Improved Protocol for the Propagation of Narcissus in vitro

Merel Langens-Gerrits* and Shigeru Nashimoto†.

* Bulb Research Centre and Centre for Plant Tissue Culture Research, PO Box 85, 2160 AB, Lisse, The Netherlands; † University of Tokyo, Japan

Narcissus is propagated in vitro using ‘twin-scales’ (two longitudinal segments cut from adjacent scales, connected by the basal plate) as initial explant. Shoot clusters or bulblets regenerating on these twin-scales were used for further propagation. When shoot clusters were subdivided, the propagation rate decreased after some cycles, possibly because the basal plate, from which the shoots originate, lost the capacity to regenerate. The propagation rate was improved by an intermediate bulb formation phase. For this, shoots were cultured separately on bulbing medium. From the bulblets, ‘mini twin-scales’ were cut: depending on the size, two to four twin-scales per bulblet. On every twin-scale two to three new shoots regenerated. The procedure of mini twin-scales can also be used for bulblets regenerated on the original explant. The propagation rate of longitudinally cut bulblets was very low. After propagation, bulblets were planted in soil. A very important factor for good performance after planting was the presence of roots. Without roots, a cold treatment was necessary for sprouting. However, the leaves remained short (0-5-1 cm) and died after a few weeks. When roots were present at planting, bulblets sprouted and grew very well, even without a cold treatment.

In vitro Propagation of Rose Rootstock 'Sturcinq'

A.M.A. van Mil-Vieveen and H.J. van Telgen.
Centre for Plant Tissue Culture Research, PO Box 85, 2160 AB Lisse, The Netherlands

Rose rootstock Rosa canina ‘Inermis’ clone ‘Sturcinq’ gives a very high production of good quality flowers of the scion cultivar grafted upon it. Propagation of ‘Sturcinq’ by cutting or grafting is difficult due to a low rooting percentage. In many woody plants, rooting of cuttings from tissue-cultured stock-plants is greatly improved compared to cuttings from conventional stockplants. Therefore a tissue culture protocol for rose rootstock ‘Sturcinq’ has been developed.

For initiation, surface-sterilized axillary buds were placed on full strength MS-medium solidified with 7 g/l agar, containing 45 g/l sucrose, 0-1 mg/l 6-benzylaminopurine (BAP) and 96 mg/l FeEDDHA (Van der Salm et al. (1994): Plant Cell Tiss. Org. Cult. 37: 73-77). Glucose was slightly negative for initiation. There was no difference in yellowing of the plantlets growing on medium with NaFeEDTA or with an equimolar amount of FeEDDHA.

Outgrowth of axillary buds of the shoots was barely increased by adding higher concentrations of BAP. Propagation was strongly stimulated by adding liquid MS-medium with 45 g/l sucrose on top of the solid medium (with 1 mg/l BAP). The highest fresh and dry weights were obtained if 4 ml liquid medium per tube were added after 2 weeks of culture. The highest propagation rate was obtained when 3 ml were added after 2 weeks.

Shoots were rooted on 1/2 MS, 20 g/l sucrose, 48 mg/l FeEDDHA, 7 g/l agar and 0-3 mg/l indole-3-butyric acid (IBA). Even without addition of auxin all shoots rooted. With 0-1 mg/l IBA significantly more roots per plantlet were formed. Shoots could be rooted directly in rockwool plugs. The highest rooting percentage (97%) was obtained after rinsing the plugs with 0-1 mg/l IBA. The highest percentage of acclimatized plants (91%) was reached at 0-05 mg/l IBA.

Effect of Cytokinins on Axillary Bud Growth of ‘Madelon’ Roses

Janneke A. Dieleman. DLO-Research Institute for Agrobiology and Soil Fertility (AB-DLO), PO Box 14, 6700 AA Wageningen, The Netherlands

An in vitro single node system was used to study the response of axillary buds of ‘Madelon’ roses to the cytokinin free bases BA, Z, iP, (RS)DHZ, R(+)/DHZ, S(−)/DHZ and their ribosides. Addition of cytokinins was necessary for the bud to grow out to a fully developed shoot. Bud break, expressed as the number of days until the first compound leaf was visible, was not affected by the presence or the type of cytokinin.

Cytokinin ribosides were equally active as their cognate free bases. When the cytokinin concentration in the medium was raised from 0 to 32 μM, the dry weight of the total plant increased. Depending on the type of cytokinin added, the optimum was reached at 3-2 or 32 μM. The number and dry weight of secondary and tertiary shoots increased with exogenous cytokinin concentrations. The cytokinin activity was in the decreasing order

Z=BA>iP>S(−)/DHZ>(RS)DHZ>R(+)/DHZ.

Anthraxinone Production in Agrobacterium rhizogenes Transformed Roots and Cell Suspension Cultures of Rubia and Morinda


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*

M. Stalman, A.M. Koskamp, A.F. Croes and G.J. Wullems. Department of Experimental Botany, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

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. This reaction is the branching point of anthraquinone biosynthesis and the primary shikimate pathway. The isochorismate synthase, which catalyzes the conversion of chorismate into isochorismate, is 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 Activity in Anthraquinone-producing Cell Cultures of *Rubia tinctorum*

L.J.P. van Tegelen*, R.J.M. Bongaerts†, A.H.W. Toebe*, A.F. Croes*, R. Verpoortef and G.J. Wullems*. † Department of Experimental Botany, University of Nijmegen; *Leiden/Amsterdam Center for Drug Research, Department of Pharmacognosy, University of Leiden, The Netherlands

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-phenylpropanoid 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 branching point of anthraquinone biosynthesis and 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 substantial production of anthraquinone, 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 synthase 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