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Roots of *Rubia tinctorum* and *Morinda citrifolia* are used to study the role of cellular differentiation in anthraquinone biosynthesis. Root cultures of *Rubia* and *Morinda* transformed with *Agrobacterium rhizogenes* have been established and they provide a structured *in vitro* system that will be used to relate the biochemical events to processes in the intact plant. Anthraquinone accumulation was quantified and the distribution in the root tissue was studied. The localization of anthraquinones at the (sub)cellular level was determined with confocal laser scanning microscopy. Cell suspension cultures of *Rubia* and *Morinda* which are inducible by changes in the composition of the medium were studied for comparison. These cell cultures are also well suited to investigate the effects of rapid anthraquinone accumulation on cellular metabolism.

Future studies will focus on immunolocalization of the important key enzymes of the shikimate pathway and of anthraquinone biosynthesis, in an attempt to specify the cell types in which this biochemical process occurs.

Effects of Glyphosate on Cell Suspensions of *Morinda citrifolia*

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One important question in secondary metabolite research is how plants divide available resources over primary and secondary metabolic routes. Many biosynthetic routes have several branching points where precursor molecules are channelled either into secondary routes or remain in primary pathways. Secondary metabolites known as anthraquinones are produced by a route which branches off the shikimate pathway at the point of chorismate. Chorismate remaining in the shikimate pathway is converted into the aromatic amino acids tryptophan, tyrosine and phenylalanine. This means that chorismate, being a common precursor for both secondary and primary metabolites, marks an important regulatory point in the flow of precursors through the shikimate pathway.

In order to understand more about the metabolic regulation of this system, the activities of several important enzymes are being investigated in both anthraquinone-producing and non-producing cells. The first enzyme of interest is isochorismate synthase, which catalyzes the conversion of chorismate into isochorismate, the first committed step in the production of anthraquinones. Chorismate mutase converts chorismate into prephenate (a precursor of the amino acids tyrosine and phenylalanine) and phenylalanine ammonia lyase is involved in the conversion of phenylalanine into trans-cinnamic acid, from which many secondary compounds are formed via the phenylpropanoid pathway.

When investigating enzyme regulation an interesting feature of the shikimate pathway is the possibility to block the formation of chorismate via the herbicide glyphosate (N-(phosphonomethyl)-glycine). Adding glyphosate to the culture medium results in cells no longer producing aromatic amino acids and therefore reduces growth. However, it may be possible to reverse the growth inhibitory effect of glyphosate by providing the cells with aromatic amino acids in the growth medium. In that case it would be possible to study metabolic regulation of shikimate pathway enzymes in normal growing cells while part of the pathway is blocked.

Effect of Elicitation on Isochorismate Synthase in Anthraquinone-producing Cell Cultures of *Rubia tinctorum*

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Cell cultures of *Rubia tinctorum* produce substantial amounts of secondary metabolites in contrast to most cell cultures. These secondary metabolites are called anthraquinones. In the Rubiaceae, anthraquinones are synthesized via the shikimate-o-succinylbenzoic acid pathway. One important step in this pathway is the conversion of chorismate into isochorismate, a reaction catalyzed by the enzyme isochorismate synthase. This reaction is the branchpoint of anthraquinone biosynthesis and the primary shikimate pathway and is therefore a potential site for regulation of flow into secondary metabolism. We investigate whether this enzymatic conversion is indeed a rate-limiting step in the biosynthesis of anthraquinones.

Elicitation with a fungal extract resulted in a substantial increase in anthraquinone production, which is preceded by a large rise in isochorismate synthase activity. Application of inhibitors of translation or transcription annihilates the effect of elicitation on isochorismate activity and anthraquinone production. These results indicate that elicitation requires *de novo* RNA synthesis.

Partial purification revealed the presence of at least two isoenzymes. Native PAGE showed a molecular weight of about 95 kD for both enzymes. The

enzymes are characterized with respect to kinetic properties.

Genetically Stable Cell Lines of Cucumber for the Large-scale Production of Diploid Somatic Embryos

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We studied the initiation of embryogenic cell lines from excised radicles of cucumber (*Cucumis sativus* L.) cultured in liquid medium. The culture regime, explant density and type and concentration of hormones were adjusted so that pro-embryogenic masses (PEMs) were formed within about 8 weeks. The established cucumber cell lines were maintained for several years without loss of embryogenic and genetic stability. The ploidy level of somatic embryos from different cucumber cell lines was either diploid or tetraploid and depended on the ploidy level of the cell line. Cucumber cell lines that produced only diploid embryos were obtained by selecting completely diploid explant material and growth in the dark during the initiation phase. Mixoploid explants could lead to tetraploid or mixoploid cell lines. Isolation and additional selecting and subculturing of single PEMs resulted in either completely diploid or completely tetraploid cell lines, indicating that all cells of individual PEMs are either diploid or tetraploid. The embryogenic cucumber cell lines differing only in ploidy level were indistinguishable in growth rate and embryogenic potential and were genetically stable over several years.

MEETING OF THE SECTION FOR PLANT SYSTEMATICS AND GEOGRAPHY ON 15 MARCH 1996

On the Genus *Trichosanthes* (Cucurbitaceae) in Java

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Cucurbitaceae is a middle-sized family of pantropical and temperate areas. All species are susceptible to frost. Most are trailing. There are *c.* 120 genera with 850 species, indicating that most genera contain few species. Cucurbitaceae are at present being studied in Leiden for Flora Malesiana. In that area 27 genera occur (including those known only in cultivation), with 60–70 species. One of the larger SE Asian genera is *Trichosanthes* L., with some 50 species, ranging from India and China (and Japan) through Malesia into the Pacific and Northern Australia. *Trichosanthes* is readily recognizable by the longly fringed petals. Malesian *Trichosanthes*, some 20–25 species, will be revised in Bogor, in cooperation with Leiden.

Some characters of Cucurbitaceae, also present in *Trichosanthes*, are dioecism, night-flowering, the presence of a pro-bract, the typical lateral insertion of the tendril and the typical construction of the androecium. Pollen and seed morphology will probably be of great importance taxonomically, but sufficient research is still lacking.

Study of the taxonomy of the species of *Trichosanthes* in Java will result in accepting nine species, compared with eight species in Backer (*Flora of Java* **1,** 1963) and 10 species in Blume (*Bijdrage Fl. Ned. Ind.*, 1826). However, Backer's revision contains important differences: *T. trifoliata* (L.) Merr. should be renamed as a new species because of misinterpretation of the type; *T. anguina* L. should be regarded as a cultivar of *T. cucumerina* L., and the species accepted in Backer (1963) under the name *T. bracteata* (Lam.) Voigt (a continental SE Asian species), appears to represent three different quite distinct species, especially evident when examining living specimens in the field: *T. tricuspidata* Lour., *T. pubigera* Blume and *T. quinquangulata* (A. Gray).

Morphological Variation of Recent Invaders in Northern Central America: the case of *Malmea* (Annonaceae)

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Cluster analysis was used to reveal patterns of morphological variation in a species complex of *Malmea* (Annonaceae), distributed in eastern Mexico, Guatemala, Belize and Honduras. Initially *M. depressa*, *M. gaumeri*, *M. leiophylla* and *M. guatemalensis* belonged to this species complex.

In total 53 characters were used for the cluster analysis, among which were 50 leaf, flower and fruit morphological characters. Of these, 24 characters significantly determined clustering of 238 herbarium specimens into 12 clusters. No cluster is exclusively specified by any character or combination of characters, nor can any geographical pattern be detected, except for the clustering of specimens from Los Tuxtlas Biological Station, Veracruz, Mexico. A new