



Voorjaarsymposium Spring Symposium

Nederlandse Vereniging voor Plantenbiotechnologie en -Weefselkweek
Netherlands society for Plant Biotechnology and tissue Culture

28 Maart 2003

WICC-IAC – Wageningen

- 9:30 Registration and coffee
- 10:00 Opening by the chairman: **Andre Schram**
- 10:05 **Kim Boutilier** (Plant Research International, Wageningen)
Expression Profiling of *Brassica napus* Microspore Embryo Cultures
- 10:35 **Geert-Jan de Klerk** (PPO-Lisse)
New Plant Growth Regulators for tissue culture: A Survey
- 11:05 **Asaph Aharoni** (Plant Research International, Wageningen)
Strawberry and Beyond: A novel and comprehensive investigation of fruit maturation and ripening
- 11:35 **Jaques Hille** (University of Groningen)
Apoptosis and leaf senescence
- 12:05 **Christa Testerink** (University of Amsterdam)
Lipid signal transduction in higher plants
- 12:35 **Lunch & NVPW Ledenvergadering**
- 14:15 **Paul Condliffe** (Plant Research International, Wageningen)
Coming up Roses: the biotech approach to floriculture
- 14:35 **Stefan Royaert** (Catholic University, Nijmegen)
Analysis of the MADS-box gene family in *Petunia hybrida*
- 14:55 **Bea Pauw** (Leiden University)
Stress signal transduction involved in the production of pharmaceutical important alkaloids
- 15:15 **Ronald Snijder** (Plant Research International, Wageningen)
Breaking breeding barriers in *Zantedeschia*
- 15:35 **Announcement winner of the travel fellowship**
- 15:45 **Coffee and tee**

Expression profiling of *brassica* microspore embryo cultures

Kim Boutilier

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Androgenesis, microspore- and pollen embryogenesis are different terms that describe the process in which haploid embryos are formed *in vitro* from developing male gametophyte. Haploid embryo culture has been used for many years as a tool to rapidly generate material for true breeding lines and mapping studies. More recently haploid embryo cultures have been used to study aspects of plant development ranging from cell competence and determination, to early embryo development and seed maturation.

We are using the well-defined *Brassica napus* microspore embryo culture system as a model to identify gene expression programs that are associated with the initiation and determination of embryo development in culture. This culture system is based on the ability of the microspore- or vegetative cell nucleus of an immature pollen grain to develop into an embryo after application of a heat-stress treatment. Previously we used differential screening approaches to identify individual genes that are upregulated during the switch from pollen to embryo development in culture. This approach was successful in that it led to the identification of genes such as *BABY BOOM*, which has been shown to play a central role in the initiation of embryo development. We are now using a more comprehensive approach to obtain a broader perspective on the molecular pathways that accompany to the switch from pollen to embryo development, and to classify temporal patterns of gene expression during early embryo development. Our goal is not only to gain insight into the molecular control of early embryo development, but also to use the genes we identify as tools to overcome bottlenecks encountered during *in vitro* embryo culture.

To identify expression profiles associated with microspore embryo development, we developed a dedicated *B. napus* microarray carrying cDNAs for genes expressed at different time periods during embryo culture. Suppression Subtraction Hybridisation (SSH) was applied to some samples to normalize the mRNA abundance and to enrich for cDNAs that are up or down regulated at specific stages of culture. We used the expression profiles of the 2000 arrayed cDNAs to characterize the development of three types of cultures: embryogenic cultures (3 time points), control pollen cultures (2 time points), and cultures containing suspensor-bearing embryos (2 time points).

Our preliminary analysis of the microarray data focussed on identification of genes that are present in embryogenic cultures, but absent in pollen cultures.

Approximately 200 embryo-expressed genes were identified and further subdivided into five temporal categories. A number of these embryo-expressed genes have been previously shown to play a role in embryo development, however the majority of the genes we identified have not been assigned a function, nor have they been annotated as being embryo ESTs. The next challenge is to translate these "guilt by association" profiles into a meaningful understanding of the regulatory networks and interactions that occur during early embryo development in plants.

New plant growth regulators for tissue culture: a survey.

Geert-Jan de Klerk and Frans Krens

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In plant tissue culture, plant growth regulators (PGRs) have an essential role. They are required to obtain growth and to control the direction of development. The PGRs used in tissue culture usually belong to the cytokinin- or the auxin-type. The other three classical PGRs (gibberellins, abscisic acid and ethylene) and the newly discovered PGRs (such as brassinosteroids, NO and polyamines) are considered only occasionally. In this presentation, an overview is given of new tissue-culture applications related to both the classical and the new PGRs. Treatments may result in improved growth and development, or in protecting tissue-cultured plants from stress associated with tissue culture (wounding stress in particular in small explants, and acclimatization).

Strawberry and Beyond: A Novel and Comprehensive Investigation of Fruit Maturation and Ripening

Asaph Aharoni

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Functional-genomics tools such as Expressed Sequence Tags (ESTs) and whole genome sequencing, gene expression using macro and micro arrays and generation of mutant populations contributed largely to the research on model plants, mainly *Arabidopsis thaliana*. A substantial portion of this "tool-box" can also be utilized successfully for research on non-model plants, which are more difficult and time consuming to deal with, but allow investigations of unique biological processes such as fruit flavour biogenesis. As a first phase in a strategy to investigate strawberry fruit maturation and to identify key genes associated with fruit quality traits (in particularly aroma and flavour), we generated a collection of more than 1000 ESTs from ripe fruit cDNA library (cv. *Elsanta*). Combining information on the putative identity of the ESTs and gene expression studies was subsequently used to select candidate genes for further investigation. In early studies we used RNA gel-blot to analyse levels of 50 selected genes (selected based on homology). More than 15 transcripts showed ripening - regulated expression pattern and included *FaMYB1*, a member of the R2R3 MYB family of transcription factors. The *FaMYB1* gene was subjected to a more profound investigation, and the results suggested it to function as repressor of late flavonoid biosynthesis genes in the ripe strawberry fruit. To perform a more comprehensive study of gene expression we constructed DNA microarrays representing 1700 strawberry cDNAs and compared gene expression both during fruit development and between receptacle and achene tissues. A major finding in this study was the identification of the *SAAT* gene encoding the ester-forming enzyme from strawberry. Volatile esters are major components of the aroma profiles of most fruit, including strawberry. We also generated a second, dedicated set of arrays, comprising only 384 probes selected on the basis of the first hybridisation results including mainly ripening regulated and receptacle associated cDNAs. This set was used to analyse gene expression in fruit treated with auxin and fruit under oxidative stress conditions. Taken as a whole, microarray experiments have provided us with an extensive and novel insight into the transcriptional programs active in strawberry fruit during maturation. They also led to the identification of several other flavour associated genes which are currently being characterised. As a complementary step for the large-scale analysis of gene

expression using microarrays we conducted a set of experiments aimed at identifying key metabolic changes in strawberry fruit during development using a Fourier Transform Ion Cyclotron Mass Spectrometry (FTMS)-based method. The analysis identified changes in the levels of a large range of masses corresponding to known fruit metabolites and revealed novel information on the metabolic transition from immature to ripe fruit. The integration of emerging functional genomic practices will be an invaluable approach both for gene discovery and for understanding the biology of non-model plant species such as strawberry.

Coming up Roses: the biotech approach to floriculture

Paul Condliffe

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The production of cut-flower roses makes up a large proportion of the global floriculture industry. Breeding programs are driven by the consumers need for novel and improved phenotypes coupled to the producers requirement of optimal production and disease and pest resistance. Traditionally new cultivars have been developed through sexual hybridisation, this process is time consuming and in introducing one useful trait another may be sacrificed. For example the quest to improve flower form and production over the centuries has resulted in the loss of scent from many modern cut rose cultivars.

The introduction of specific genes to known cultivars could enable the production of new lines with improved agronomic traits, without the problems associated with conventional breeding. Phenotype, yield and resistance to pests and diseases are all characteristics that can be manipulated on an individual basis.

Agrobacterium tumefaciens-mediated transformation of somatic embryo cultures appears the most promising method of gene introduction. There are several problems associated with this method which must be overcome prior to its large-scale application. For example many existing embryogenesis protocols are only effective within a limited number of cultivars. Experiments were initiated in an attempt to develop a method of somatic embryogenesis and transformation for a number of different cultivars. Results indicate that a general protocol can be arrived at and therefore that the manipulation of individual traits by genetic engineering within existing cultivars is possible on a commercial scale.

Analysis of the MADS-box gene family in *Petunia hybrida*

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MADS-box genes form a large family of transcription factors that so far mainly has been shown to be involved in quite diverse aspects of floral development. The plant MADS-box proteins typically contain a MADS-box (DNA-binding), the non-conserved I-box (intervening region), the K-box (Keratine-like, important for dimerization) and a variable C-terminal part. We perform a comparative analysis of the MADS-box gene family in *Petunia* and *Arabidopsis*, one aspect being the analysis of redundant functions. Using Transposon Display and a very efficient way of screening, the Family Screening approach, we identified 27 MADS-box genes in *Petunia*, which are spread over all identified subfamilies. The first emphasis is on the elucidation of the B function subfamily genes which provide the specificity for

the development of petals and stamen. In both *A. thaliana* and *A. majus*, a single pair of genes has been described, the products of which act as a heterodimer to determine the developmental fate of whorl two and three meristems. Consequently, mutations in either one of these genes result in similar phenotypes: the homeotic conversion of petals into sepals and of stamen into carpels. In *Petunia*, the gene pair has duplicated which apparently led to specialization of its members towards a function in the development of either petals or stamen. We discuss the necessity of a fourth B-function gene to explain all observed phenotypes and have identified a candidate gene for this function: *PhTM6*. Although its sequence clearly identifies it as a B-function gene, its expression pattern does not fit the classical ABC model.

Stress signal transduction involved in the production of pharmaceutically important alkaloids

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Plant physiology and plant responses to stress are important not only for agriculture and horticulture, but also for industries using raw plant materials. In modern culture techniques, plants are exposed to a variety of stress factors, that often have a negative effect on product yield and quality. On the other hand, stress factors can stimulate plants to produce high quality chemicals. *Catharanthus roseus* produces the secondary metabolites terpenoid indole alkaloids (TIAs), some of which have pharmaceutical applications. Several TIA biosynthetic genes, including strictosidine synthase, are coordinately induced by stress, e.g. yeast elicitor. This induction is mediated via several signaling steps including the biosynthesis of jasmonic acid via the octadecanoid pathway, and the activation of Octadecanoid-Responsive Catharanthus AP2-domain (ORCA) transcription factors. We investigated the possible role of mitogen-activated protein kinases (MAPKs) as signaling intermediates in this system. Yeast elicitor treatment was shown to induce at least 3 different MAPKs. MAPK inhibitor treatment blocked elicitor-induced ORCA and TIA gene expression, indicating that one or more MAPKs are required for the activation of these genes. Five MAPKs, belonging to 3 different MAPK classes, were isolated from *Catharanthus*. The mRNA and protein levels of one of them, CrMPK1, were induced by YE treatment. To determine the role of CrMPK1 in the elicitor-induced signal transduction pathway leading to activation of TIA biosynthesis, transgenic plants inducibly over-expressing this MAPK were generated. However, over-expression of CrMPK1 does not lead to active CrMPK1 protein and (consequently?) does not induce ORCA2 gene expression. Cell lines over-expressing other CrMPKs will also be generated. If one or more CrMPKs play an essential role in this pathway, activation of these MAPKs may improve metabolite productivity.