INVESTIGATION ON IN VITRO LATICIFER DIFFERENTIATION IN THEVETIA PERUVIANA L.¹

ANJANI KUMAR & PRAMOD TANDON

Plant Biotechnology Laboratory, Department of Botany, School of Life Science, North Eastern Hill University, Shillong 793 014, India

Abstract

Amongst different combinations of auxins and cytokinins used, the optimum initiation and subsequent development of laticifers were observed in the callus tissue of Thevetia peruviana L. grown on MS medium supplemented with 1 mg/l 2, 4-D. This study was designed to follow the in vitro differentiation of laticifers, their long-term preservation and chemo-differentiation of glucoside thevetin, the active principle of the plant.

Keywords: Thevetia peruviana, callus cultures, laticifer, differentiation.

Laticifers are present in a large number of species and genera belonging to about twenty families (Metcalf 1966) and they accumulate many substances of high therapeutic value. Thevetia peruviana L. (Apocynaceae) contains a milky latex which is highly poisonous due to the presence of a water soluble glucoside thevetin which on hydrolysis gives rise to theveresin. Both these compounds possess digitalis like action on the heart which is an indication of its possible clinical application (Chopra et al. 1983).

Despite the fact that cultured cells subjected to growth regulators differentiate specialized cell types (Biesboer 1983), there has been limited success in growing laticifers in culture (Fahn 1979). Wilson & Street (1975) reported fragments of laticifers in callus cultures of Hevea stem and Biesboer (1983) detected some cells with a laticifer-like metabolism in Asclepias syriaca suspension cultures. However, the earlier workers were not successful in maintaining any stock callus material for preserving laticifers for longer period, by repeated subculturing. The present work, is therefore, an attempt to study cytodifferentiation leading to laticifer formation in cultures of Thevetia peruviana, their long-term preservation and also cytochemo-differentiation of active principles.

Material and Methods

Callus cultures were initiated from discs punched from young leaves of T. peruviana. Murashige & Skoog (1962) medium supplemented with auxins (indoleacetic acid, IAA; indole butyric acid, IBA; 2, 4-dichlorophenoxyacetic acid, 2, 4-D; and naphthalene acetic acid, NAA) and cytokinins (benzylaminopurine, BAP; and kinetin, Kn) alone or in combinations (at a range of concentrations 0.1 - 2.0 mg/l) were used for callus cultures and differentiation of laticifers. Cultures were maintained by regular subculturing at 15-day intervals and at 25 ± 2°C under 16 hr photoperiod (2,000 lux cool white fluorescent tubes).

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The Liebermann & Burchard (LB) and Kedde test for spot analysis of glucosides were done as described by Brower et al. (1972) and Harborne (1973), respectively. Other histochemical tests (Table 1) were performed to identify and characterise the contents of laticifers. The laticifers were isolated from the callus and stained following the techniques of Zhao Xin Qian (1987) and Inamdar & Murghan (1987). Laticifer development was followed in friable callus of parenchymatous cells subcultured for long period even beyond 200 days.

**Observations and Conclusions**

It is evident that growth and differentiation of laticifers occurs in cultures older than 80 days and start degenerating after 160 days (Fig. 1). However in 2, 4-D treatments laticifer formation initiated after 40 days and maximum of laticifer differentiation was recorded on 160 days. The degeneration of laticifers was slow in cultures grown in medium supplemented with 2, 4-D, BAP and Kn (both at range of concentrations 0.1-2.0 mg/l), in conjunction with 2, 4-D (1mg/l), in the medium brought about poor laticifer differentiation as compared to other treatments. The present finding on *Thevetia* shows a close relationship between phytohormone induced initiation of laticifers and age of the cultures.

![Graph showing laticifer formation percentage against culture age](image-url)
The non-articulated laticifers present in *Thevetia* originate as continuously growing single cell and subsequently develop into long tube like structures which may branch but do not undergo anastomosis (Fig. 2 A-D) which is contrary to reports of Milanez & Neto (1956) and Milanez (1959) on formation of non-articulated laticifers through fusion of cells. Young laticifer cells grow more rapidly than neighbouring cells. Early stages of laticifer development are characterised by the presence of dense cytoplasm. The walls of laticifers become thickened except at the extreme ends, as pointed out by Fahn (1979); the walls become wavy at maturity. The glucoside is supposed to be the main constituent of the latex (Chopra et al. 1983). Some important constituents of latex in certain plants are sugars (Compositae), starch grains (*Euphorbia* spp.), tannins (*Musa* sp.), alkaloids (*Papaver*...
somniferum), protein crystals (Taraxacum bicornre) (Mahalberg 1975, Fahn 1979, Uzabakiliho et al. 1987, Craig 1988). In Thevetia, cardiac glucoside, mainly thevetin, and osteoid starch grains are observed in the latex of laticifers differentiated in vitro. A few parenchyma cells adjacent to the laticiferous cells showed the presence of the active principles in traces which may be attributed to intercellular transport. However, it could not be detected in other parenchymatous cells. The cardiac glucoside was found in the cultures of Thevetia after laticifers were initiated. The quantity of glucoside increased with the age of the cultures (Table 1). Hence, cytochemo-differentiation especially laticifer differentiation

| TABLE 1 — HISTOCHEMICAL COLOUR REACTIONS OF LATICIFERS IN CULTURE |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| REAGENTS USED               | TEST FOR                     | LATICIFERS*                  | OTHER CELLS                  |
|                             |                             | 60d                         | 120d                         | 60d                         | 120d                         | Remarks                                    |
| Liebermann & Burchard (LB)  | Cardiac glucosides          | ++                          | +++                          | +                            | +                            | Strong positive test for cardiac glucosides and complex latex constituent |
| Kedde                       |                             | ++                          | +++                          | –                            | +                            |                                           |
| Millon's                    | Protein                     | +                           | +++                          | ++                           | +                            | Black violet colour was noted only in laticifers |
| KOH (10%) followed by chromic acid (10%) | Latex constituents | ++                          | +++                          | ++                           | ++                           |                                           |
| KI Solution                 | Starch grain (Osteoid type) | ++                          | ++                           | ++                           | ++                           |                                           |

+++ , intense; +++, appreciable; +, moderate; +, traces; – undetectable.

* Laticifers in culture grown on MS medium with various growth regulators.

and related cardiac glucoside biosynthesis in culture is possible. Moreover, as active principle of the plant is confined to these specialised cells i.e., laticifers, their enhanced differentiation regulated by hormones would certainly favour production of useful compounds in culture.

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