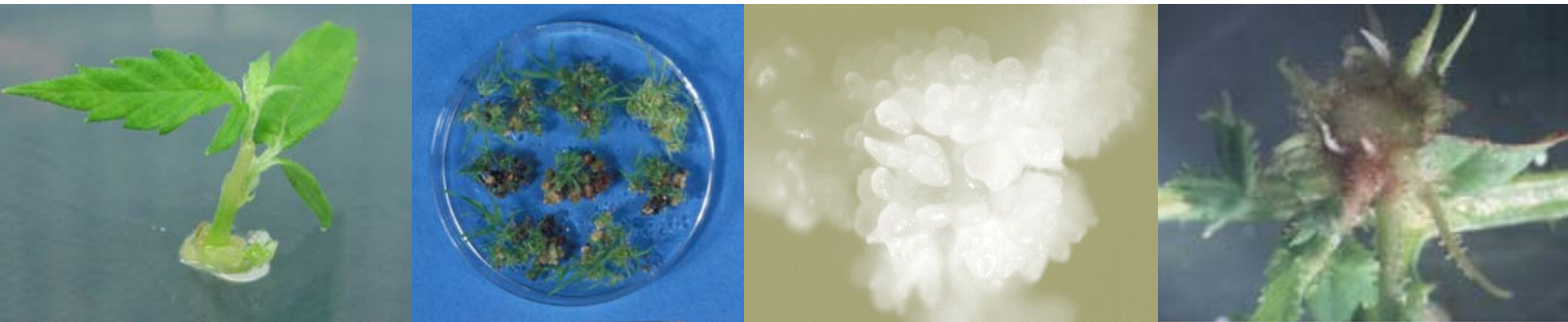


NVPW
anniversary 25 years symposium



WICC Wageningen
17 March 2006

Venue

Wageningen International Congress Centre

Lawickse Allee 9
6701 AN Wageningen
the Netherlands

For a route description see page 9 & 10.

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The program is sponsored by

Snijders Scientific

Wageningen Tissue Culture Centre

Bejo Zaden

Duchefa

Nunhems Netherlands

SBW International

Vitrocom Holland / Pothos Plant

Program

9.00 Registration

9.45 Opening

10.00 Pierre Debergh (Belgium) History of tissue culture in the last 25 years, with focus on applications

10.40 Sara von Arnold (Department of Plant Biology and Forest Genetics, SLU, Uppsala, Sweden) Somatic embryogenesis in conifers

11.20 Ivana Machácková (Czech Academy of Sciences, Praha, Czech Republic) Hormones and tissue culture, including the use of novel hormones in regeneration

12.00 Lunch

The lunch is included in the registration fee. During lunch you can visit demonstrations by Snijders Scientific and Wageningen Tissue Culture centre (WTCC)

13.00 Rob Verpoorte (Institute of Biology, Leiden University) The plant cell factory: From blueprint to production of secondary metabolites

13.40 Maritza Escalona Morgado (Centro de Bioplantitas, Cuba) Propagation in tissue culture using temporary immersion

14.20 Sergio Ochatt (INRA Centre de Dijon, France) Protoplast research for multiplication and breeding

15.00 Coffee break

You can visit demonstrations by Snijders Scientific and WTCC.

15.30 Paul Hooykaas (Institute of Biology, Leiden University) Improvement of plant properties with focus on technologies (e.g. homologous recombination, TILLING, RNAi, *A. tumefaciens* and particle-gun transformation)

16.10 Rainer Fischer (RWTH Aachen & Fraunhofer IME, Germany) Using plants and plant cells for the manufacturing of protein-based biopharmaceuticals

16.50 Closing

16.55 NVPW ledenvergadering (in Dutch)

Annual general assembly for NVPW members

17.30 Drinks

Offered to all participants by the NVPW to celebrate its 25th anniversary.

18.30 Buffet

For participants who registered for the buffet

Abstracts

History of tissue culture in the last 25 years with focus on applications

Prof. Em. Pierre Debergh,

Belgium

Before the eighties tissue culture technology grew up to adolescence, with most applications in the field of horticulture. Over the past 25 years it reached maturity, with commercial business in all plant related sectors. This development was possible because of the exemplary intercourse between fundamental and applied research and commercial operations, especially in the field of micropropagation, but also for other applications.

Some points I want to highlight are listed hereafter:

- The assortment enlarged, but there are still some recalcitrant species
- The centres of production shifted from the Western world to other locations
- A better understanding of the system became available, especially because of the more fundamental research approach of the ecophysiology prevailing in a tissue culture container (hyperhydricity, phytohormones, headspace, mixotrophic behaviour, stress enzymes,)
- Liquid media were not favoured, but there is a regain in interest, e.g. TIB
- Recently we witness a major interest in breeding related applications (haploids, polyploids, embryo rescue, protoplasts,)
- There is a regain in interest for phytopathology
- HACCP has also made his introduction
- After a dip in the interest for secondary metabolites in the seventies, it becomes again an important item, as well as the possibilities for the manufacture of biopharmaceuticals
- So far public acceptance hampered the breakthrough of genetic engineering technologies, but the molecular techniques they developed played already a major role in the elucidation of somaclonal problems and in fingerprinting
- Although somatic embryogenesis was already developed in the fifties, it did not yet become a routine commercial application, but it is still a promising possibility for mass propagation and genetic engineering.
- Automation and application of robots never made the promised breakthrough

Somatic embryogenesis in conifers

Sara von Arnold

Department of Plant Biology and Forest Genetics SLU, Uppsala, Sweden

Somatic embryos of conifers have become a useful tool for clonal propagation. Somatic embryos are also valuable for studying regulation of embryo development. The somatic embryo system includes a stereotyped sequence of developmental stages, resembling zygotic embryogeny, which can be synchronized by specific treatments, making it possible to collect a large number of somatic embryos at specific developmental stages. The genetic regulation of embryonic pattern formation has been studied in the model plant *Arabidopsis*. In contrast, hardly anything is known about the regulation of embryo development in gymnosperms. Gymnosperms have several disadvantages as experimental organisms. They have large genomes, about 200 to 400 times bigger than *Arabidopsis*. Furthermore, they have large size and long generation time. Molecular data suggest that extant seed plants (gymnosperms and angiosperms) share a last common ancestor about 285 million years ago. From an evolutionary

point of view it is important to learn more about the regulation of embryogenesis in gymnosperms.

The sequence of embryo development in gymnosperms can be divided into three phases: proembryogeny, early embryogeny and late embryogeny. The pathway of somatic embryo development resembles zygotic embryogeny. Embryogenic cultures of Norway spruce are established from zygotic embryos. The cell lines proliferate as proembryogenic masses (PEMs) in the presence of auxin and cytokinin. Somatic embryos differentiate from PEMs after withdrawal of auxin and cytokinin. This stage corresponds to early embryogeny. The embryos develop further, to a stage corresponding to late embryogeny, in the presence of abscisic acid.

The variation in gene expression during somatic embryogenesis in Norway spruce suggests that the level of DNA methylation is important for the differentiation of somatic embryos from PEMs. Furthermore, down-regulation of genes involved in auxin metabolism is important for apical-basal polarization during early embryogeny and down-regulation of genes involved in cell wall formation is important for differentiation of primary meristems.

Formation of the apical-basal embryonic pattern during early embryogeny proceeds through the establishment of three major cell types: the meristematic cells of the embryonal mass, the embryonal tube cells and terminally differentiated suspensor cells. The suspensor cells are degraded by programmed cell death. Metacaspase is essential for maintaining the proper balance between cell proliferation and programmed cell death. Differentiation of the outer cell layer in the embryonal mass is regulated by *PaHB1*, which encoded protein is highly similar to those from the HD-GL2 angiosperm counterparts. Suggesting similarities in the definition of the outer cell layer in seed plants. Like in angiosperms, a proper function of the outer cell layer in Norway spruce requires a specific expression pattern of a lipid transfer protein gene. During late embryogeny *PaHB2* participates in the maintenance of the radial pattern by specifying cell identity in the cortical layers. The pattern of expression of *Pavp1* during maturation of Norway spruce somatic embryos is similar to that of angiosperm VP1 homologues during somatic and zygotic embryogenesis. Taken together, the regulation of embryo formation has many similarities in gymnosperms and angiosperms, but there are also differences which have to be studied more in detail.

Hormones and tissue culture

Ivana Machácková

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The talk will bring a brief, general overview of use of hormones in tissue cultures. First, a brief explanation of how levels of hormones are regulated both in planta and in vitro will be given. The talk will concentrate on auxins and cytokinins, their main effects in tissue cultures as well as on uptake and metabolism (with some examples mostly from our own work). Also interaction of auxins and cytokinins will be mentioned with some examples. Role of ethylene and phenolic substances will be briefly mentioned. With couple of examples use of new regulators - brassinosteroids and jasmonic acid - will be demonstrated.

Last, but not least, several examples how tissue cultures are useful in hormone research will be given.

The plant cell factory: From blueprint to production

R. Verpoorte

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In the past 25 years plant cell biotechnology has been one of the themes of plant cell, tissue and organ culture research. It aimed at the production of fine chemicals such as medicines by means of plant cells. Originally it was thought that plant cells would be difficult to grow in bioreactors because of shear forces caused by stirring. These shear forces would make the cells to collapse. However, studies on this aspect showed that plant cells are reasonable shear resistant, and large bioreactors are presently used for the production of among others taxol by plant cells. Calculations on the economy of the system showed that at a production of 0.3g/l per 14 days, costs would be 1500 €/kg. This seems high, but compared to prices of compounds like taxol and vincristine, in fact, is quite low. First all efforts were focused on improving production of cell cultures for the desired compounds by screening for high producing cell lines and by optimizing growth and production media. However, in many cases this did not result in the required improvement. Poor understanding of the regulation of the biosynthesis of the products was a major reason to change the focus to studying biosynthesis in more detail. Identification of intermediates and the enzymes eventually led to the cloning of the genes encoding various steps in the pathways. This opened the way to further studies of the regulation and to metabolic engineering. These new possibilities have been applied both to cell and organ cultures, but can also be applied to the plant. Several interesting results were obtained, but not sufficient improvement of production to increase the number of applications. Still too many steps in the pathways remain unsolved, and it is now recognized that also compartmentation, and thus transport do play an important regulatory role. To further elucidate the pathways novel approaches have to be developed, as in many cases the intermediates are not known, or not available. Functional genomics, using transcriptomics, proteomics and metabolomics are presently the major approaches used for unraveling the production of secondary metabolite in cell cultures. The cell cultures are a quite useful system for this purpose as they are easy to use for feeding experiments, and the number of cell types is limited whereas in plant tissues many different cell types are present, and consequently the -omes of all cell types are mixed. Plant cell cultures will thus be a major tool in the years to come for studying biosynthesis.

References

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Propagation in tissue culture using temporary immersion

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Temporary immersion has been shown to reduce problems usually encountered in liquid culture. Based on this concept, a collective of researcher belong to Bioplant Center adapted a semi-automated system for large-scale propagation of plants. This bioreactor has been named as Twin Flasks system (BIT[®]) and grouped into the systems with complete immersion by pneumatic driven transfer of liquid medium without medium replenishment. BIT[®] is relatively simple and easy to use. They enable contact between all parts of the explants and the liquid medium. The culture environment is renewal by forced ventilation during each immersion period. For special type of plants, a forced aeration in the culture recipient can be used. The injection of CO₂ permits to improve the photomixotrophic culture. BIT[®] has been used for *in vitro* commercial propagation of a wide range crops: *Ananas*, *Saccharum sp*, *Musa sp*, *Colocasia sp*, *Araceae*, *Eucalyptys sp*, *Rosaceae*, *Bromelias*, *Paeony*. In order to establish a micropropagation procedure and increase the efficacy of BIT[®] -technology, different parameters should be optimized. Among them, the immersion time, immersion frequency, the volume of nutrient medium, the volume of culture container, the duration of proliferation phase, the use of plant growth retardant, the number of cycle in BIT[®]. Plants regenerated by BIT[®] have not showed somaclonal variation detected by molecular probes and evaluations in the field. The simplicity and low cost of BIT[®] is compatible with large-scale propagation. It permits important lower labor, better biological yield and consecutively reduces production cost.

Protoplast research for multiplication and breeding

S.J. Ochatt

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The term protoplasts, refers to all components of a plant cell, excluding the cell wall, but they do not simply represent "wall-less cells" as originally envisaged. It is now nearly 50 years since protoplasts were first isolated enzymatically and, ironically, it is precisely such a treatment of cells with crude enzyme preparations that leads to a deleterious effect on protoplast viability, due mainly to the many undefined contaminating compounds inevitably found in such preparations. In addition, the high osmotic pressure conditions required for early stage cultures and the accumulation of metabolic products during culture also adversely affect cell viability. Thus, although protoplasts can now be isolated from virtually any plant species and any type of tissue source, the ability to isolate protoplasts capable of sustained mitotic division with subsequent callus proliferation and plant regeneration is still limited to a relatively small (but nonetheless increasing) number of species. As a result, the use of protoplasts has now become a baseline for the successful genetic improvement of many species. Thus, protoplasts can be used for *in vitro* selection studies and for direct gene transfer although both these approaches have somewhat been eclipsed by the possibility to induce stress tolerance using explant-derived calluses and to obtain transformed plants following co-culturing of explants with disarmed vectors of agrobacteria, respectively. There is, however, one field where protoplast technology remains unparalleled by most other biotechnological approaches to plant breeding, i.e. protoplast fusion and somatic hybridisation techniques which provide the opportunity for the bypassing of reproductive isolation barriers and, therefore,

facilitating gene-flow between species. To achieve such goals, the development of efficient protoplast to plant systems stands as a prerequisite. Finally, other applications of protoplast technology (not necessarily requiring plant regeneration) include using protoplasts as the material for the understanding of mechanisms underlying several host-stress interaction studies or for more fundamental research on the fate of DNA in cultured cells. All these aspects of protoplast technology will be briefly outlined and their implications for breeding will be discussed.

New technology for improving plant properties

Paul J.J.Hooykaas

Clusius Laboratory, IBL, Leiden University

We celebrate the 25 years of existence of the Netherlands Society for Plant Biotechnology and Tissue Culture. At the date of establishment of the Society it had just been discovered that the soil bacterium *Agrobacterium tumefaciens* uses genetic engineering to exploit the biosynthetic capacity of plants in nature. These special properties of the bacterium have since been used for the genetic engineering of many crop plants. However, there are also still some unsolved problems associated with plant transformation, which limit the use of its full potential. These relate for instance to the use of antibiotic resistance selection markers and to the integration of the DNA at random position in the genome resulting in position effects and possibly insertion- mutation. Nevertheless, over the years the tool box of the plant genetic engineer has become more and more sophisticated. And also the knowledge about plant genes and plant genomes has increased tremendously culminating in the sequencing of the complete genomes of *Arabidopsis thaliana* and rice. In the lecture I shall report on the new tools and methodologies that are being developed for and applied in plant genetics and focus on methods for gene targeting and targeted mutation.

Using plants and plant cells for the manufacturing of protein based biopharmaceuticals

Prof. Dr. Rainer Fischer

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Antibodies, vaccines and cytokines are critical tools in human and animal health care, enabling us to treat many life-threatening diseases. Until now, the engineering and largescale production of recombinant biopharmaceuticals has been time consuming and expensive, prohibiting the wide spread use of these proteins throughout medicine. Recent developments in genomics, proteomics and cellomics have provided novel targets for development of small molecule and protein based pharmaceuticals. Furthermore, protein engineering and production technologies have contributed to overcome many of these problems. Using microbes, animal and plant cells heterologous protein production has become feasible. Of particular interest is molecular farming, i.e. the production of recombinant proteins in transgenic organisms, cells or transiently infected plants, which allows the use of plants to synthesize biopharmaceuticals on an agricultural scale. This technology will help to bring recombinant antibody and protein therapeutics down in cost, without sacrificing their quality or safety, enabling us to broaden our concept of what they can be used for.

Different antibody variants and vaccines are all produced in an active form and they join a growing list of recombinant proteins that can be functionally expressed in heterologous systems. The highest production yields can be seen

with recombinant proteins which are retained within the cell's secretory pathway, and the lowest yields are seen in the cytosol.

This presentation will focus on discussing the challenges involved in the yield maximization of protein based pharmaceuticals through molecular as well as cellular approaches and downstream processing technologies. Our longterm perspective is that recombinant protein production in heterologous production hosts including crop plants and plant cells may create an opportunity to distribute these recombinant proteins beyond the developed and into the developing world to combat the three grand challenges and other infectious and tropical diseases.

How to reach the WICC

By Car:

Coming from Arnhem/Nijmegen (=Motorway A50):

Take exit "Renkum/Wageningen/Oosterbeek". At the traffic light take direction Renkum/Wageningen (N225). Go straight on till you come to a roundabout. At the roundabout go straight on, and you will enter Wageningen. At the 4th traffic light turn left (= "Stationstraat"). Take the first turn to the right (= "Plantsoen"). At the end of this road follow the curve and at the crossing (without traffic lights) turn right. At the traffic light turn right again (= direction Renkum (N 225): "Lawickse Allee"). After 10 metres take the road parallel to the main one. After 75 metres at your right hand you will see the parking lot of the WICC.

Coming from Utrecht/Ede/Apeldoorn (=Motorway A12):

Take exit "Wageningen/Ede/Bennekom". At the end of the exit go direction Wageningen (= "Dr. Dreeslaan"). In Wageningen turn right at the first traffic light (= "Nijenoord Allee") follow this ring road direction Rhenen. At the 3rd traffic light (you will see the Agro Business Park in front of you) turn left (= "Kortenoord Allee"). At the 1st traffic light, after roundabout, turn left (N225), direction Renkum (= "Lawickse Allee"). After 10 metres take the road parallel to the main one. After 75 metres at your right hand you will see the parking lot of the WICC.

Coming from Rotterdam/Breda (=Motorway A15)

Take exit "Ochten/Kesteren". Immediately after the exit turn right (follow N233, Rhenen). Over the bridge at Rhenen (river Rhine) turn right (follow N225, direction Wageningen/Renkum). After approximately 6 kilometres at the roundabout straight on (N225). After the first traffic light, take the road parallel to the main one. After 75 metres on your right-hand side you will see the parking lot of the WICC.

