Plant Tissue Culture & Biotechnology

Netherlands Society for Plant Biotechnology and Tissue Culture
&
Belgian Plant Tissue Culture Group

3 October 2003

Van der Valk Hotel, Maastricht
Nijverheidsweg 35, 6227 AL Maastricht

9.30 **Reception & registration** (with coffee and tea)

10.15 **Opening**

10.30 **Marc van Montague** (Ghent University, Belgium)
Agrobacterium: thirty years later, still so much to learn

11.10 **Rob Dirks** (Rijk Zwaan, Netherlands)
Reverse Breeding: a good alternative for apomixis?

11.40 **Wim Rook** (Royal van Zanten, Netherlands)
Commercial micropropagation

12.10 **Andreas Mordhorst**
Analysis of mutant somatic embryos: zygotic and somatic embryogenesis share the same developmental program

12.40 **Lunch**

14.00 **Amos Richmond** (Ben-Gurion University, Israel)
The case of microalgae agriculture

14.30 **Rene Wijffels** (Wageningen University, Netherlands)
High-value compounds from sun and seawater

15.00 **Heinz Martin Schumacher** (Deutsche Sammlung von Mikro-organismen und Zellkulturen, Germany)
Strategies for the maintenance of undifferentiated plant cell lines as tools for research

15.30 **Henri van Onckelen** (University of Antwerp, Belgium)
Cytokinins and cell cycle progression

16.00 **Closing** (coffee and tea thereafter)

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Abstracts:

Reverse Breeding: a good alternative for apomixis?

Rob Dirks
Rijk Zwaan, Netherlands

The suppression of recombination during meiosis followed by doubled haploid technology results in surprising new applications for plant breeding.

Analysis of mutant somatic embryos: zygotic and somatic embryogenesis share the same developmental program

Andreas Mordhorst

Embryogenic cell cultures of three *Arabidopsis thaliana* mutants lacking an embryonic shoot apical meristem (SAM), *stm*, *wus* and *zll*, were established. Somatic embryos recapitulated the mutant phenotype of the respective zygotic equivalent. These results provide genetic evidence that the developmental program of somatic and zygotic embryos is indistinguishable. In addition, somatic embryogenic cell formation does not depend on functional SAMs.

Strategies for the maintenance of undifferentiated plant cell lines as tools for research

Heinz Martin Schumacher
Deutsche Sammlung von Mikro-organismen und Zellkulturen, Braunschweig, Germany

Apart from great efforts for the large scale production of secondary metabolites by fermentation of plant cell suspension cultures during the 80's and 90's, undifferentiated plant cell cultures were more and more used as tools for research - for the search of novel secondary compounds but most often for the biochemical study of metabolic pathways including cellular and sub-cellular transport mechanisms.

In 1990 the DSMZ plant cell culture collection was founded to maintain the undifferentiated plant cell lines which have been initiated in previous years in Germany and supply them to clients in the scientific community. Today the collection maintains more than 700 cell lines of more than 400 different plant species and supplies between 100 and up to 500 cell lines per year to clients worldwide. More than 80 % of the cell lines are supplied to clients outside Germany and more than 80 % to clients representing profit-institutions. In most cases the use that clients make of the cell lines is not known to the collection. The major challenge for a public collection is therefore to maintain these cell lines in a stable form not only with respect to one specific trait. This is even more problematic since - although deep freezing of plant cells is possible in general - no long term preservation method can be applied routinely. Cell lines still have to be maintained in the growing state. DSMZ therefore established a system for sub-culturing which minimizes the risks for loss of cultures by laboratory failure and microbial infection and at the same time facilitates the observation of growth and phenotype of the cell lines.

Previous experiments with slow growth procedures showed that the application to such a big range of taxonomically and physiologically different cell lines as present at DSMZ, does not essentially reduce work, although it exposes the cell lines to undesired selection pressures and phenotypic changes. To date different approaches of deep freezing for plant cells have been developed and established at DSMZ. Controlled-rate-freezing and encapsulation/dehydration turned out to be the best suited techniques for undifferentiated cell lines in terms of practical application. Nevertheless, the different parameter of each method have to be optimized for each specific cell line. Methods to facilitate this optimization process for controlled-rate-freezing have therefore been set up.

For reasons of collection management, like authentication of cell lines, as well as for stability testing after cryo-preservation, methods for the characterization of cell lines have to be developed. For practical reasons it was not possible to measure all different parameter the cell lines are maintained and deposited for. Furthermore, as mentioned earlier, clients use the cell lines for different purposes. A single generally applicable characterization method should therefore be applied. Since cell lines are maintained in the living state, tests have to be repeated on a regular basis. Besides a simple database for phenotypic characterization, a simple system of "chemical fingerprints" from the HPLC analysis of methanolic extracts is applied at DSMZ. At the same time we started to build up an authentication system based on RAPD techniques. We hope the approach will finally provide a simple method for the authentication of cell lines on the DNA basis.

Cytokinins and cell cycle progression

Harry A. Van Onckelen.
University of Antwerp, Department of Biology, B-2610 Antwerp, Belgium

The importance of N_6-isoprenoid cytokinins in the G_2-M transition of *Nicotiana tabacum* BY-2 cells was investigated. Both cytokinin biosynthesis and entry in mitosis were partially blocked by application at early or late G_2 of lovastatin (10 µM), an inhibitor of mevalonic acid synthesis.
LC-MS/MS quantification of endogenous cytokinins proved that lovastatin affects cytokinin biosynthesis by inhibiting HMG-CoA reductase. Out of eight different aminopurines and a synthetic auxin tested for their ability to override lovastatin-inhibition of mitosis, only zeatin was active. Our data point to a key role for a well-defined cytokinin (here, zeatin) in the G_2-M transition of tobacco BY-2 cells.

Theories on the importance of cytokinins in G_1/S transition control are manifold and contradictory. By establishing a double Aφ-PZ block, maximal synchronization of a BY-2 suspension culture was obtained to investigate the effect of cytokinin depletion on G_1/S transition. Lovastatin was used as a specific inhibitor of cytokinin biosynthesis. Flow cytometry showed that the G_1/S transition occurred regardless of the cytokinin drop. This observation indicates an extremely low dose requiry for that stage of the cell cycle. It is very likely that precisely the downregulation of zeatin-type cytokinins matters for the G_1/S transition to occur, since cytokinin addition at early G_1 blocked the cycle at G_1/S.

These results allowed us to elaborate a model on the action of cytokinins on cell cycle progression in BY-2 synchronised tobacco cells. The validity of this model will be confronted with several *in planta* observations.