Phytomorphology **40** (1&2), pp. 113-117, 1990

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 initiatiation 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-differentitaion 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 (Metcalfe 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 butryic 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).

1. Received for publication : September 6, 1989.

The Lieberamann & 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

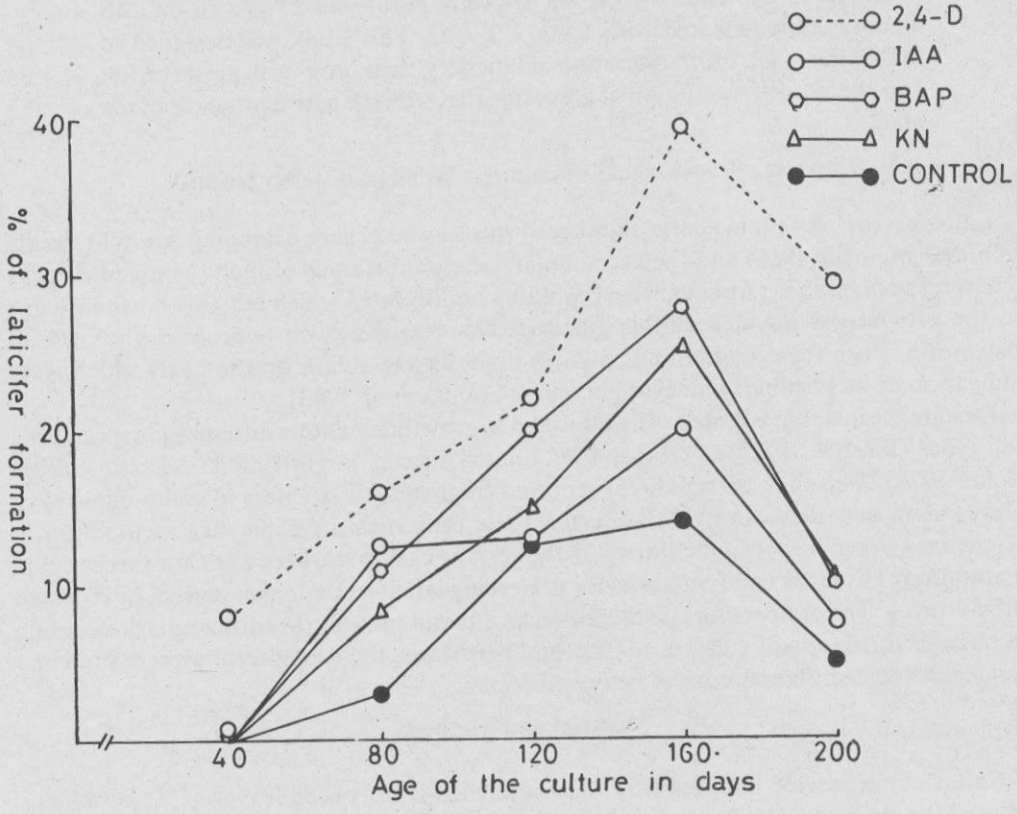

Fig. 1 — Laticifer differentiation in *T. peruviana* in response to growth regulators applied at 1 mg/l (optimum concentration).

with 2, 4-D. BAP and Kn (both at range of concentrations 0.1-2.0 mg/l), in conjunction with 2, 4-D (lmg/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.

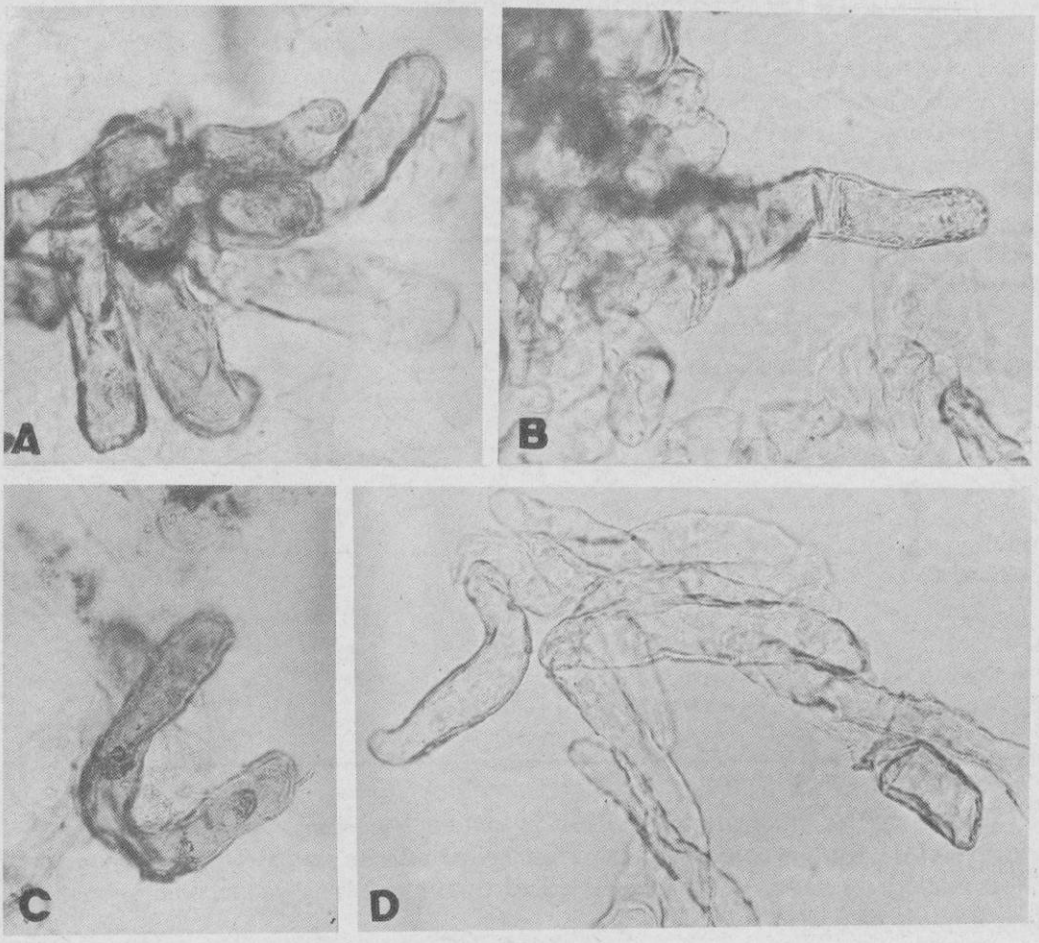

Fig. 2 A-D— In vitro differentiation of laticifers in *T. peruviana.* **A.** Initiation of laticifers from parenchymatous cells. **B.** Further elongation of the differentiated cells. **C.** Isolated non-articulated laticifer with contents. **D.** Further development and elongation leading to laticiferous tubes.

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 bicorne*) (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 paraenchymatous 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-diferentiation especially laticifer differentiation

TABLE 1 — HISTOCHEMICAL COLOUR REACTIONS OF LATICIFERS IN CULTURE

Reagents used	Test for	Laticifers*		Other Cells		Remarks
		60d	120d	60d	120d	
Liebermann & Burchard (LB)	Cardiac glucosides	+	++++	+	+	Strong positive test for cardiac glucosides and complex latex constituent
Kedde	"	++	++++	–	++	
Millon's	Protein	+	++++	++	++	
KOH (10%) followed by chromic acid (10%)	Latex constitutents	++	++++	++	++	Black violet colour was noted only in laticifers
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.

Literature Cited

Biesboer D D 1983 The detection of cells with a laticifer like metabolism in *Asclepias syriaca* L. suspension culture; *Pl. Cell Rep.* 2 137-139

Brower L P, Mc Evoy P B, Willaiamson K L & Tlannery M A 1972 Variation in Cardiac glycoside content of monarch butterflies from natural population in Eastern-North America; *Science* **177** 426-429

Chopra R N, Badhwar R L & Ghosh S 1983 *Poisonous Plants of India.* Vol. I pp 665-669 (Jaipur: Academic Publishers).

Craig L Nessller 1988 Comparative analysis of the major latex proteins of opium poppy; *J. Pl. Physiol.* **132** 588-592

Datta S K & Datta P C 1980 Laticifer in *Euphorbia rivulia; Cell Chro. News Lett.* 3 46-47

Fahn A 1979 *Secretory Tissues in Plants;* (London: Academic Press)

Harborne J B 1973 *Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis;* (London: Chapman & Hall)

Inamdar J A & Murghan R B 1987 Staining methods for the detection of laticifers in plant tissue; *J. Microse.* (OXF) **147** 347-352

Mahlberg P G 1975 Evolution of the laticifer *Euphorbia* as interpreted from starch grain morphology; *Am. J.* Bot. **62** 577-583

Metcalfe C R 1966 Distribution of latex in the plant kingdom. Notes Fodrell Lab. III

Metcalfe C R 1983 Laticifer and Latex: In *Anatomy of Dicotyledons* Vol. II; pp 70-81 eds C R Metcalfe & L Chalk (Oxford: Clarendon Press)

Milanez F R 1959 Contribucao ao conhecimento anatomico de. *Cryptostegia grandifolia I; Embriao Rodriguesia* **18-19** 351-395

Milanez FR & Neto HM 1956 Origem dos laticiferos do embariao de *Euphorbia pulcherrima* Wild.; *Rodriguesia* **18-19** 351-359

Murashige T & Skoog F 1962 A revised medium for rapid growth and bioassay with tobacco tissue culture; *Physiologia Pl.* **15** 473-497

Uzabakiliho B, Largean C & Cassadevall E 1987 Latex constituents of *Euphorbia candelabrum, Euphorbia grantii, Euphorbia tirucalli* and *Synaderim grantii; Phytochemistry* **26** 3041-3046

Wilson H M & Street H E 1975 The growth anatomy and morphogenetic potential of callus and cell suspension culture of *Hevea brasiliensis; Ann. Bot.* **39.** 671-682

Zhao Xin Qian 1987 The significance of the structure of laticifer in relation to the exudation of latex in *Hevea brasiliensis; J. Nat. Rubber Res.* **2** 94-98