lower concentration, multiple shoots were formed. With NAA alone, callus formation was followed by profuse root formation and simultaneous shoot an root elongation. ON BAP and NAA together, with increase in the concentrations, profuse callus was formed. With BAP at lower concentration, together with varying concentrations of kn, multiple shoots were formed. With increase in BAP concentration callus formation was faster than shoot development.

1.4 USE OF ORDINARY TABLE SUGAR AS CARBON SOURCE FOR *IN VITRO* CULTURE OF *Dendrobium joanie OSTENHOLT*

Madhuri Sharon and Manisha Sharon

C C Shroff Research Institute Excel Estate, Goregaon (W), Bombay - 400 062

Calli of *Dendrobium joannie* Ostenholt which were growing for nearly 24 months on Vacin and Went (1949) ,media supplemented with 15% coconut milk and 2% sucrose as carbon source were taken as explant and subcultured on the same media except that carbon source was ordinary table sugar instead of sucrose Four different concentrations of sugar were tried. Growth was analysed using following parameters - increase in fresh and dry weight, total chlorophyll content, mitotic index and glucose and sucrose- content of growing callus It has been noticed that there is no alteration in growth in initial stages

1.5 ALKALOID PRODUCTION IN SUSPENSION CULTURES OF *Holarrhena antidysenterica* WALL.

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Steroidal alkaloid formation in cell suspension culture of *Holarrhena antidysenterica* Wall is reported here for the first time Root, stem and leaf of *Holarrhena* seedling were used as a source of explant Among the three types of cell suspension cultures, root cells produced maximum amount of total alkaloids. Five different steroidal alkaloids were identified by TLC from cells as well as from spent medium of cell suspension cultures.

1.6 *IN VITRO* STUDIES ON EXCISED ROOT CULTURES OF *Boerhaavia diffusa* L.

Neeta Shrivastava and M.A. Padhya

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Boerhaavia diffusa (Nyctaginaceae) is known as 'Punarnava'. It is known for its medicinal importance due to the alkaloids accumulated in the roots.

The present investigation deals with the establishment of *in vitro* excised root cultures of *Boerhaavia diffusa* The nutritional/hormonal levels of the Murashige and Skoog's (1962) medium were standardised and with the application of IBA, profuse lateral roots were induced to the main root without its further development Biomass production of excised roots were obtained, which showed the presence of the alkaloids, specific to this plant. Enlarged parenchymatous cells were observed during their anatomical studies

FREE COMMUNICATIONS

29th SEPTEMBER, 1992 TIME : 5.00 to 6.30 P.M.

AR01 STANDARDIZATION OF A HERBO-MINERAL IMMUNOREGU LATORY FORMULATION

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Pharmacognostical & clinical evaluation of a herbo-mineral product has been carried out Immunomodulatory agents of plant origin have been claimed to be effective in infections disease and immunodisorder Incorporation of such product in herbal formulation is the modern trend Hence such studies were undertaken. The details of investigations would be presented

AR02 IN VITRO EXCISED ROOT CULTURES OF BOERHAAVIA DIFFUSA : A SOURCE OF ALKALOID.

Neeta Shrivastava and M. A. Padhya

Tissue Culture Lab. Dept. of Botany, Faculty of Science, The M. S. University of Baroda

One of the biotechnological applications in the field of tissue culture is excised organ culture for the production of medicinally important plant products *Boerhaavia diffusa* L (Family . Nyctaginaceae) is very well known for its use in Ayurvedic system of medicine

The present study deals with the establishment of continuous cultures of excised roots of *Boerhaavia diffusa* which gave positive indication for the alkoloid production Murashige and Skoog's (1962) medium containing sucrose (2%) supplemented with kinetin (1 μ M/l) and Indole-buteric acid (2 μ M/l) was found ideal for optimal biomass of excised root production The presence of alkoloid was confirmed by squash method and testing it with Dragandorffs reagent. The results will be discussed in details

AR03 INVESTIGATION OF PLANT DRUGS FOR ANTI-PEPTIC ULCER ACTIVITY

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University Dept. of Chemical Technology, Bombay University, Matunga (E) BOMBAY-19

Peptic ulcer is an exconated area of the mucosa caused by the digestive action of gastric juice A peptic ulcer can be caused by the excess secretion of acid and pepsin by the gastric mucosa and by the diminished ability of the mucosa to protect the stomach wall The Ayurvedic system, has identified the disease and prescribed a number of effective formulations for not only the symptomatic relief but also for permanent cure The clinical experience on the empencal use of these preparations are quite encouraging However, there are no reports on the pharmacology of these drugs as anti-peptic ulcer agents

The present study deals with the screening of plant drugs for anti-ulcer activity in rats Ulcers were developed by inducing HCl - Ethanol mixture The results obtained with more than 40 plant drugs is the subject matter of this investigation.

'Punarnavine' profile in the regenerated roots of *Boerhaavia diffusa* L. from leaf segments

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Roots were regenerated from the leaf segments of *Boerhaavia diffusa* L. when cultured *in vitro* on MS (Murashige and Skoog) medium containing sucrose and indole-3-acetic acid (IAA). At the end of four weeks, about 17 roots, 10 cm long and containing 0.15% of the alkaloid punarnavine, developed from leaf segments of the third leaf (in serial order of development from the apex) when the IAA level of the medium was 0.5 μM/l. Increase in IAA levels of the culture media not only reduced the number of roots regenerated from the leaf segment but also reduced their length and alkaloid content. Replacing IAA by α-naphthaleneacetic acid caused no morphogenic response. However, treatment with 2,4-dichlorophenoxyacetic acid induced the development, from leaf segments, of callus which differentiated into roots having no capacity for alkaloid accumulation. Histological observations revealed that the roots were initiated from the phloem parenchyma of the cultured leaf segments.

BOERHAAVIA diffusa L. (family Nyctaginaceae) is a perennial herb commonly known as *punarnava*. Root extracts of this plant find applications as antihepatotoxic[1] and antiviral agents[2]. It cures corneal ulcers and night blindness[3]. The active principle lies in the alkaloid fraction – known as *punarnavine*[4] – of the root extract. Experimental work done on screening of the roots from garden-grown *in vivo* plants of *Boerhaavia diffusa* of different ages has shown that maximum alkaloid content (2%) was accumulated in the roots of three-year-old mature plants[5]. Due to extensive industrialization in and around Baroda, the high alkaloid yielding roots of *Boerhaavia diffusa* are scanty. An alternative possibility which is gaining importance nowadays, is producing roots *in vitro*. There are reports about *in vitro* root regeneration from excised leaves of the aerial organs of *Lycopersicon esculentum* Mill and *Begonia*[6, 7].

The present paper describes the possibility of *in vitro* regeneration of roots from excised leaf segments of *Boerhaavia diffusa* L. The alkaloid profile of these regenerated roots has been examined.

Healthy leaves, first to fifth from the apex of *Boerhaavia diffusa* plants grown in Botanical Gardens of the M S University of Baroda, were collected. They were washed under tap water, surface-disinfected with mercuric chloride (0.1%) for 1–2 minutes, washed with sterile distilled water and inoculated on to (30 ml) Murashige and Skoog's (MS)[8] medium containing sucrose (BM) supplemented with one of the auxins such as indole-3-acetic acid (IAA), α-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) at various levels (0.5–4.0 μm/l). BM was used as the control. The pH of the test media was adjusted to 5.7 before gelling with agar (0.8%). Culture flasks, six replicates per treatment, were incubated in culture room at

Table 1. Regeneration of roots from the third-leaf segments of *Boerhaavia diffusa* L. and their punarnavine content recorded after 4 weeks of culture period

	Level of auxin (μm/l)	Number of regenerated roots	Length of root in cm	Response (%)	Alkaloid content on dry-wt basis (%)
	00	–	–	–	–
IAA					
	0.5	17	10 ± 1	80	0.15
	1.0	9	5 ± 1.5	80	0.05
	2.0	7	3.5 ± 0.5	90	0.02
	4.0	4	1.5 ± 0.2	80	–
NAA					
	0.5	–	–	–	–
	1.0	–	–	–	–
	2.0	–	–	–	–
	4.0	–	–	–	–
2,4-D					
	0.5	Callus + roots	–	–	–
	1.0	Callus + roots	–	–	–
	2.0	–	–	–	–
	4.0	–	–	–	–

Results of six replicates
– No response

25 ± 2°C with 16 h photoperiod (1000 lux)

For histological studies paraffin blocks were prepared from leaf pieces with root primordia, which were fixed in FAA (40% formalin. glacial acetic acid 50% alcohol in 5:5.90 ratio) and dehydrated in alcohol–xylene series[9] Sections of 10 µm were cut using a rotary microtome and were stained with toluidine blue. Mounting was done in Canada balsam Photomicrographs were taken with Carl–Zeiss microscope with automatic photographic equipment. The presence of alkaloid in the root squashes was detected by Dragandroff's reagent and its quantification was done according to the method described by Huber[10] The total alkaloid content was estimated by titrating the extract of oven-dried powder (0.2 g) in glacialacetic acid mixed with acetic anhydride, using crystal violet as an indicator against 0 1 N acetus perchloric acid (prepared by mixing 8 5 ml of perchlorrc acid with 500 ml of glacialacetic acid and 21 ml of acetic anhydride. The final volume was made to 1000 ml by adding glacialacetic acid). The total content of the alkaloid punarnavine was expressed on dry-weight basis

Excised leaf segments, from first to fifth leaf in their serial order of development, cultured on BM (control) turned brown within four weeks of incubation This indicated that exogenous supply of a growth regulator was necessary for root regeneration from leaves.

Segments of the third and fourth leaves cultured on BM + IAA (0 5 µm/l) medium regenerated roots within one week of the culture period (Figure 1 *a*) The highest number of roots, up to 17, were produced from the segments of third leaf at the end of four weeks of the experimental period (Figure 1 *b*) The results recorded in Table 1 show that these roots reached a length of 10 cm and accumulated a maximum of 0.15% alkaloid (dry-weight basis) at the end of four weeks Further increase in the IAA levels to 1 and 2 µm/l of the culture medium not only reduced the number of roots to 9 and 7 but also reduced their length to 5 and 3.5 cm, respectively (Figures 1 *c*, *d*) This was associated with a sharp decline in the quantity of alkaloid accumulated in the roots from 0 15% to 0.05% and 0 02%, respectively Callus along with few short roots was regenerated when the level of IAA in the culture medium was 4 µm/l (Figure 1 *e*), with a further decline in alkaloid production. Thus, the quantity of IAA present in the culture media showed pronounced effect on the morphology of regenerated roots and on their biosynthetic potential for alkalo profile It appeared that in *Boerhaavia diffusa*, rege eration of roots from leaf segments cultured *in vitro* w an autonomous process, as observed in *Haplopapp revnil*[11] In *Nicotiana tobacum cv Xanthi nc* from tl fourth leaf, *in vitro* rhizogenesis was observed with the application of IBA[12]

Leaf segments cultured on BM + NAA (0.5–4.0 µm/l) failed to regenerate roots (Table 1) However, in the presence of 2,4-D (0 5 and 1 µm/l) in the medium, white callus was produced within one week of culture perio which regenerated roots at the end of four week (Figure 1 *f*) These roots contained traces of alkaloi These results suggest that the quality and quantity o auxins supplemented to the cultures of leaf explants a fected the type of regeneration as well as secondar metabolite production, as has been reported in severa other organ cultures[13–15]

Histological observations confirmed that roots wer initiated from the phloem parenchyma of the veins o leaf segments (Figure 1 *g*) Similar results have bee noticed in *Lycopersicon esculentum* Mill[6]

The present study clearly demonstrates that leaf ex plant of *Boerhaavia diffusa* L possessed root regenera tion capacity which was triggered by the application o IAA The alkaloid punarnavine profile in the regener ated roots was directly affected by the IAA level in th culture medium.

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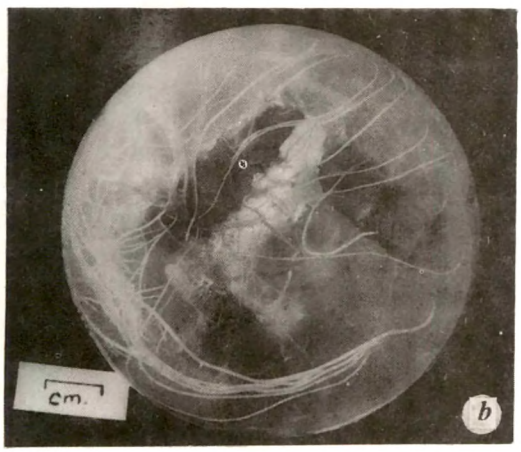
Figure 1 *a–g*. *a*, Roots (R) regenerated from third-leaf segments on BM + 0 5 µm/l of IAA, after one week of culture period, *b*, Profuse rhizogenesis on BM + 0 5 µm/l of IAA, after four weeks of culture period, *c*, Pattern of rhizogenesis on BM + 1 0 µm/l of IAA, after four weeks of culture period, *d*, Short roots regenerated on BM + 2 0 µm/l of IAA, after four weeks of culture period, *e*, Callus (C) with short roots on BM + 4 0 µm/l of IAA, after four weeks of culture period, *f*, Profuse growth of callus (C) with roots from excised leaf in response to 2,4-D (0 5 µm/l, after four weeks of culture period), *g*, Section of cultured leaf segment showing root initiation (R) from phloem parenchyma (ph) (160 ×) EP – upper epidermis, P –palisade, VB – vascular bundle


a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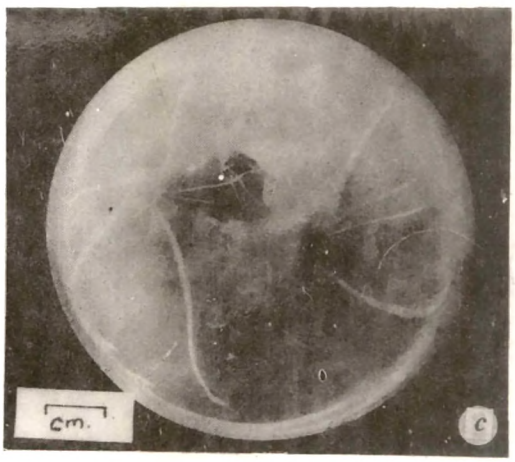
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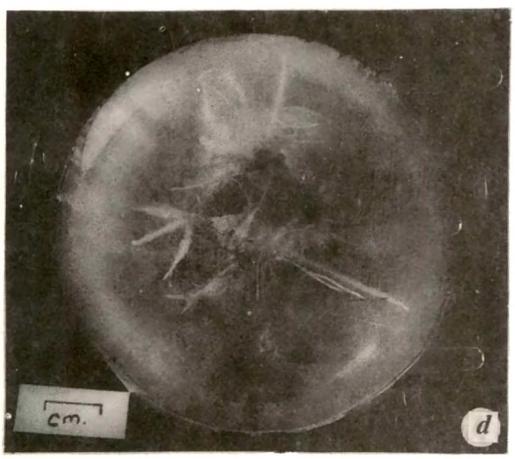
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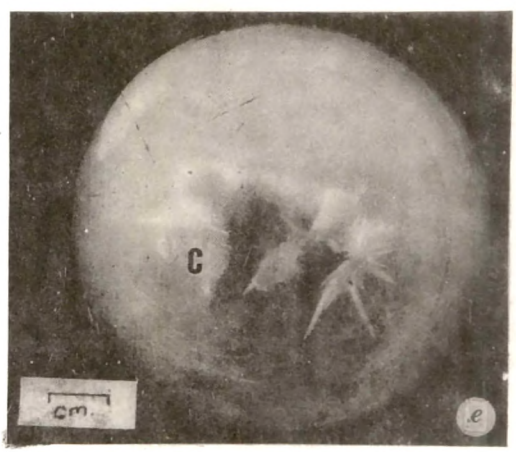
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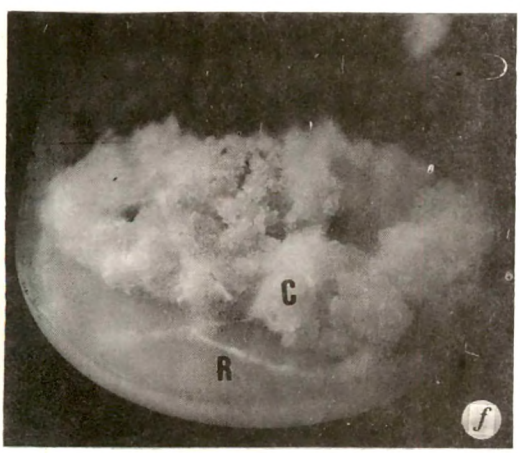
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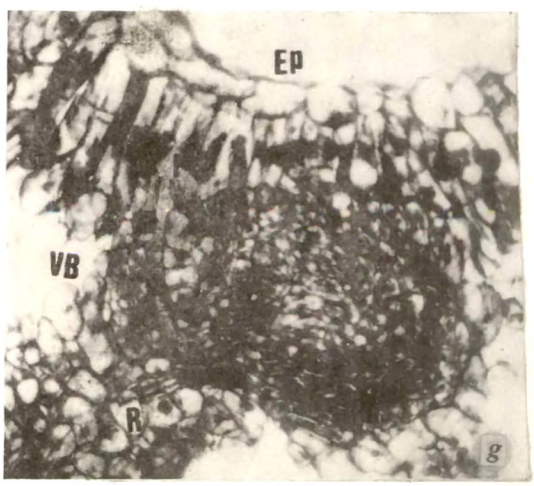
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