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Histological Marker to Differentiate Organogenic Calli from Non Organogenic Calli of *Gloriosa superba* L.

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Abstract

A histological study of the indirect organogenesis from internodal cultures of *Gloriosa superba* L. showed that the callus initiated from the subepidermal cells. The organogenic and non organogenic callii are the result of hormonal variation in the medium. In non organogenic callus cells redifferentiated into xylem elements forming clusters of nest like structures. In organogenic callus, cells redifferen-tiated into nodules of meristemoids which further differentiated into shoot apical meristem.

Introduction

Glory lily (*Gloriosa superba* L.) is among some of the modern important medicinal plants which actually facing local extinction (Dhushara 2004). Different parts of the plant have a wide variety of uses especially within Indian traditional medicine from time in immemorial. Tubers and seeds of *Gloriosa superba* are an expensive export commodity. All parts of the plant contain colchicine and related alkaloids.

Successful *in vitro* techniques for micropropagation of *Gloriosa superba* has been reported using shoot tips, axillary buds, eye buds of corms, root primordial and even by culturing embryos (Sayeed and Shyamal 2005, Somani et al. 1989, Finnie and Van Staden 1989, Samarajeewa 1993, Custers and Bergervoet 1994). Similarly somatic embryos were also induced from the callus developed from shoot primordia (Jadhav and Hedge 2001) and directly from leaf explants (Manju and Joy 2008).

This paper reports on the histological events leading to shoot regeneration from internode-derived callus of *Gloriosa superba* L. The main objective of the present study was to have an understanding of the histological events taking place during organogenesis and also to explore the reasons why certain callus cells despite of various experimentation with various hormonal combination failed to induce callus.

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Materials and Methods

Internodal explants from healthy and profusely growing *Gloriosa superba* were collected from botanical garden of Sacred Heart College, Thevara, Kochi, India. The explants were washed thoroughly under running tap water. They were washed in detergent solution 'Labolene' for five minutes followed by ten minutes soaking in 0.1% mercuric chloride solution for ten minutes for surface sterilization followed by three to five rinses with sterile distilled water.

Surface sterilized internodal explants were cut into small pieces (1 - 1.5 cm) and placed horizontally on media containing MS basal salts and vitamins with 3% (w/v) sucrose, 0.8% (w/v) agar. Plant growth regulators 2,4-D (1.0, 4.0 mg/l), IAA(1.0 - 4.0 mg/l), BAP (0.5 - 3.0 mg/l), Kn (0.5 - 3.0 mg/l), for callus induction (with high auxin to cytokinin ratio) and shoot regeneration (with high cytokinin to auxin ratio) were used. Histological studies of cultures were carried out at uniform time intervals of five - 25 days. Free hand thin sections were then stained in saffranin and toludine blue for microscopic observations. Photographs were taken on Carl Ziess photomicroscope.

Results and Discussion

MS supplemented with 3.0 mg/l 2,4-D and 0.5 mg/l BAP induced callus. The sequence of histological events occurring during organogenesis from internode was traced. Callus started to develop from the cut ends of the explants. Induction of callus initiated from the collenchymatous hypodermal cells. As the development continued the epidermal tissues bulged at points encasing the callus cells inside (Fig. 1a). Proliferation of callus cells resulted in rupturing of epidermis. The callus showed the presence of scattered xylem elements (Fig. 1b). These scattered xylem elements later developed into prominent nest like structures (Fig. 1c).

When callus was subcultured on different concentrations and combinations of growth regulators the emergence of shoot initials were observed only in MS supplemented with 2.0 mg/l Kn and 1.0 mg/l 2,4-D. This callus cells were characterized by presence of meristamoids with dense cytoplasm and conspicuous nuclei (Fig. 1d). These meristematic points (Fig. 1e) were quite distinct from the adjoining callus cells. The meristematic points after repeated division gave protrusions forming compact nodule like structures which developed further into shoot buds (Fig. 1f). These meristematic points showed asynchronous development.

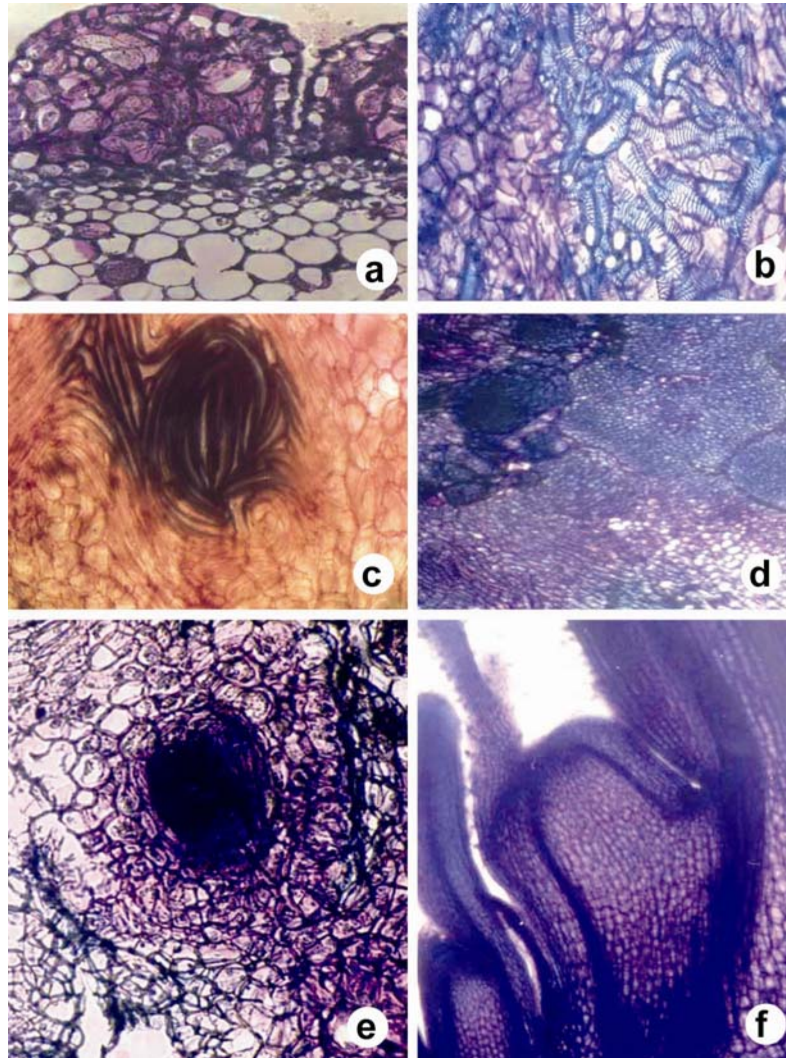

Fig. 1. (a) The epidermal tissues bulged at points encasing the callus cells. (b and c) non organogenic callus. (b) Scattered xylem elements in non organogenic callus. (c) Prominent nest like structures. (d, e, f) organogenic callus. (d) Meristamoids with dense cytoplasm and conspicuous nuclei. (e) Meristematic points and callus cells. (f) Compact nodule like structures developing into shoot buds.

The formation of densely stained highly cytoplasmic meristematic points in organogenic calli is reported earlier (Vijayan et al. 2000, Murch et al. 2000). According to Chen and Galston (1967) callus cultures contain vascular elements and parenchyma cells together called vascular nodules. The formation of vascular nodules in callus cultures may represent or be associated with an early stage of development of shoot meristems. These shoot primordia were very tightly clustered and could not be separated individually without cutting, which is in contrast to the somatic embryos induced in 2,4-D supplemented medium, where embryos could be easily separated (Liu et al. 1992).

The reason for the high xylogenesis in non organogenic calli is attributed to the plant growth regulators in the medium. The xylem elements were lignified and physiologically dead. The cell autolysis is the general feature after secondary wall thichenings due to the loss of nucleus and cytoplasmic contents in the cultured cells. This process leading towards cell death seems to be programmed at the beginning of the secondary wall thickening. There is every reason to believe that if these cells were not programmed for such cytodifferentiation it would have been destined for meristemoid development.

The fact that the organogenic cultures generally do not show these nest like structures and non organogenic cultures do show such xylogenesis suggest that presence of xylem elements can be a histological marker to distinguish non organogenic callus from organogenic callus. The fate of callus can thus be predicted.

Histological abnormalities were not observed among the callus regenerants in *Gloriosa* and were successfully rooted and planted out with more than 80% success.

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