notch was formed. In germinated somatic embryo, the shoot apex was connected with the root by the vascular bundle strands. This is an important characteristic of somatic embryo. The result led us to the conclusion that rice somatic embryos underwent the same structural and developmental pattern as that of rice zygotic embryo.

Keywords*: Rice, somatic embryogenesis, light microscopy*

Abstrak
Pada studi ini, kami menggunakan mikroskop cahaya untuk mengamati pola struktur dan perkembangan selama embriogenesis somatik langsung pada padi (Oryza sativa L.) Inisiasi embriogenesis somatik mengungkapkan asal mula embrio somatik, yaitu dari satu sel epidermis skutelum embrio zigotik. Polaritas embrio somatik terjadi ketika embrio yang memanjang menjadi datar pada ujung apikalnya. Pada awal perkembangan skutelum terbentuk takik skutelum. Pada embrio somatik yang berkecambah, tunas apikal dihubungkan dengan akar oleh untaian ikatan pembuluh. Hal ini merupakan ciri yang penting dari embrio somatik. Hasil ini mengarahkan kami pada kesimpulan bahwa embrio somatik padi mengalami pola struktur dan perkembangan yang sama seperti pada embrio zigotik padi.

Kata kunci : *Padi, embriogenesis somatik, mikroskop cahaya*

1. Introduction

Somatic embryogenesis is a process by which somatic cells undergo a developmental sequence similar to that seen in zygotic embryos. This process is an important plant propagation technique[1)] and provides an essential tool for basic research into plant embryo development[2,3)] and other aspects of plant physiology[4)].

Living organisms are known for the high degree of order in their constituent parts, so it is not surprisingly to find that embryos undergo characteristic morphological and anatomical changes during their development. Therefore, a study of the pattern of development will provide a better understanding of the order-generating process[5)].

In rice (*Oryza sativa* L.) cv. Nipponbare, we have observed the development of somatic embryo by scanning electron microscopy. We observed changes in surface structure during direct somatic embryogenesis in rice scutellum[6)]. However, there have been no report on observation of direct somatic embryogenesis in rice (*O. sativa* L.) cv. Nipponbare by light microscopy.

The objective of this study was to characterize the structural and developmental patterns observed during direct somatic embryogenesis in rice (*O. sativa* L.) cv. Nipponbare, by light microscopy.

2. Materials and methods

2.1 Materials

Immature caryopses, which were used as the materials of this experiment, were obtained from rice (*Oryza sativa* L.) cv. Nipponbare plants grown in a greenhouse.

3. Methods

3.1 Immature zygotic embryo culture

The immature caryopses were surface sterilized in 70% alcohol for 1 min followed by 1% Na-hypochlorite for 20 min. The caryopses were then rinsed 4 times with sterilized, distilled water and inoculated on half-strength Murashige and Skoog[12)] basal medium. After 4 days of culture, the immature embryos were dissected from germinated caryopses and transferred to embryo-induction medium (EIM) (Table 1). The immature embryos were placed with the abaxial regions in contact with the medium (scutellum region was exposed). Ten immature embryos were cultured in each petri dish. This experiment was carried out in 4 replications each with five petri dishes. After 1 week of culture, the scutellum covered by nodular translucent structures was subcultured every week on embryo-maturation medium (EMM). After 2 or 3 subcultures, depending

on the development of somatic embryo, maturing embryos were transferred to embryo-germinating medium (EGM) and cultured for 2 to 4 weeks. EIM, EMM and EGM[9] with a modification on carbon source and gelling agent in EGM[13] were used for inducing somatic embryo from scutellum of immature zygotic embryo explant. (Table 1). The cultures for induction, maturation and germination of somatic embryos were kept in the dark at 25° C.

Table 1. The composition of media for inducing somatic embryos from the rice scutellum of immature zygotic embryos[6]

Media	Basal Media	Hormone	Carbon Source	Gelling Agent
EIM	MS*)	2 mgL^{-1} 2,4-D	3% sucrose	0.8% agar
EMM	MS	1 mgL^{-1} 2,4-D	3% sucrose	0.8% agar
EGM	MS	-	1% sucrose 3% sorbitol	0.4% Gelrite

*) Murashige and Skoog (1962)

3.2 Light microscopy

Somatic embryos from the globular to germinated stage were prefixed in 0.1 M cacodylate buffer pH 7.2 containing 5% glutaraldehyde for 24 h, rinsed 3 times in cacodylate buffer, postfixed in 2% osmium tetroxide diluted in cacodylate buffer for 12 h, rinsed in cacodylate buffer once and distilled water twice, and gradually dehydrated in an alcohol series, all at 4° C. The samples were then infiltrated and embedded with Spurr resin at room temperature. The embedded samples were polymerized in an oven at 70 ° C for 24 h.

Semi-thin sections (0.9 to 2µm) were made with an ultramicrotome apparatus. The sections were then stained using Auramine O[7] or toluidine blue[8]. The sections stained with Auramine O were viewed under Nikon fluorescence microscopy, using UV light and B filter. The sections stained with toluidine blue were viewed on a light microscope, Optihot.

4. Results and discussion

4.1 Iniation of somatic embryogenesis

The somatic embryo in this study derives from the somatic cells of scutellum, which were termed as the Pre-Embryogenic Determined Cells (PEDC). These cells have already expressed an embryogenic gene expression program. This is seen in Figure 1 that show the globular somatic embryo formation on the surface of scutellum dorsal directly without intervening callus stage. This process will reduce somaclonal variation.

Auxin, which affects the intracellular interaction, could induce PEDC. Early event during the induction of somatic embryogenesis is the disruption of tissue integrity. This results in the isolation of cells or groups of cells and the severance of plasmodesmata. The disruption of tissue integrity causes the cell connection between neighbouring cells to become disrupted[6]. The isolated cells or groups of cells may be free of influences from surrounding tissues. Accordingly, physical treatment that results in cell isolation may stimulate embryogenesis. Under SEM, we observed that the proembryo formed after the cell was isolated from the surrounding tissue[6].

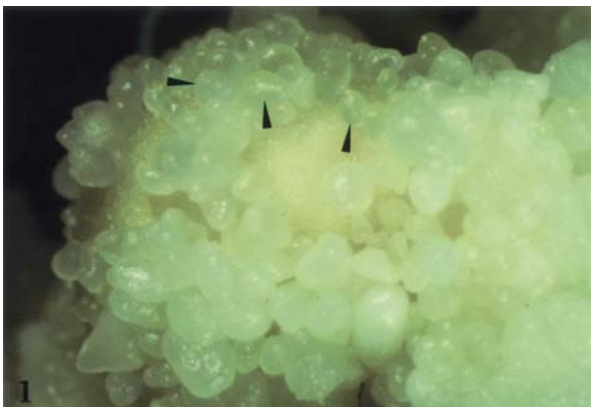

Figure 1. The globular somatic embryo (head arrow) formed on the surface of scutellum dorsal without intervening callus stage.

4.2. Origin of Somatic Embryo

Semi-thin section in Figure 2 shows that the proembryo differentiated from the scutellum epithelial cells. It also shows that the proembryo was derived from unicellular origin. Unicellular origin means that the somatic embryo was derived from a single cell. Therefore, it will reduce somaclonal variation as well. In addition, this advantage will be important in applying the somatic embryo in plant genetic transformation.

A shift in the direction of cell division can be an important early indicator for somatic embryogenesis[5]. In rice, some epithelial cells have been induced to divide by 24 hr[9]. The first cell division signals the beginning of somatic embryo formation, i.e. the induction of an epithelial cell to become a determined embryogenic cell occurs within 24 hr.

Somatic embryogenesis continues by a series of random divisions of the proembryo cell to produce a small globular embryo (Figure 3). In *Trifolium repens*, the first sign of somatic embryo induction is a

shift from anticlinal to iregular, periclinal and oblique quantal divisions[10].

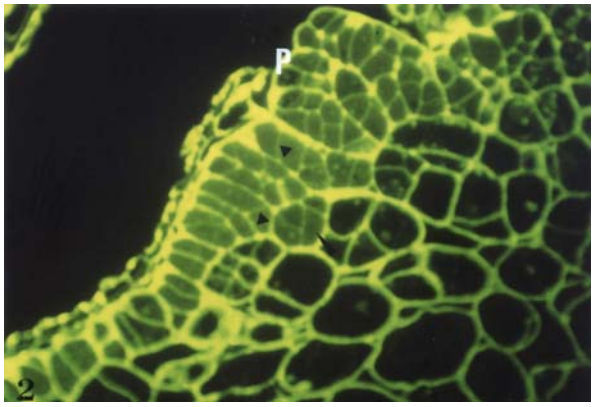

Figure 2. The proembryo (P) differentiated from the scutellum epithelial layer. Note the unicellular origin of the proembryo (big head arrow) and the shift in the direction of cell division (small head arrow).

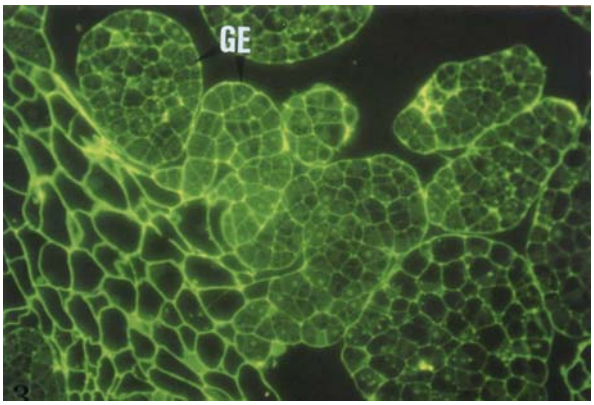

Figure 3. A small globular embryo (GE), which was produced by a series of random divisions.

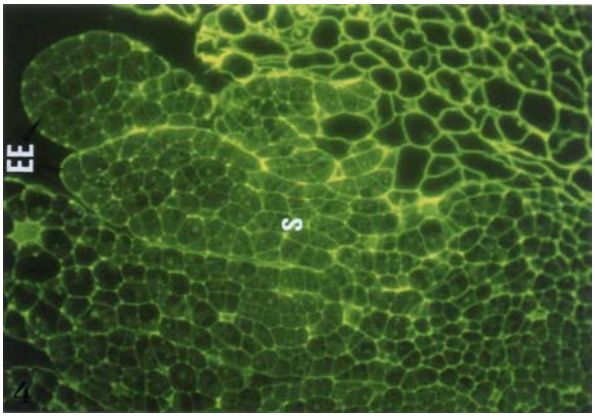

Figure 4. Elongated embryo (EE). Note the suspensor (s) on the basal of embryo

At an early stage, suspensors were observed on the elongated somatic embryo (Figure 4), because the somatic embryo was unicellular origin. PEDC tissue gave rise to embryos with unicellular origins. This explains why many immature cells of explant induced to form proembryo that differentiated into somatic embryo.

4.3. Polarity establishment

As the elongate embryo continues growing, it becomes flattened at the apical end, on the onset of scutellum development (Figure 5.). It was seen that the apical cells were much bigger and vacuolated and the protoderm has differentiated. The differentiation of the protoderm is one of the most important structural features at this developmental stage[5]. This developmental structure was also observed in rice zygotic embryo[11]. The procambial-like cells differentiated in the central core towards the root pole.

Figure 6 shows the scutellar stage with the early formation of scutellar notch. The epidermis has developed well and scutellar vascular bundle was seen longitudinally. The scutellum vascular bundle that was observed in rice zygotic embryo 6 days after anthesis, shows the similarity between somatic and zygotic embryo[8].

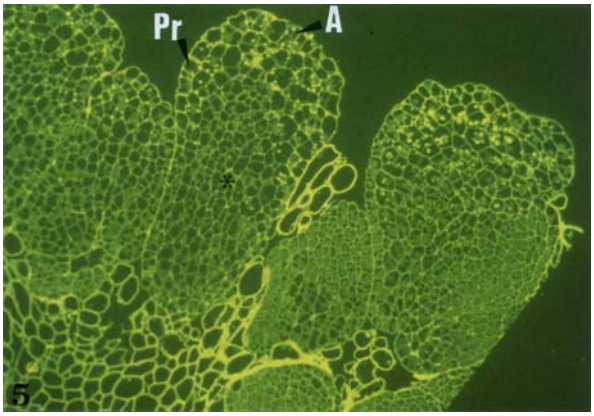

Figure 5. Embryo flattened at the apical end, on the onset of scutellum development. It was seen that the apical cells (A) were much bigger and vacuolated. The protoderm (Pr) has differentiated. The procambial-like cells (*) differentiated in the central core towards the root pole.

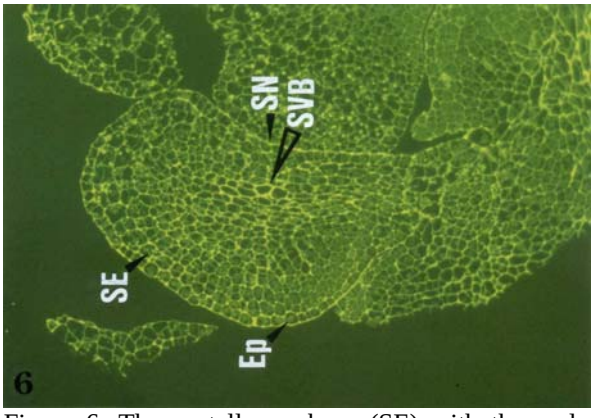

Figure 6. The scutellar embryo (SE) with the early formation of scutellar notch (SN). The epidermis (Ep) has developed well. Scutellar vascular bundle (SVB) was seen longitudinally.

4.4. Vascular Connection

Mature somatic embryo germinated after 2 weeks on embryo germination media, as shown in Figure 7. Shoot apical meristem differentiated, followed by the differentiation of root. It can be seen that the shoot apex was connected with the root by the vascular bundle strands. This is an important charactistic of somatic embryo. Shoot apex and root initial in the rice zygotic embryo are connected with provascular bundle strands before 6 days after anthesis[8].

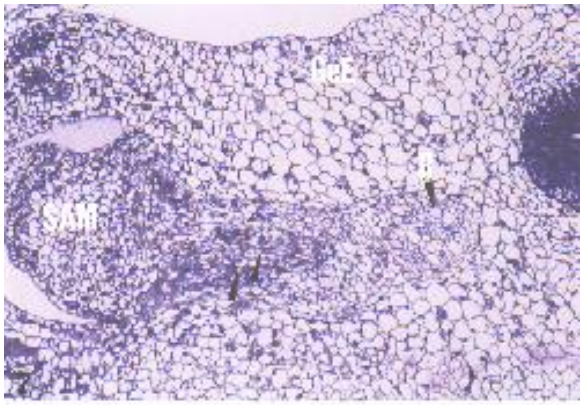

Figure 7. Germinated embryo (GeE) after 2 weeks on embryo germination media. Shoot apical meristem (SAM) differentiated, followed by the differentiation of root (R). It can be seen that the shoot apex are connected with the root by the vascular bundle strands (head arrow).

4.5. Germination of somatic embryo

Germinating somatic embryos were often seen in clusters. Usually, germinating somatic embryos could be separated easily from the mother explant, scutellum zygotic embryo.

5 Conclusion

Rice somatic embryo formed directly on the scutellum surface of zygotic embryo explant. Rice somatic embryo formed on the immature zygotic embryo explant was of unicellular origin. Rice somatic embryos developed structural and developmental patterns as zygotic embryo, these were proembryo, globular, elongated, scutellar and germinated stages. The formation of protoderm and its differentiation into epidermis was an important developmental pattern at the early somatic embryogenesis. In rice somatic embryo, the shoot apex connects with the root by the vascular bundle strands, as in the rice zygotic embryo.

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