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JP-2000

In Vitro Propagation and Quantification of Rotenoids in Callus of Derris sp. J.E.B.P. PINTO; H.E.O. Conceição; N.E.A. Castro; E.J.A. Santiago, and O.A. Lameira. Laboratory Tissue Culture, UFLA Cx.P.37, LAVRAS-MG, 37200-000 BRAZIL. E-mail: jeduardo@ufla.br

The Amazonian ecosystems are rich in plants with insecticide properties. In this work, in vitro techniques of propagation and quantification or rotenoids in callus of Derris sp were applied. In vitro propagation of na endangered insecticide plant was achieved by culturing the nodal segment explant. Nodal segment containing two axilar buds showed better development in number and size of the shoots than one axilar bud. Shoots were rooted on MS/2 basal medium and soaked for 30 seconds in indole-3-butyric acid (IBA) at 2,000 mg/L with pH adjusted to 4.5. Derris sp did not show any multiple shoots type. This species showed multiplication through nodal segments. Plantlets with a morphologically normal appearance were transferred to soil and acclimated in the growth chamber for 30 days. Callus culture were established from root segment of seedlings germinated in vitro on Murashige and Skoog (MS) basal medium supplemented with 1.6 mg/L naphthalenecaccetic acid (NNA) + 1.0 mg/L benzylaminopurine (BAP). The most efficient culture medium of maintanance of callus was provided on basal MS medium supplemented with 2.0 mg/L NNA + 2.0 mg/L BAP. Callus from root segment presented positive response to biosynthesis of rotenoid compound.

JP-2001

Extraction and Detection of Kavapyrones from In Vitro Cultures of Kava (*Piper methysticum* Foster). H. Kobayashi, M.A.L. Smith, M. Gawienowski, and D. Briskin. Dept. of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801. E-mail: hkobayas@uiuc.edu

The roots and rhizomes of kava (*Piper methysticum* Foster), a South Pacific medicinal herb, are used as a phytomedicinal treatment for anxiety, tension, agitation, and insomnia. Slow maturity, sterility, and diseases threaten the supply of this medicinal herb. The objectives of this study are to develop kava micropropagation and kavapyrone production *in vitro* to support conventional kava production and future bioreactor-based production of kava phytomedicinals. Young, expanding leaves from greenhouse kava plants ('Awa' and 'Makea') were introduced to modified 1/2 Murashige and Skoog media containing Plant Preservative Mixture (PPM, 2.0 ml L^{-1}), and, in mg L^{-1}, 2,4-dichlorophenoxyacetic acid (2,4-D, 2.0), or a-naphthalenacetic acid (NAA, 0.1) and N6-benzylaminopurine (BA, 0.5). Despite severe and persistent contamination, callus initiation subsequently occurred on media with 2,4-D after four weeks, and formation of protuberances resembling embryos were observed within two months. Root regeneration occurred after transfer of calli to within one month to 1/2 MS media with NAA at 2.0 mg L^{-1}. High Performance Liquid Chromatography and Thin Layer Chromatography analyzed the methanolic extraction of callus and regenerated roots from callus, along with roots of greenhouse plants. The amount of kavapyrones detected from the callus sample by HPLC was significantly less than that of kava roots from the greenhouse, while the amount of kawain from regenerated roots was comparable to that of roots *in vivo* on the TLC plate.

JP-2002

Light Does Not Regulate All Steps in the Mevalonat way of Terpenoid Biosynthesis. F. SOURET, P. Weatl partments of Biology and Biotechnology, and Chen istry, Worcester Polytechnic Institute, Worcester, ! souret@wpi.edu

Two distinct terpenoid pathways have been character leading to the biosynthesis of isopentenyl diphosphat cytosolic mevalonate pathway and the recently chara independent pathway, postulated to be located in pla demonstrated that the mevalonate independent patl involved in sesquiterpene production that normally o *Artemisia annua* transformed hairy roots produce a terpene lactone, with effective anti-malarial activity. portance of sesquiterpenes as natural products anc roots of *A. annua* as a unique biotechnological moc ulation of terpenoid biosynthesis, we decided to inv zymes involved in the mevalonate independent pat interest is the enzyme performing the first committe onate independent pathway, 1-deoxyxylulose-5-phosp ase (DXPR). Using RT-PCR, we have isolated a p that was then used to screen a cDNA library. We isola cDNA clone characterized by a 1.4 kb ORF encodi of 471 aa. Bacterial expression of DXPR cDNA confi encodes a protein of roughly 50 kDa. Northern blot a DXPR was constitutively expressed in normal greer and in transformed roots and was not affected by cu while light exposure caused a significant transcriptio DXPS, the enzyme immediately upstream of DXP scription levels of DXPR was unchanged upon ligh currently investigating other factors that could influ levels in our transformed roots.

JT-2003

In Vitro Effects of Semipure Protease Inhibitor Fractions Malignant Cell Survival. T. GARCIA-GASCA, L. A. Salaz Olaya, C. Aguirre and A. Blanco-Labra. Department of Bio nology, Cinvestav Unit for Biotechnology and Genetic E School and Medicine School, Queretaro Autonomous Univ Queretaro 76010, Qro. MEXICO. E-mail: alter@sunserver.

Protease inhibitors (PI) have been described as the first die cancerigen potential. Among them, the one that has receive soybean Bowman-Birk inhibitor, which presently is under its suitability as a therapeutic agent. However, few PI form so far been investigated on this context. Here, we report semipure PI fractions extracted from seeds of chan (*Hyptis* amaranth (*Amaranthus hypochondriacus*) (A-PIF), on the s cells. Protein fractions with PI activity, obtained after pu extract of those two seeds through a G-75 Sephadex colum using three different cell lines: HeLa cells, a human tran epithelial origin; a murine transformed fibroblastic cell line a normal fibroblastic 3T3 cell line. Normal and transformed tained in Dulbecco's modified Eagle medium (DMEM) wit whereas for HeLa cells, the same conditions were used but added; all cell lines were incubated at 37° C under a 10% C atmosphere. Cells were seeded (0.8 or 1.0 x 10^4 cells /well with 5% CS DMEM. After 48 h, medium was removed an with 100, 500, 750 and 1000 UI/mL of C-PIF or A-PIF in bovine serum albumine (ASB). Two control treatments v supplemented with 1% BSA and DMEM added with 5% 72 h, cell number was estimated. Dose-response curves sl HeLa cells was not affected by C-PIF, however A-PIF decr up to 26%. Proliferation of transformed 3T3/Ha-ras fibrob to 59% and 49% in the presence of C-PIF and A-PIF re normal 3T3 fibroblasts were not affected by any of the two tr show that both, C-PIF and A-PIF affected cell survival of cells, and that such effect is dependent on cell lineage and