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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