



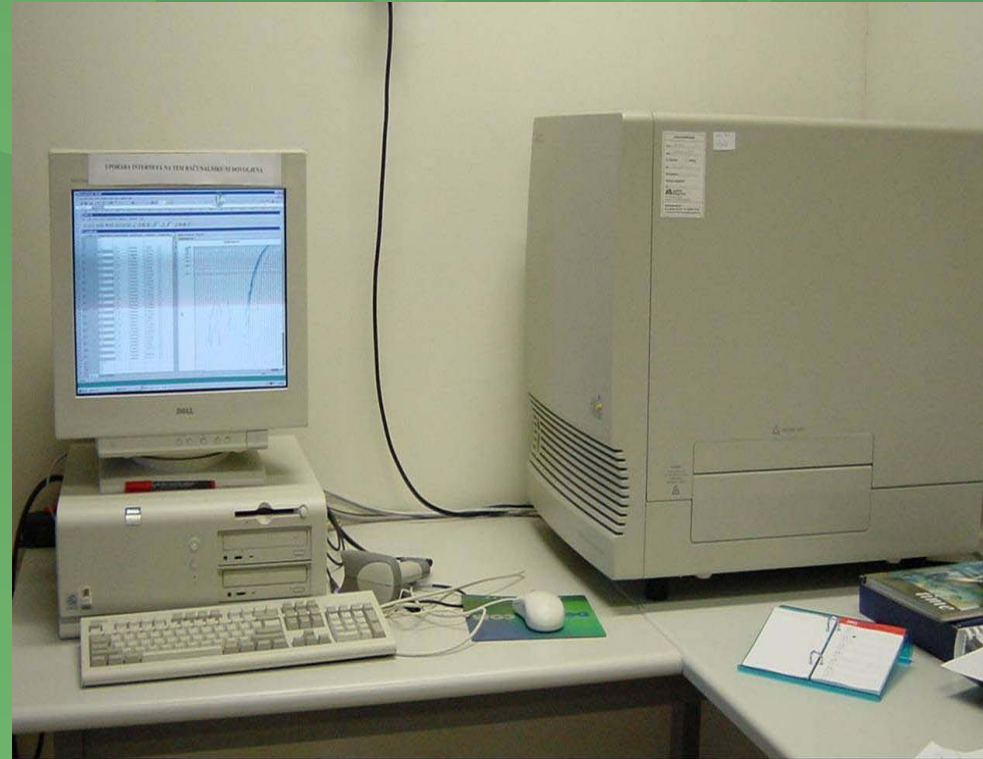
**Department of Plant Physiology and Biotechnology,
National Institute of Biology**

**Nucleic acid microarray application to potato and
grapevine pathogen interaction studies**

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Kristina Gruden et al**

INSTRUMENTAL PLATFORM

- Instrumental CENTER
PLANTA NIB
Real time PCR, EM,
Growing chambers and
Quarantine greenhouse
- Slovenian Center of
Excellency: platform for
Microarray applications,
- Scener, plotter



MAIN RESEARCH AREA

Department of Plant Physiology and
Biotechnology

- Plant-pathogen and plant-pest interactions
- Development of high through-put methods (QPCR, microarrays), for diagnosis and different gene expression studies

STUDIES OF BIOINTERACTIONS

Main fields:

- potato – virus (PVY^{NTN})
- potato- colorado beetle - PVY
- grape-vine - phytoplasma interactions

in order to discover mechanisms of disease development on one side and resistance mechanisms on the other.

Our long-term aim is to identify the genes, proteins and signalling molecules, which are involved in resistance mechanisms of potato and other plants to pathogens

Study of potato – PVY^{ntn} interaction

- Mechanism of PTNRD development and resistance to PVY^{NTN} is not well understood:
- We are following:
 - Time course of differential gene expression after PVY^{NTN} infection using microarrays
 - Use of QPCR for confirmation



Resistant/sensitive

Resistant	No symptoms	No tuber necrosis	Sante Carlingford
Tolerant	Mild symptoms, systemic	No tuber necrosis	Pentland squire
Sensitive	LL and mosaic, systemic	1/3 tuber necrosis	Desiree
Highly sensitive	Severe LL and mosaic, systemic	100% tuber necrosis	Igor

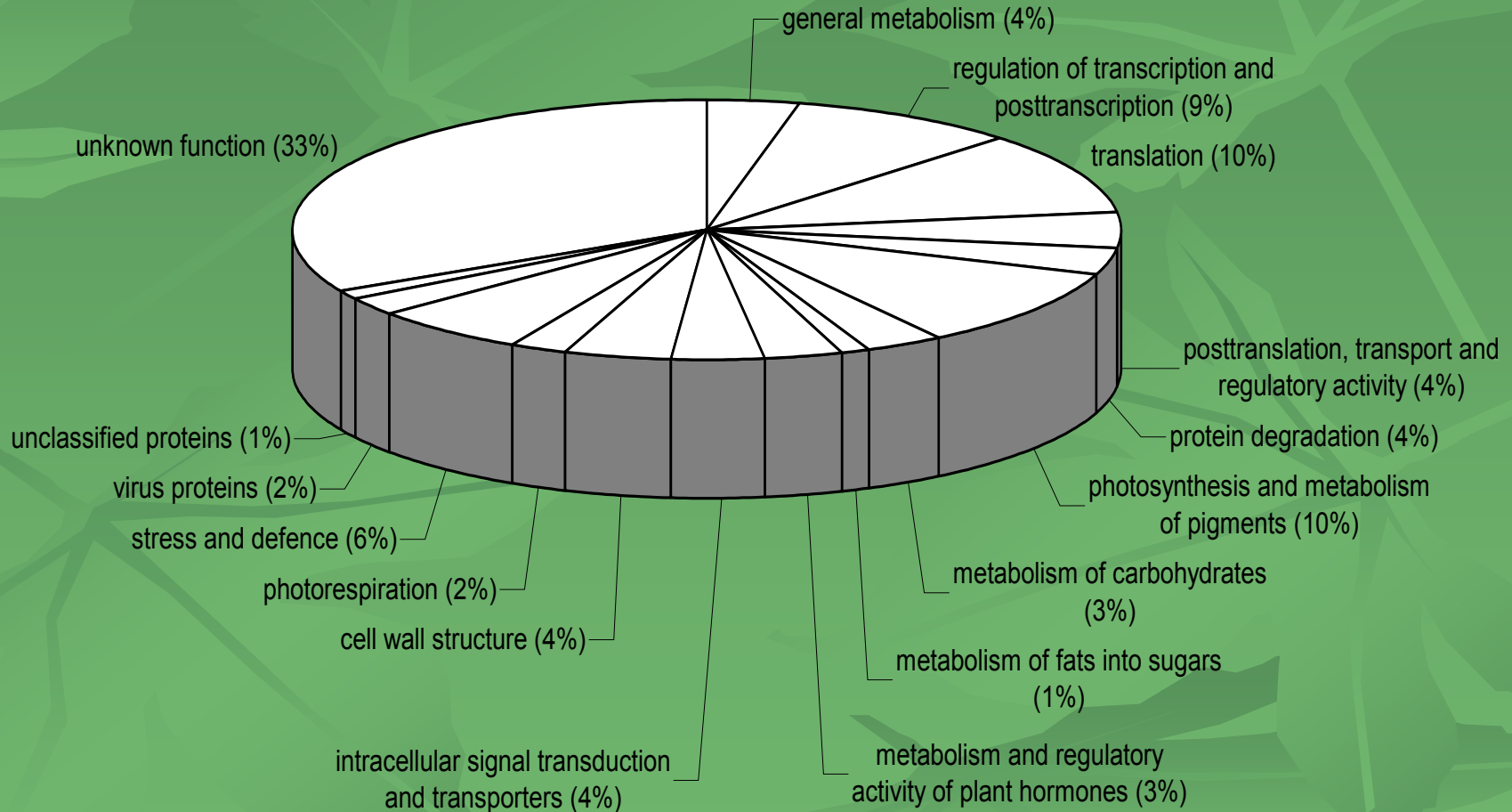
Parallel studies of: plant hormones / enzymes / immunolocalisation of proteinases and PI

- Our first investigations showed that virus infection is related to changes of **cytokinins**. After infection in sensitive cv. Igor cytokinins are present in the form of inactive glucosides in contrast to resistant cv. Sante.
- **Jasmonic acid** and **salicylic acid**,
- **photosynthetic pigments**,
- **peroxidases** and **proteinase inhibitors**
- changes in **morphology** were observed also on cellular level

Hybridization of cDNA microarrays enabled observation of differences in gene expression

- We constructed library of differentially expressed genes in infected and noninfected potato leaves of cv. Igor
- Sequenced 400 clones and prepared DNA microarrays
- **175 genes** were identified
- Expression was followed **after local lesions appearance** (7 days), **in systemic infection** (14 days) and in plants with **established infection** (plants grown from infected tubers)

Sensitive cv. Igor



Sensitive cv. Igor

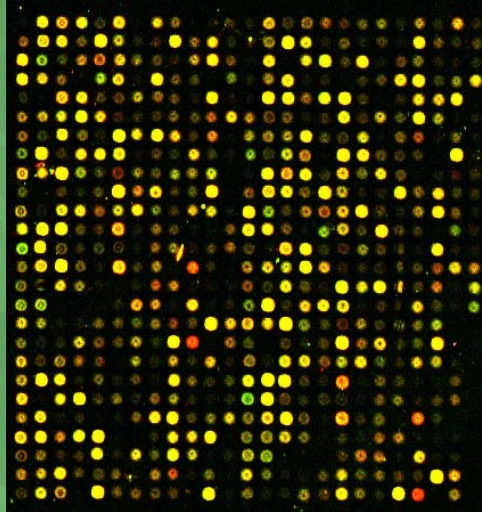
- The most pronounced changes in gene expression were detected in systemically infected leaves **14 days after infection (35)** and in **plants grown from infected tubers (26), less 7 days after infection (18,7)**
- Different sets of genes are activated in different stages of disease development (1/2 of genes responded)
- Expression of several stress related genes was observed like **heat shock proteins (HSP 70↑)**
catalase ↓, β -1,3-glucanase↑

- microarrays proved to be very sensitive (more than ELISA and EM) for **detection of PVY^{NTN}** coat protein in early stages after infection what was confirmed using qPCR. We can detect viruses in noninoculated leaves 5 days after inoculation
- The largest amount of coat protein gene was found in plants grown from infected tubers

Study of potato – PVY interaction



Study of gene expression by microarrays



Cooperation with **Jožef Stefan Institute**, Ljubljana (Borut Štrukelj)
PRI, Wageningen (Maartin Jongsma)

Lately:

Rikilt Institute of Food Safety, Wageningen (Ester Kok)

CSL, York (Neil Boonham and Ian Barker) and

Bristol University (Gary D. Foster)

Potato microarrays in progress

- Study of gene expression (cvs. Igor, Sante) **early after infection** (30min, 1, 8, 12, 24h) using TIGR microarrays
- A library of **differentially expressed genes** in infected and mock inoculated plants of **resistant cv. Sante** and further gene expression studies
- A study of **differences in gene expression** in sensitive cvs. Igor and Nandine after infection with PVY^{NTN} and PVY^N isolates

Expression of potato genes involved in gene silencing by microarrays and real time PCR

- Searching for **mechanism of RNAi** in transgenic potato plants resistant to PVY^{NTN} is in progress.
- **TIGR microarrays** in combination with **qPCR** (**RdRp, Dicer, RISC genes**) are used in order to evaluate resistance in plants with inserted viral coat protein.

Transgenic plants production

- PVY resistant potato
- Introduction of PVY coat protein in cv. Igor



STANIČ RACMAN, Darja, MCGEACHY, Kara, REAVY, Brian, ŠTRUKELJ, Borut, ŽEL, Jana, BARKER, Hugh. Strong resistance to potato tuber necrotic ringspot disease in potato induced by transformation with coat protein gene sequences from an NTN isolate of Potato virus Y. *Ann. Appl. Biol.*, 2001, vol. 139, p. 269-275

Cooperation with **SCRI**, Dundee, Hugh Barker

Study of potato – Colorado beetle interaction using microarrays and QPCR

- Colorado beetle's adaptation to plant's defense mechanism is studied:
 - Time course of the adaptation
 - Tritrophic interaction:
PVY / potato / Colorado beetle



- We characterised digestive enzymes from guts of larvae

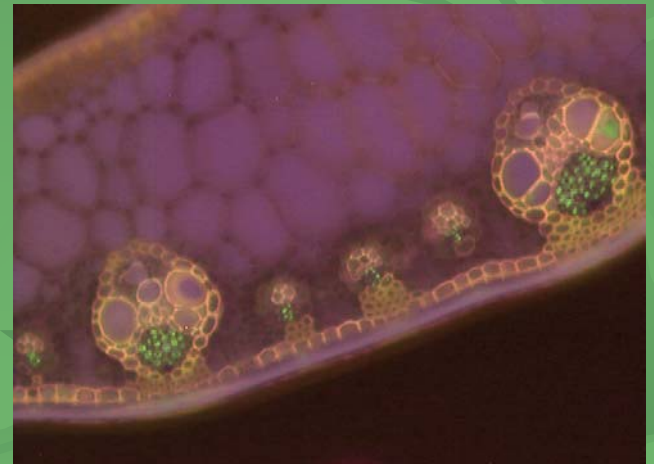
- Three structural different groups of **cysteine proteinases** were identified.
- Intestatin3 can cleave potato proteinase inhibitors
- The complex analysis of the insect adaptation process was performed.
- GRUDEN, Kristina, KUIPERS, Anja G. J., GUNČAR, Gregor, SLAPAR, Nina, ŠTRUKELJ, Borut, JONGSMA, Maarten A. Molecular basis of Colorado potato beetle adaptation to potato plant defence at the level of digestive cysteine proteinases. *Insect biochem. mol. biol.*, 2004, 34, p. 365-375

Plant-phytoplasma interaction – in progress

- Phytoplasmas are not well characterized intracellular pathogens
- Differences in gene expression are studied using
- Oligo microarrays , Genoplant

Cooperation:

- Serge Delrot, Univ. Poitiers
- Nancy Terrier INRA, Montpellier
- Charles Romieu INRA, Montpellier
- University of Udine (Rugero Osler)



QPCR is used for investigation of :

- ADH1, HSP 70, SSH
- and genes specific for grapevine phytoplasmas



In previous studies we have shown the impact of sucrose synthase in disease development in sweet corn

Diagnosis of viruses, bacteria and phytoplasmas

QPCR:

Port Check QPCR

Development of QPCR for *Xylophylus ampelinus*,
CaMV, ToMV, CSNV

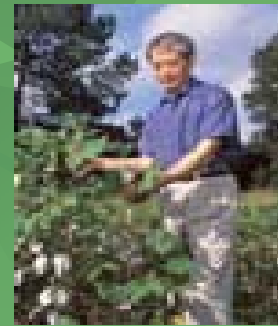
Mycroarrays

Ring testing



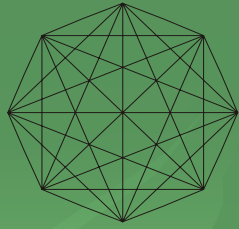
GMO detection

CO-EXTRA project, 6th EU project: development of microarrays and QPCR for GMO detection



- NIB department of Plant Physiology and Biotechnology is accredited for qualitative and quantitative detection of GMOs





2005 Event

European networking summer school

Genomics and Bioinformatics: Exploiting Microarrays in Plant Physiology

August 22nd - 31st, 2005
Ljubljana, Slovenia

- Program:
 - Focus on use of micrarray hybridizations and data analysis in plant physiology and genomics.
 - a theoretical part (lectures): open to public
 - practical part (lab and computer sessions): open to the selected participants only.

The event is organized by:

- Federal Ministry of Education and Research in Germany (BMBF),
- Slovenian Ministry of Higher Education, Science and Technology,
- German Plant Genomics Program GABI,
- Max-Planck-Institute of Molecular Biology, Potsdam-Golm, Germany,
- University of Potsdam, Germany,
- National Institute of Biology, Ljubljana, Slovenia,
- National Centre for Functional Genomics and Microarray Technology, Ljubljana, Slovenia

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