PLANT SYSTEMS BIOLOGY AND MEDICINAL PLANTS

GA Symposium, April 24-25, 2008

Leiden, The Netherlands

Organizing committee
Prof. Dr. Hans Scheffer
Prof. Dr. Rob Verpoorte
Prof. Dr. Brigitte Kopp

PLANT SYSTEMS BIOLOGY AND MEDICINAL PLANTS

GA Symposium, April 24-25, 2008

Leiden, The Netherlands*

Venue: Museum Naturalis, Pesthuis Leiden, The Netherlands
5 min walk from Leiden Central Station, 20 min by direct train from Amsterdam Schiphol Airport

Deadlines

Abstracts for posters and short lectures:	March 1
Late registration:	After March 15

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Systems biology is revolutionizing science. It means a different approach to study biological systems, *i.e.* studying the systems in a more holistic way. In complex organisms such as plants this offers many new opportunities and many challenges. For studying plant resistance against pests and diseases, or studying the effect of medicinal plants in living systems (cells, animals or humans), systems biology will be of great value. Key technologies in systems biology are transcriptomics, proteomics and metabolomics. Particularly the latter technology is of great interest for finding compounds that correlate with biological activity, e.g. in resistance against insects, or a pharmacological activity. Metabolomics is also an important tool for quality control of plant products, e.g. showing equivalence of GMOs, or for control of consistency of the composition of botanicals.
The 2-days symposium will highlight a number of aspects of plant systems biology in relation to the above mentioned applications. The symposium will be in Naturalis, Museum of Natural History, one of the best visited museums in The Netherlands.

*on the occasion of the retirement of Prof. Dr. Hans Scheffer

INVITED SPEAKERS

The invited speakers include:

J. van der Greef (Director Systems Biology, TNO, Zeist, The Netherlands)
"Systems biology, the future of Medicine and Personal Health"

Mei Wang (SU Biomedicine, Zeist, The Netherlands)
"Systems Biology: Scientific evidence and a novel quality control for Traditional Chinese Medicine (TCM)"

K.-M. Oksman-Caldentey (VTT, Espoo, Finland)
"Plant cells as efficient factories to produce high-value secondary metabolities"

A. Goossens (Department of Plant Systems Biology, VIB, Gent, Belgium)
"Transcriptomics and jasmonates, a potent combination to unravel the regulation of plant secondary metabolism"

Y.H. Choi (Section Metabolomics, IBL, Leiden University, Leiden, The Netherlands)
"*Potential and limitations of NMR-based metabolomics in plant science"*

R. Hiltunen (Faculty of Pharmacy, Helsinki, Finland)
"From essential oils to plant phenolics"

A.C. Figueiredo (Centro de Biotecnologia Vegetal, University of Lisbon, Portugal)
"Volatiles, hairy roots and metabolomics: what future?"

R. van der Heijden (LACDR, Leiden University, The Netherlands)
"*Metabolite profiling of herbal drugs by high-resolution LC-MS"*

A. Fernie (Molecular Physiology, Max-Planck-Institute of Molecular Plant Physiology, Potsdam-Golm, Germany)
"*Flux profiling - going beyond the steady state"*

E. Holmes (Chemical Biology, Imperial College, London, UK)
"*Metabonomics"*

REGISTRATION

Registration Information

GA Symposium Plant Systems Biology, April 24-25, 2008, Leiden

Registration costs include coffee and tea during breaks and lunch during both days. The registration also includes a free ticket to the Naturalis Museum.
On Friday evening, there will be an excursion to the beautiful park “Keukenhof”, famous for flowering bulbs. The symposium dinner will take place in the park (costs € 80, all in).
Abstracts for posters and oral presentations will only be accepted after payment of the registration.
The number of participants is restricted to 200. The first come-first serve principle will be used for the registration.

Costs of registration*

Students early registration (before March 15)	€ 200
Students late registration	€ 250
Others early registration (before March 15)	€ 250
Others late registration	€ 350
Excursion to Keukenhof and Symposium Dinner, evening April 25, also including transport	€ 80

* **Grants**
Graduate students who like to present their scientific results in a poster or short oral presentation during this meeting, are eligible for a grant. The applicants have to submit the following documents:
• Abstract
• Letter of recommendation by their supervisor, confirming their PhD student status
The number of grants, which covers at least registration costs, is limited.

Course in Metabolomics
In connection with the meeting we will organize a Course in Metabolomics, April 21-23. Costs € 400, not including the meeting. Further information can be obtained from R. Verpoorte (address see below)(http://www.fcog.nl/wiki).

Abstracts for posters and short oral presentations (20 min, limited number) can be submitted to the organizers. The topic does not necessarily have to be related to the specific theme of the meeting. Your name, address, title and abstract (maximum size 1 page A4, using preferably Times Roman 12) of the presentation have to be submitted **before March 1**, so the organizers can select the speakers and make the final program.

Registration form (and abstract) should be sent to:
R. Verpoorte, Department of Pharmacognosy, Section Metabolomics, IBL
P.O. Box 9502, 2300 RA, Leiden, The Netherlands
tel. +31 71-5274528/5274510, fax +31 71-5274511
E-mail: FCOGSYMP@LACDR.LeidenUniv.NL

REGISTRATION

Registration form

I want to participate in the GA Symposium Plant Systems Biology, April 24-25, Leiden, The Netherlands

Name: .....

Institute: .....

Address: .....

Postal code: .....

City: .....

Country: .....

Tel: ..... Fax: ..... E-mail: .....

poster: no/yes, title.....

oral presentation: no/yes, title.....

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Excursion to Keukenhof and Symposium Dinner, evening April 25, also including transport.....	€ 80
	_____+
Total €	

The registration costs have been paid in EURO, with all costs for participant to:
Account number: 5533817 of Symposia Farmacognosie
Address: Gloxiniadal 24, 2317 HB Leiden, The Netherlands
IBAN NL25PSTB0005.5338.17
BIC PSTBNL21
Address of the bank: Postbank N.V. Foreign Operations,
PO Box 1800, 1000 BV Amsterdam, The Netherlands

Form (and abstract) should be sent to:
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tel. +31 71-5274528/5274510, fax +31 71-5274511

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Systems biology, the future of Medicine and Personal Health

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In recent decades various scientific domains ranging from physics, biology to cosmology have been focusing towards a *systems-based view*. The key research topic being the *interconnectivity* of systems and the study of the *organizing principles*, realizing that new properties emerge at different levels of complexity. This focus is initiated in Systems Biology research.
Systems biology has developed in recent years from a technology driven enterprise to a new strategic tool in Life Sciences, particularly for innovative drug discovery and drug development. Combining the ultimate in systems phenotyping with in-depth investigations of biomolecular mechanisms, will enable a revolution in our understanding of disease pathology and will advance translational medicine, combination therapies, integrative medicine and personalized medicine. A pre-requisite for deriving the benefits of such a systems approach is a reliable and well-validated bioanalytical platform across complementary measurement modalities, especially transcriptomics, proteomics and metabolomics, that operates in concert with a megavariate integrative biostatistical/bioinformatics platform.
The opportunities, challenges and strategies in the development of *herbal medicine* will be discussed including a future outlook on personalized health.

1. Van der Greef J, Martin S, Juhasz P, Adourian A, Plasterer T, Verheij ER, McBurney RN. The art and practice of systems biology in medicine: mapping patterns of relationships.
J Proteome Res. 2007 Apr;6(4):1540-59.

2. Van der Greef J, Hankemeier T, McBurney RN. Metabolomics-based systems biology and personalized medicine: moving towards n = 1 clinical trials? Pharmacogenomics. 2006 Oct;7(7): 1087-94

3. Van der Greef, J and McBurney, RN. Rescuing Drug Discovery and Drug Development: In Vivo Systems Pathology and Systems Pharmacology, Nature Reviews Drug Discovery 2005 Dec; 4: 1-7

Systems Biology: Scientific evidence and a novel quality control for Traditional Chinese Medicine (TCM)

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Key for worldwide registration and acceptance of Traditional Chinese Medicine (TCM) is the ability to provide scientific evidence in combination with a quality control system based on the bioactive ingredients. A Plant-to-Patient platform is described that is comprised of varies novel approaches based on Systems Biology to tackle these issues

The use of indigenous herbs is a very important concept for TCM materials medica production and specific areas are known to produce the highest quality herbs especially when Good Agricultural Practice (GAP) is practiced. In relation to the QC aspect a number of factors need to be considered besides the geographical source, such as variation in climate and soil factors which can influence the growth of herbal plants and consequently the composition patterns. In addition, the processing of TCM materials medica is also a very important part which has a history as long as traditional Chinese herbal medicine itself but needs to meet modern Good Manufacturing Practice. It underlines the importance of tools to accurately measure the composition of TCM products in order to guarantee the quality of herbs.

Modern scientific technology tools are now available to accomplish standardization of TCM products in order to achieve a high level of efficacy and safety, enhancing the introduction into the international markets. The complexity of ingredients and the aspect of synergistic bioactivities in TCM, limited the analysis for quality control so far only on major components for each herb and had no evidence for a direct relation with bioactive components. The systems Biology approach, a multi dimensional chemical and pharmacological approach enables linking of the complex metabolic profile of herbs with biological effects and is therefore a key for quality control of TCM material medica, while providing simultaneous scientific evidence for the underlying efficacy. This is crucial for registration of herbal medicinal products such as under the new EU Herbal Medical Products guideline.

Transcriptomics and jasmonates, a potent combination to unravel the regulation of plant secondary metabolism

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Plants are capable of producing an overwhelming variety of secondary metabolites, many of which display interesting biological activities. The potency of the plant hormone jasmonate to elicit secondary metabolism in plant cells has converted them into powerful tools to unravel this complex cellular process. Combined transcript and metabolite profiling approaches to tackle plant secondary metabolism allowed unraveling the biosynthesis of compounds from model plants such as *Arabidopsis* and *Medicago* but also from a variety of valuable medicinal plant species such as *Taxus* and *Catharanthus*. Yet, the molecular insights into plant secondary metabolism remain limited, which hampers the design of effective metabolic engineering strategies.

To address this issue we employ a functional genomics based technology platform that enables comprehensive investigations and large-scale gene discovery programs in plant metabolism. The platform relies primarily on the integration of genome-wide transcript profiling with metabolite profiling and is applicable to any plant species or system to map the biosynthesis of any metabolite. The platform starts from the rationale (i) that transcripts corresponding to genes involved in a particular metabolic pathway will be 'enriched' in cells in which the particular metabolites are produced and (ii) that patterns of co-expression can reveal associations between metabolic pathway genes. Profiling is followed by extensive functional analysis programs for the de novo discovered genes. Functional analysis relies principally on the investigation of effects of gain- and loss-of-function; both in transient expression assays and in stable transformed plants or plant cells; and both in the plants from which the genes are derived and in heterologous plant systems.

Integrated profiling of jasmonate elicited plants or plant cells of amongst others *Arabidopsis thaliana, Catharanthus roseus, Medicago truncatula,* and *Nicotiana tabacum*, has been undertaken, providing a gene platform that allowed establishing conserved and specific themes in the molecular mechanisms involved in the universal role of jasmonates as elicitors of plant secondary metabolism.

Metabolite profiling of herbal drugs by high-resolution LC-MS

Rob van der Heijden, Hongwei Kong, Jiangshan Wang, Chunxiu Hu, Theo Reijmers, Mei Wang, Thomas Hankemeier and Jan van der Greef

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Plant metabolomics provides one of the pillars to study the relationship between the composition of complex and variable mixtures of plant-derived remedies and their –also complex- biological effects. Plant metabolomics starts with the analysis of as many as possible detectable individual components that are present in the material. Extracts made from individual herbs/plants, total mixtures or combinations of individual herbs/plants and extraction/mixing/preparation-methods as used in traditional Chinese medicine (TCM) can be analyzed by means of different techniques (LC-MS, GC-MS, NMR, etc.), resulting in total metabolite profiles. The aim of this project is to develop new analytical methods for the profiling of TCM herbs and formulations, which are able to detect the (expected) hundreds of metabolites with good sensitivity. The discovery of relevant metabolites and fingerprints allow the introduction of an appropriate QA/QC in TCM formulations. Various analytical aspects, including those related to bioavailability, will be illustrated by the analysis of popular herbs as ginseng and *Aconitum.*

Reference

Angelova N, Kong H, van der Heijden R, Yang SY, Choi YH, Kim HK, Wang M, Hankemeier T, van der Greef J, Xu G and Verpoorte: Recent Methodology in the Phytochemical Analysis of Ginseng. Phytochemical Analysis 19: 2–16 (2008).

Flux profiling - going beyond the steady state

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Genomics approaches aimed at understanding metabolism currently tend to involve mainly expression profiling, although proteomics and steady-state metabolite profiling are increasingly being carried out as alternative strategies. These approaches provide rich information on the inventory of the cell. It is, however, of growing importance that such approaches are augmented by sophisticated integrative analyses and a higher-level understanding of cellular dynamics to provide insights into mechanisms that underlie biological processes. This is inherently difficult but two alternate strategies are emerging for the determination of broad range flux analysis - steady state or dynamic labeling. These will both be briefly discussed and the attainability of fluxomics will be addressed. On a more positive note the utility of these approaches in understanding systematic responses to metabolic perturbations and even the structure of metabolic pathways themselves will be presented.

Potential and limitation of NMR-based metabolomics in plant science

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Any endo- or exogenous stimuli are likely to be reflected in organisms by changes in such biochemical pools as transcriptome, proteome, or metabolome. Studying metabolites has a long history compared with those of genes and proteins but ironically metabolomics is the youngest 'OMICS' technology and only recently it became an area of major research interest. It might be because metabolomics has to handle large number of different compounds with highly dynamic levels.

In any metabolomic research, the first step is the selection of an appropriate analytical method aiming at unbiased profiling of metabolites present in a target organism. For the selection of analytical methods, one should consider several factors, e.g. range of detected metabolites, sensitivity, signal robustness, and efficiency in terms of time and cost. Up to date, among the possible candidate technologies, nuclear magnetic resonance spectroscopy (NMR) is currently considered one of the primary approaches for the metabolomics.

In the past, NMR spectroscopy has been regarded as complementary to other analytical methods. However, as a tool for metabolomics, NMR has some unique advantages over other analytical methods. It can provide a detailed analysis on the biomoleculear composition (diverse group of metabolites) very quickly with relatively little sample preparation. It is a universal detector for all molecules containing NMR-active nuclei, particularly protons in 1H-NMR. For all proton-bearing molecules, the intensity of all proton signals is absolutely proportional to the molar concentration of the metabolite. In addition, NMR signals are uniquely based on physical properties. It results in unsurpassed signal robustness which makes it easier to perform following multivariate data analysis.

However, despites these clear advantages of NMR, one has hesitated to use it as an analytical method for metabolomics. Low sensitivity, mol in NMR compared with nmol in MS spectrometry, is one of the most important obstacles to do comprehensive profiling of metabolites, which results in low number of metabolites detected in NMR-based metabolomics. In most cases NMR analysis shows 20-50 metabolites while several hundreds of metabolites can be identified in MS-based metabolomics. Morover, complexity of the spectra is another problem. In general NMR-based metabolomics does not use a preseparation step (non-chromatographic method). In a certain range of NMR spectra, there might be many congested signals. Many splitted NMR signals are detected even for a single molecule, further contributing to the complexity of NMR spectra. Therefore, prior to the interpretation of NMR spectrum, signal sorting and purity confirmation is required. Although many multidimensional methods have been developed for this purpose, the long experimental time for a multidimensional NMR spectrum is still an unsolved problem.

In reality, there is no single analytical method that meets the most crucial requirement of metabolomics, profiling all metabolites. To make metabolomics a comprehensive profiling of metabolites, in a final stage all the results obtained from diverse analytical methods should be integrated in order to provide a real metabolomic data set. There is no doubt that NMR will be a major tool in metabolomics with many benefits in spite of its inherent limitations.

In this presentation, the potential and limitation of NMR will be discussed using case studies of tobacco, *Arabidopsis*, and *Senecio* plants.

Volatiles, hairy roots and metabolomics: what future?

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Volatiles and essential oils are complex mixtures of secondary metabolites, mainly composed by mono-, sesquiterpenes and phenylpropanoids, but diterpenes and polyacetylenic compounds, among others, are also frequently present. Several environmental, political and social factors hamper, in nature, a homogeneous and continuous production of secondary metabolites leading industry to search for alternative procedures to overcome these problems. *In vitro* plant cultures appear, in this context, as a potential approach to explore.

Nevertheless, *in vitro* volatiles production is only known for a limited number of plant cell cultures, mainly due to low secretion and also, in some cases, poor solubility. In most cases, these cultures were unable to produce the same compounds as found in the volatiles from the *in vivo* plant, or the production was rather low. As an example, the yield of the volatiles from cell suspension cultures of *Achillea millefolium*, *Artemisia dracunculus*, *Coleonema album*, *Cryptomeria japonica*, *Foeniculum vulgare* and *Pimpinella anisum* varied from 0.001% to 0.01%, which was about 100 to 1000 times less than that obtained *in vivo* (references in 1). In general, the composition of the oils from these cultures was quite different from that of the parent plant volatiles, and in some cases the production of quite unusual products was achieved. Several procedures have been tested in attempts to surmount cell suspension culture problems, and hairy root cultures appeared to be useful to increase the volatiles production.

Hairy roots are autonomous roots obtained by transformation with *Agrobacterium rhizogenes*. Many dicotyledonous species have been successfully transformed by *A. rhizogenes*. Transformed roots are fast growing, sometimes 10 times faster than the cell suspension cultures; they are laterally highly branched and show a massive biomass increase over relatively short periods of time, in the absence of exogenous phytohormones, because the Ri T-DNA regulates the balance of endogenous hormones.

Our study has focused mainly on the volatiles production by hairy roots of yarrow (*A. millefolium*), anise (*Pimpinella anisum*), dill (*Anethum graveolens*) and lovage (*Levisticum officinale*). All these cultures, maintained already for over 10 years each, with a fortnight or a three-week interval subculture, have shown a high biosynthetic stability. The hairy roots volatiles yield showed major differences when compared to the cell suspension cultures counterparts and is, in some cases, equal to or higher than that obtained with the parent plants. This capacity was strictly correlated with the differentiated state of the cultures, the level of production being severely impaired or lost when the hairy root phenotype was lost.

Several attempts have been made to enhance productivity, but no general procedure is known to be effective in all cases. The outcome of these studies may help to understand the hairy root culture behaviour and reveal some advantages of this *in vitro* system for the production of the different types of secondary metabolites that can be found in plant volatiles.

INVITED SPEAKERS - ABSTRACTS

1. Figueiredo AC, JG Barroso, LG Pedro, JJC Scheffer (2006) In: Teixeira da Silva JA (Ed.), *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues*, Global Science Books, UK.