International Conference
Genetic control of plant pathogenic viruses and their vectors: Towards new resistance strategies

Puerto de Santa María (Cádiz, Spain), 23rd – 27th November 2008

Book of Abstracts

http://www.richalia.es/congreso/index.html

ResistVir

Congress Presentation

Plant viruses and their vectors cause serious economic losses, limit worldwide crop production, and have negative effects on the quality and safety of food. The use of genetic resistance is one of the best ways to control plant viruses and their vectors. **ResistVir** (http://www.resistvir-db.org/) is a Coordination Action funded by the 6th EU Framework Program which aims at improving co-ordination of research on genetic resistance to plant pathogenic viruses and vectors in European crops. This will ultimately result in new sources of sustainable resistance to plant viruses and vectors, being used in conventional and GM crops, and in decreased pesticide usage.

ResistVir is now organizing an International Congress dedicated to current research activities on genetic resistances to plant pathogenic viruses and their vectors. The meeting will cover aspects related to (i) important/emergent plant virus diseases, (ii) factors required for virus multiplication and spread, (iii) plant responses to viruses and/or their vectors, (iv) innovative approaches, including transgenic resistance, to control plant viruses and vectors, (v) and resistance durability. A Technology Tranfer round table, in which significant companies in the field are going to be represented, has also been included in the programme. We hope the meeting will provide the opportunity for the scientists working in plant genetic resistance to viruses and their vectors and related research areas to meet and discuss their work and establish new international collaborations.

The Organising Committee would like to thank Chairs of the scientific sessions and members of the Scientific Committe for their help in preparing the programme, as well as to our colleague Amelia Sánchez-Pina for reviwing this booklet of abstracts. We would also like to thank our sponsors for kindly providing support to this meeting.

We hope you enjoy the Resistvir meeting on Genetic control of plant pathogenic viruses and their vectors: Towards new resistance strategies, and your stay in El Puerto de Santa Maria (Cadiz).
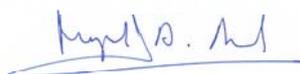
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18:30-19:30   Welcome reception
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Day 2 – Monday 24th November 2008

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9:40-10:00 "*Two monopartite begomoviruses associated with eudicots endemic to Puerto Rico*". **Judith K.Brown**, University of Arizona, USA

10:00-10:20 "*The synergistic interactions between SLCV and WmCSV – a new danger to cucurbit crops in the Middle East*". **Moshe Lapidot**, Volcani Center - ARO, Israel

10:20-10:40 "*Emergence of new strains of Watermelon mosaic virus in South-Eastern France: evidence for limited spread but rapid local population shift*". **Cécile Desbiez**, INRA-Avignon, France

10:40-11:00 Invited talk: "*The evolutionary genetics of plant viral emergence*". **Santiago F. Elena**, CSIC-UPV, Valencia, Spain

11:00-11:30   **Coffee break**

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Chairperson: Lesley Torrance

11:30-12:10 Keynote lecture: *"Both plant and insect factors are specifically required for the success of CaMV spread through vector-transmission".* **Stéphane Blanc**, INRA-CIRAD-ENSAM, Montpellier, France

12:10-12:30 "*Tubulin and transmission of Cauliflower mosaic virus*". **Martin Drucker**, INRA-CIRAD-ENSAM, Montpellier, France

12:30-12:50 "*The major nucleolar protein, fibrillarin, is required for a plant virus multiplication and spread*". **Michael Taliansky,** Scottish Crop Research Institute, Dundee, United Kingdom

12:50-13:10 *"Role of plasmodesmata located proteins in the intra- and inter-cellular trafficking of Grapevine fanleaf virus movement protein and implication in virus movement"*. **Khalid Amari**, IBMP – CNRS, Strasbourg, France

13:10-13:30 "*Phloem limitation of potato leafroll virus is mediated by the readthrough P5 and is selected for by the virus"*. **Peter Palukaitis**, Scottish Crop Research Institute, Dundee, United Kingdom

Symposium II (cont.): **Factors required for virus multiplication and spread**
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16:10-16:30 "*Structural analysis of eIF4E, a susceptibility factor for Pea seed-borne mosaic potyvirus*". **Jamie Ashby**, John Innes Centre, Norwich, United Kingdom

16:30-16:50 "*Common and distinct features of eIFiso4G-mediated resistance to Rice yellow mottle virus compared to eIF4E-mediated resistance to potyviruses*". **Eugénie Hébrard,** IRD, Montpellier, France

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17:10-17:30 "*Sobemoviruses possess a common genome organization and a highly modified VPg protein*". **Erkki Truve,** Tallinn University of Technology, Estonia

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18:00-19:30
Important/emergent plant virus diseases
Factors required for virus multiplication and spread
Plant responses to viruses and/or their vectors

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9:40-10:00 "*Is the silencing suppressor activity of P0 targetting ARGONAUTE enough to establish a successful infection of poleroviruses in Arabidopsis?*" **Véronique Ziegler-Graff**, CNRS, Strasbourg, France

10:00-10:20 "*Cymbidium Ringspot Virus harnesses RNA silencing to control the accumulation of the virus parasite SatRNA*". **Vitantonio Pantaleo**, Istituto di Virologia Vegetale del CNR, Italy

10:20-10:40 "*Viroid-induced symptoms are dependent of rdr6 activity, a key component of the RNA silencing pathway*". **Gustavo Gómez**, CSIC-UPV, Valencia, Spain

10:40-11:00 "*ASSYMETRIC LEAVES1 is a receptor of betaC1, the pathogenesis factor of TYLCCNV*". **Nam-Hai Chua**, Rockefeller University, New York, USA

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12:10-12:30 "*Aphid and virus resistance triggered by the CC-NBS-LRR Vat melon gene*". **Catherine Dogimont**, INRA-Avignon, France

12:30-12:50 "*Plant-virus-insect interactions: a new role for the 2b protein of cucumber mosaic virus?*". **Jack Westwood**, University of Cambridge, United Kingdom

12:50-13:10 "*Differential effects of 2b protein from two Cucumber mosaic virus strains and satRNA co-infection in the alteration of microRNA-regulated gene expression in tomato*". **Fabrizio Cillo**, CNR, Istituto di Virologia Vegetale, Bari, Italy

13:10-13:30 "*Genetic and functional characterization of the RTM-mediated resistance*". **Frédéric Revers**, UMR GDPP, INRA-Bordeaux, France

Symposium IV: **Innovative approaches, including transgenic resistance, to control plant viruses and vectors**
Chairperson: Margit Laimer da Camara Machado

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16:10-16:30 "*RNAi-mediated resistance to Potato spindle tuber viroid in transgenic tomato expressing the viroid hairpin DNA construct*". **Gabi Krczal**, RLP Agroscience GmbH, AlPlanta, Neustadt, Germany

16:30-16:50 "*Molecular breeding for virus resistance in cereals*". **Frank Ordon**, Julius Kuehn-Institute, Quedlinburg, Germany

16:50-17:10 "*Subcellular visualisation of homologous and heterologous protein-protein interactions of the 2b protein of CMV, and their significance to its suppressor of gene silencing function*". **Tomás Canto**, CSIC, Madrid, Spain

17:10-17:30 "*Analysis of the effects of high selection pressure on recombination between RNA3 of Cucumber mosaic virus and transgene mRNAs*". **Marco Morroni**, ICGEB Biosafety Outstation, Italy

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20:00   **Social evening**

Day 4 – Wednesday 26th November 2008

8:00   **Registration opening**

Symposium IV (cont.): **Innovative approaches, including transgenic resistance, to control plant viruses and vectors**
Chairperson: Jari Valkonen

9:00-9:40 Keynote lecture: *"Host targets of viral proteins and their manipulation in transgenic plants".* **Uwe Sonnewald**, Friedrich-Alexander University Erlangen-Nürnberg, Germany

9:40-10:00 "*SharCo FP7 European initiative: Testing new strategies of resistance to Plum pox virus*". **Véronique Decroocq**, IBVM, INRA-Bordeaux, France

10:00-10:20 "*Geminiviruses: when VIGS defeats PDR*". **Mario Tavazza**, ENEA, Dipartimento BAS-biotec, Roma, Italy

10:20-10:40 "*The high and wide-ranging action of PPV 5'UTR/P1 hairpin construct to confer resistance to Plum pox virus*". **Vincenza Ilardi**, CRA-Research Center on Plant Pathology Rome, Italy

10:40-11:00 "*Evaluation of resistance conferred to Italian isolates of TYLCSV and TYLCV by Ty-1 and Ty-2 resistance genes and application of new CAPS markers for introgression in a traditional Italian tomato variety*". **Gian Paolo Accotto**, Istituto di Virologia Vegetale, C.N.R., Torino, Italy

11:00-11:30 **Coffee break**

Symposium III (cont.): **Plant responses to viruses and/or their vectors**
Chairperson: Alan Schulman

11:30-12:10 Keynote lecture: *"Anti-viral responses induced by plant NB-LRR proteins involve Argonaute-dependent control of viral transcript translation".* **Peter Moffett**, Boyce Thompson Institute for Plant Research, Ithaca, NY, USA

12:10-12:30 "*Characterization of the interaction between Rx and Potexvirus coat protein: what can we learn from the elicitor side?*". **Bénédicte Sturbois**, URGV-INRA-CNRS-UEVE, Evry, France

12:30-12:50 "*Plant virus infection induced persistent host gene down regulation in systemically infected leaves*". **Zoltan Havelda**, Agricultural Biotechnology Center, Hungary

12:50-13:10 "*RNase III encoded by a plant RNA virus suppresses antiviral defence in sweetpotato plants*". **Jari P.T. Valkonen**, University of Helsinki, Finland

13:10-13:30 "*Functional domains in Helper-Component of ZYMV involved in suppressor activity and symptom expression*". **Amit Gal-On**, Volcani Center-ARO, Israel

Symposium V: **Resistance Durability**
Chairperson: Hervé Lecoq

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16:10-16:30 "Constraints on evolution of virus avirulence factors predict the durability of corresponding plant resistances". **Benoît Moury**, INRA-Avignon, France

16:30-16:50 "*African rice cultivated species, Oryza glaberrima, opens new perspectives for analysis and breeding of resistance against Rice yellow mottle virus*". **Laurence Albar**, IRD/CNRS/Université de Perpignan, Montpellier, France

16:50-17:10 "*Base-by-base analysis of siRNA production by a plant inverted-repeat transgene giving viral resistance*". **Victor Gaba**, Volcani Center - ARO, Israel

17:10-17:30 "*Broad-spectrum resistance to Turnip mosaic virus in Brassica rapa (Chinese cabbage)*". **John A. Walsh**, University of Warwick, United.Kingdom

Poster Session II

Chairpersons: Mark Tepfer & Emanuela Noris

18:00-19:00
Innovative approaches, including transgenic resistance, to control plant viruses and vectors
Resistance Durability

Presentation of ResistVir document

19:00-19:40 "*Suggested guidelines and recommendations for future research policy*". **Andy Maule**, John Innes Centre, Norwich, United Kingdom

20:00   **Banquet diner**

Abstracts of lectures

Symposium I: Important/emergent plant virus diseases

Chairperson: Denis Fargette

(L1) Emergence of whitefly-transmitted viruses: recombination and synergism

Enrique Moriones

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Viruses cause the largest fraction of emerging diseases of plants. Emergence of whitefly-transmitted viruses is paradigmatic and is occurring worldwide after the emergence of their insect vectors. As a consequence, severe economic damage is caused for example by begomoviruses (genus *Begomovirus,* family *Geminiviridae*) and criniviruses *(*genus *Crinivirus*, family *Closteroviridae*) in vegetable crops, tomato among others. Changes in host ecology and environment or in host genetics are factors that can drive virus evolution and emergence. Human activities can affect these factors and may favour virus emergence. Thus, global trade in recent times is contributing to the expansion of the geographical ranges of viruses and movement to new ecological niches. Moreover, human manipulation of domesticated plants, reducing genetic diversity and using resistant genotypes might contribute to the emergence of viral diseases. Viral populations are complex and mixed infections can occur in nature. Then, genetic mutation, reassortment or recombination processes might help virus diversification and result into the emergence of better adapted variants. Furthermore, mixed infections in single plants can occur and result in synergistic interactions that may favour virus adaptation and emergence. Understanding the basis of emergence of viruses is essential to prevent and control new emergence, to support policy making and to design of more durable and efficient control practices. This aspect will be discussed under the light of the knowledge acquired from the study of epidemics occurred in intensive vegetable crops of southern Spain during the last decade.

(L2) Two monopartite begomoviruses associated with eudicots endemic to Puerto Rico

J.K.Brown, J. Bird, and A.M. Idris

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In a previous study, using PCR amplification, we discovered a new bipartite begomovirus of tomato, named *Merremia mosaic virus* (MeMV). The virus also was detected in two uncultivated eudicot species, *Merremia aegyptia* (L.) Hallier f.and *M. quinquefolia* (L.) Urb. belonging to the plant family *Convolvulaceae*, which is endemic to Puerto Rico (Idris et al., 2002). The latter species exhibited bright yellow mosaic symptoms of Merremia mosaic disease (MeMD). Recently we revisited these samples to pursue the possibility of undiscovered etiological agents, because disease symptoms observed in field grown tomato could not be reproduced using infectious MeMV clones. We employed rolling circle amplification (RCA) (Haible *et al.*, 2006; Inoue-Nagata *et al.*, 2004), which uses Φ29 DNA polymerase to amplify circular ssDNA, to amplify all possible begomoviral ssDNA templates from plant extracts. The high molecular weight products were cloned following restriction endonuclease digestion, and the DNA sequence was determined. Results indicate the presence of two distinct *Merremia*-infecting monopartite begomovirus species belonging to the genus *Begomovirus*: a new natural recombinant species provisionally designated Merremia leaf curl virus (MeLCV) and an isolate of *Sweet potato leaf curl virus* (SPLCV), designated SPLCV-PR. The recombinant isolate represent a new species for which relatives from sweet potato have been reported from Spain and in the U.S.A. These three begomoviruses, MeLCV, SPLCV and MeMV, also were identified in mix-infection in single *Merremia* spp plants showing typical MeMD symptoms. These findings demonstrate that several plant species indigenous to Puerto Rico serve as hosts of genetically diverse begomoviral populations that are of economic importance in tomato and sweet potato crops. Whether the monopartite genomes discovered here from *Merremia* species are introduced and endemic viruses is not yet known. This is the first report of two monopartite begomoviruses in the New World.

(L3) The synergistic interactions between SLCV and WmCSV – a new danger to cucurbit crops in the Middle East

Tali Sufrin and Moshe Lapidot

Dep. of Vegetable research, Inst. of Plant Sciences, Volcani Center, ARO, Bet Dagan, P.O.Box 6, 50250, Israel. E-mail: lapidotm@volcani.agri.gov.il

Towards the end of 2002, two new severe diseases were identified in cucurbits in Israel – one in watermelon (*Citrullus lanatus*) and a second in squash (*Cucurbita pepo*). The causal agents were two whitefly-transmitted geminiviruses (begomoviruses), new to the region. The viruses were cloned, sequenced and identified as *Watermelon chlorotic stunt virus* (WmCSV) and *Squash leaf curl virus* (SLCV). Both geminiviruses infect nearly all cultivated cucurbits. WmCSV induces severe symptoms and yield reduction in watermelon plants, while SLCV similarly affects squash. These viruses endanger the production of squash and watermelon in the region to the point of becoming the limiting factor in both crops, as it has become nearly impossible to grow squash and watermelon in open fields in Israel. Both viruses have been identified in Jordan (Al-Musa *et al.*, 2008, *J. Phytopathology* 156:311-316) and SLCV has also been identified in Egypt (Idris *et al.*, 2006, *Plant Disease* 90:1262). Hence, it is quite clear that both viruses are spreading, and it is only a mater of time until both viruses will be present throughout the Mediterranean region.

In the Middle East cucurbits are grown in a patchwork of small fields with different crops of different ages adjacent to each other, resulting in a year-round presence of plants that may serve as sources of virus inoculum and insect vectors. The warm climate permits a nearly year-round persistence of insect vectors, such as the whiteflies that vector WmCSV and SLCV. Moreover, it was found that squirting cucumber (*Ecbalium elaterium)*, a local wild-type cucurbit, can serve as a symptomless host for both viruses, as well as a reservoir for viral spread.

Beside the discovery of SLCV and WmCSV, a new sever disease was found in melon fields. Due to their overlapping host range, we studied whether SLCV and WmCSV may have synergistic interactions by analyzing dual infection of both viruses on melons plants. Melon plants were inoculated either with SLCV, or with WmCSV, or with both viruses simultaneously. Inoculation was done using whiteflies. As control served noninoculated plants, or plants exposed to nonviruliferous whiteflies.

Our results revealed that melon plants are hardly affected by SLCV infection, contrary to infection with WmCSV – the plants displayed clear disease symptoms and suffered yield loss of up to 30%. However, dual infection of melon plants with both SLCV and WmCSV induced the most dramatic effects. The plants displayed severe disease symptoms coupled with strong plant stunting, produced small and low quality fruits and suffered a drastic yield loss. We also studied the impact of the interaction between both viruses (SLCV & WmCSV), on viral DNA accumulation. It was found that in plants infected with both viruses, SLCV DNA accumulation strongly increased, compared with plants that were infected only with SLCV. This was opposed to accumulation of WmCSV DNA, which wasn't affected by the presence of SLCV. Hence, our results clearly demonstrate that SLCV and WmCSV are able to attack the same host simultaneously, and when doing so both viruses interact with one another.

(L4) Emergence of new strains of *Watermelon mosaic virus* in South-Eastern France: evidence for limited spread but rapid local population shift

Cécile Desbiez, Catherine Wipf-Scheibel, Benoît Joannon, Charlotte Chandeysson, and Hervé Lecoq

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Watermelon mosaic virus (WMV; genus *Potyvirus*, family *Potyviridae*) has been described in France for more than 30 years but, until recently, it caused only mild symptoms on the leaves of zucchini squash without noticeable impact on the yield and quality of the fruits. However, since 1999 strains inducing very severe symptoms on zucchini squash (mosaic and deformations on the leaves and fruit) have been responsible for important economic losses in this crop. At the molecular level, using nucleotide sequences of the C-terminal part of the polymerase and N-terminal part of the coat protein coding regions, WMV can be clustered in 3 groups, two of which (Group 1 and Group 2) were observed in France until 1999. Group 1 is predominant in all growing areas, and strains belonging to this molecular group are commonly referred as 'classical' (CL) isolates. Group 2 is more rarely encountered. Since 1999, strains belonging to the third molecular group (Group 3) have emerged in Southeastern France, frequently –but not always- associated with the more severe symptoms in zucchini squash. In order to understand the origin(s) of these emerging (EM) isolates and their spread in WMV populations, an epidemiological survey has been performed from 2004 to 2007 in the major cucurbit growing regions of France. Our results revealed the presence of 4 well-defined molecular subgroups of EM isolates, suggestive of multiple introductions, with a strong geographic structure of WMV populations: EM isolates were detected in South-eastern France only, contrary to CL isolates that were widespread around the country. Besides, there was a geographic structure at a lower geographic scale within EM isolates in South-eastern France. The geographic structure of the WMV populations remained stable over the four-year period, but EM isolates tended to replace CL isolates within a few years in South-eastern France constituting an interesting example of population shift for an RNA plant virus.

(L5) The evolutionary genetics of plant virus emergence

Santiago F. Elena

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An emerging virus is defined as the causative agent of an infectious disease whose incidence is increasing following its appearance in a new host population or whose incidence is increasing in an existing population as a result of long-term changes in the underlying epidemiology. The sources of emerging viruses are reservoir species in which the pathogen is already well established. Over the recent years, agriculture has been seriously compromised by a succession of devastating epidemics caused by new plant viruses (e.g., *Rice yellow mottle virus*, *Maize streak virus*, *Banana streak virus*, *Tomato torrado virus*, *Tomato spotted wilt virus*, *Tomato yellow leaf curl virus*, or *Pepino mosaic virus*), that switched host species, or new variants of classic viruses that acquired new virulence factors (e.g., *Cucumber mosaic virus* strains supporting necrogenic satellites).

Although viral emergence has been classically associated with ecological change or agronomical practices that brought in contact reservoirs and crop species, it has become obvious that the picture is much more complex and results from an evolutionary process in which the main players are the changes in ecological factors, the tremendous genetic plasticity of viruses, the several host factors required for virus replication (including active defence mechanisms), and a strong stochastic component.

Here, I will put viral emergence into the framework of Evolutionary Genetics and will review the basic notions necessary to understand emergence (e.g., virus mutation rates, G×E interactions on the distribution of mutational effects, basic reproductive rate, and density of susceptible hosts, among others). I will also discuss whether viral emergence requires adaptation to the new host species during the early stages of infection or it is a stochastic process that involves the transmission of a pre-existing viral strain with the right genetic background. Finally, I will discuss why certain types of viruses are more prone to emerge than others.

Symposium II: Factors required for virus multiplication and spread

Chairperson: Lesley Torrance

(L6) Both plant and insect factors are specifically required for the success of CaMV spread through vector-transmission

Stéphane Blanc

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Nearly all plant viruses use specific vectors to spread between hosts, with the most common vectors being insects, especially aphids. The predominant strategy for virus–vector interaction is non-circulative transmission, in which the virus is taken up by a vector in an infected plant, adsorbed somewhere on the cuticle lining of the inner part of the feeding apparatus, and subsequently released and inoculated into a new host plant. While the viral components involved in this phenomenon are well established in several viral species, the counterpart binding sites in the insect vector remain vastly elusive[1]. Similarly, the direct involvement of plant factors in the vector-transmission of viruses has rarely been investigated[2].

1- We have recently demonstrated the existence of a protein receptor for *Cauliflower mosaic virus* (CaMV), in the stylets of aphid vectors. A novel *in vitro* system allowed rapid visualization of the interaction between dissected stylets and the CaMV ligand protein. The receptor molecules are concentrated exclusively in a tiny area located at the extreme distal tip of the maxillary stylets, lining the bed of the common food/salivary duct. Recently, high resolution scanning and transmission electron microscopy have allowed the direct visualisation of the anatomical structure containing these receptors molecules. This anatomical structure in aphid stylets has been previously overlooked and its physiological role remains to be elucidated.

2-Regarding transmission, host cells are usually conceptualised as simple bags where the virus replicates and accumulates, and where it is eventually taken up by a vector feeding on cell contents. Several lines of evidence radically changing this simplistic view will be presented on the example of CaMV. Indeed our results demonstrate that the virus is carefully preparing its encounter with the vector, through the development of specific and astonishingly complex interactions with the plant cell. Such interactions cannot be understood through the investigation of the within plant infection process, as they appear to be entirely devoted to ulterior plant-to-plant transmission.

(L7) Tubulin and transmission of *Cauliflower mosaic virus*

Al Martinière[1], Anouk Zancarini[1], Daniel Gargani[1], Marilyne Uzest[1], Nicole Lautredou[2], Stéphane Blanc[1] and Martin Drucker[1]

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Transmission of *Cauliflower mosaic virus* (CaMV) by aphids depends on the presence of viral electron-lucent inclusion bodies (elIB) in infected plant cells. elIB contain the aphid transmission factor, the viral protein P2, and the viral protein P3. When elIB do not form, no transmission occurs even when infected cells contain functional P2 (Khelifa *et al.*, 2007*, J Gen Virol* 88, 2872-2880)*.* Thus elIB are structures specialised in transmission, hence our interest to study their formation and functions.

We transfected protoplasts with CaMV particles and followed by immunofluorescence accumulation of P2 and P3 during infection. Our results show that P2 and P3 first appear in electron-dense inclusion bodies that are thought to be the site of all viral synthesis. Then the two proteins colocalise with the microtubule network, and late in infection P2 and P3 concentrate in a huge single elIB. Inhibitor studies indicated that elIB morphogenesis requires an intact microtubule cytoskeleton and further, that P2 and P3 are actively transported on microtubules. To identify the putative motor protein, we tested an unusual kinesin, TBK5 (Goto & Asada, 2007, *Plant Cell Physiol* 48, 753-761) in transient overexpression assays. Wild type TBK5 localised to elIB whereas mutant TBK5 did not, indicating that this kinesin might play a role in elIB formation or maintenance.

In another line of experiments, we detected that stress induces import of apparently soluble tubulin into elIB. FRAP experiments indicated a high turnover of elIB-contained tubulin. In aphid transmission experiments, we found that aphids fed on stressed infected leaves transmitted CaMV better than aphids fed on control leaves, and that aphid punctures themselves induced tubulin influx into elIB. Incubation of infected protoplasts with NaN_3 induced disintegration of elIB and relocalisation of P2 and virions on microtubules, concomitant with increased CaMV transmission.

Taken together, our results indicate a double role for tubulin in transmission of CaMV: in the form of microtubules it is needed for generation of elIB, as a dynamic component of elIB, soluble tubulin might control accessibility of P2 and virions to the vector. Our results further suggest that transmission of at least CaMV is the result of highly sophisticated interactions between the host cell, the virus, and the vector.

(L8) The major nucleolar protein, fibrillarin, is required for a plant virus multiplication and spread

Sang Hyon Kim[1], Elisabetta Canetta[2], Eugene Ryabov[3], Natalia O. Kalinina[1], Jane Shaw[1], Ashok K. Adya[2], Stuart MacFarlane[1], John W.S. Brown[1], and Michael Taliansky[1]

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The nucleolus and Cajal bodies are prominent interacting subnuclear domains involved in crucial aspects of cell function such as RNA metabolism, the cell cycle and aging. Certain viruses interact with these compartments but the functions of such interactions are largely uncharacterized. In this work, we show that the ability of the Groundnut rosette virus ORF3 protein to move viral RNA long-distances through the phloem, the specialized vascular system used by plants for the transport of assimilates and macromolecules, strictly depends on its interaction with Cajal bodies, the nucleolus and the major nucleolar protein, fibrillarin. The ORF3 protein targets and re-organizes Cajal bodies into multiple Cajal body-like structures and then enters the nucleolus by causing fusion of these structures with the nucleolus. This process is mediated by the interaction between ORF3 protein and fibrillarin leading to the formation of ring-like complexes. Using atomic force microscopy we have determined the architecture of these complexes as single-layered ring-like structures with a diameter of 18-22 nm and height of 1.9-2.5 nm which consist of equal number (6-8) of ORF3 protein and fibrillarin molecules. A model of structural organization of fibrillarin-ORF3 protein complexes will be presented. It is suggested that ORF3 protein and fibrillarin can move from the nucleus to the cytoplasm in a form of these ring-like complexes. Furthermore, in the cytoplasm, these rings formed by both ORF3 and fibrillarin proteins interact with viral RNA encapsidating it and re-organizing it into helical structures, and thereby play a key role in the assembly of umbraviral RNP complexes capable of long-distance movement and systemic infection. These results demonstrate novel functions for fibrillarin as an essential component of translocatable viral RNPs and may have functional implications for other plant viruses and development of new approaches to control virus defence responses which will be discussed.

(L9) Role of plasmodesmata located proteins in the intra- and inter-cellular trafficking of *Grapevine fanleaf virus* movement protein and implication in virus movement

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Grapevine fanleaf virus (GFLV, genus *Nepovirus*, family *Comoviridae*), a major viral pathogen of grapes worldwide, possesses a bipartite genome. The RNA1-encoded proteins are involved in viral replication, whereas the movement protein (MP) and the capsid protein (CP) encoded by the RNA2 are both required for cell-to-cell movement. During infection, the MP forms tubules in plasmodesmata (Pd) through which GFLV virions pass into non-infected cells according to the tubule-guided mechanism. It has been shown previously, that ectopic expression of MP is sufficient for Pd-targeting and tubule formation. However the manner by which this protein is transported intracellularly and later assembles into tubules remains unknown.

In view of the fact that the MP is an intrinsic membrane protein and that a functional secretory pathway is required for specific MP-targeting to plasmodesmata, two possibilities exist for the intracellular transport pathway of MP: either the MP is a secretory cargo with plasmodesmata targeting signal(s) (hypothesis 1) or the MP is deprived of such signal, and thus depends on a host secretory membrane cargo specifically located at plasmodesmata for proper targeting (hypothesis 2). Using inhibition of secretion-based assays, we were able to demonstrate that only hypothesis 2 applies to GFLV MP trafficking to plasmodesmata. A family of type-I membrane proteins specifically located on the plasma-membrane at plasmodesmata, named PDLP1 (Plasmodesmata Located Protein 1), has recently been characterized (Thomas *et al.*, 2008, *PLoS Biol* 6, e7. doi:10.1371/journal.pbio.0060007, see abstract Lourdes Fernandez-Calvino). These proteins are transported along the secretory pathway. Thus, they may act as cellular partners for GFLV MP trafficking to plasmodesmata. In support of this idea, we were able to demonstrate that PDLP1 is present at the base of the tubules within modified plasmodesmata. In addition, using fluorescence lifetime imaging microscopy-based FRET, we showed that 2B but not TMV MP (30K), interact with specific PDLP1 members *in vivo*. To validate these interactions, we studied the capacity of Arabidopsis PDLP1 insertional knockout mutants to support tubule formation and GFLV movement. Thus, in PDLP1 triple knockout mutant lines, tubule formation was significantly reduced, providing genetic evidence in favor of the function of these host proteins as receptors for 2B. The potentials for PDLP1 proteins to act as receptors for other viruses using tubule-guided movement and their role in virus movement are under investigation. The knowledge of these molecular mechanisms could open new opportunities to design novel strategies for engineered virus resistance based on plant host components.

(L10) Phloem limitation of potato leafroll virus is mediated by the readthrough P5 and is selected for by the virus

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Unlike most plant viruses, viruses in the genera *Luteovirus* and *Polerovirus* in the family *Luteoviridae* are restricted to the phloem and are considered to lack either required movement proteins or plant anti-defence proteins that would allow them to access other plant tissues. The luteovirids contain two capsid proteins: a major protein, P3, of ca. 23 kDa, and an 80 kDa minor readthrough protein of P3, designated P5. Although this multifunctional readthrough protein is translated inefficiently, P5 is required for the aphid transmission of the virus and acts as a movement protein by facilitating the long-distance movement and accumulation of virus in distal phloem tissue. The analysis of a series of mutants of the P5 readthrough protein of the polerovirus potato leafroll virus (PLRV) showed that most P5 mutants were not incorporated into the virus particles, yet they could still facilitate the systemic movement of PLRV in *trans*. However, none of the PLRV P5 mutant viruses were aphid transmissible. The P5 mutant viruses also accumulated systemically at different rates. Some mutations made in the N-terminal half of the readthrough domain, which eliminated translation of the C-terminal half of the readthrough domain, resulted in reduced rates of systemic virus movement and the number of infection foci. Unexpectedly, mutant viruses unable to translate either the entire readthrough protein of the C-terminal half of the readthrough domain, were transmissible mechanically to *Solanum sarrachoides* (hairy nightshade). Moreover, these viruses were able to escape the phloem limitation and infect mesophyll cells in both inoculated and systemically infected tissues. Crystalline arrays of virions were observed associated with chloroplast membranes in mesophyll cells. By contrast, wild-type PLRV was not transmissible mechanically and was observed only in phloem-associated cells. However, the ability of the mutant viruses to move into mesophyll cells and be transmissible mechanically was temporary. Positive selection occurred for compensatory mutations that restored translation of the entire readthrough protein and re-established the phloem-specific distribution of the viruses. Therefore phloem limitation in poleroviruses is not a deficiency, but rather the selection of P5-accumulating, phloem-limited viruses may have evolved to facilitate restricted, systemic infection with the co-lateral effect of enhancing transmission by phloem-feeding aphid vectors and ensuring continued dispersal to new host plants. A model for polerovirus movement encompassing these observations will be presented.

Symposium II (cont.): Factors required for virus multiplication and spread

Chairperson: Andy Maule

(L11) Translation initiation factors: kingpin in plant resistance to RNA viruses

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In recent years, the identification of components of the translation initiation complex, chiefly eIF4E and eIF4G, as key factors in natural resistance/susceptibility to several RNA viruses in a range of plant species has enabled plant virology to take a significant step forward (Robaglia and Caranta, 2006, Trends in Plant Science). The majority of these resistances function against *Potyvirus*, although eIF4E or eIF4G were also implicated in natural resistance to *Bymovirus*, *Carmovirus* and *Sobemovirus*. A common feature is that all eIF4E/eIF4G-mediated resistances result from a small number of amino acid changes in the protein encoded by the recessive resistance allele. However, several recent results highlighted a great diversity in the specificity of use of the different proteins encoded by *eIF4E* and *eIF4G* gene families indicating that phytoviruses have developed a wide range of strategies to exploit the host's translational apparatus and to counter eIF4E/eIF4G-mediated resistance.

In order to get insights into antagonistic relationships between plants and viruses and to characterize selection pressures on *eIF4E* genes in relation with potyvirus resistance and mRNA translation functions, comparative analysis of polymorphism and functional analysis were conducted in *Arabidopsis thaliana* and *Solanaceae*. Results arguing in favour of co-evolutionary arms race between certain plant eIF4E and potyviral VPg will be presented and discussed (Charron *et al.,* 2008. Plant J.).

Another key issue of this research is to exploit fundamental data to define criteria for an efficient and durable use of eIF4E- and eIF4G-mediated resistance in breeding programs. Results on (*i*) the link between the position of amino acid substitutions in eIF4E resistance protein and durability and on (*ii*) the development of the TILLING methodology to extend the set of resistance alleles for breeding, will illustrate this topic.

(L12) Structural analysis of eIF4E, the susceptibility factor for *Pea seed-borne mosaic potyvirus*

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In a number of plant species, the eukaryotic translation initiation factor 4E (eIF4E) has been identified as a potyvirus virulence factor. In eukaryotes, eIF4E binds the 5' cap structure of mRNAs leading to the recruitment of additional factors into the translation initiation complex. In place of the cap, potyvirus genomic RNA has a viral genome-linked protein (VPg) covalently attached to the 5' end. In *Pisum sativum* L. (pea), the recessive resistance allele for the *sbm1* gene encodes a mutant eIF4E unable to facilitate the accumulation of *Pea seed-borne mosaic potyvirus* (PSbMV). Although a direct interaction of eIF4E with VPg is closely correlated successful virus infection, the precise function of eIF4E in potyvirus multiplication remains unclear. Recent work points to a role for eIF4E in cap-independent translation (Khan *et al.*, 2008 *J Biol Chem* 283:1340-1349). In addition, our previous complementation analysis indicated the involvement of eIF4E in cell-to-cell communication and/or virus movement (Gao *et al.*, 2004 *The Plant Journal* 40:376-385). With the aim of dissecting the structural determinants of eIF4E that govern these potential roles in viral genome expression and cell-to-cell trafficking, the crystal structure of recombinant pea eIF4E has been solved to 2.2Å. This is the first eIF4E structure for which resistance alleles to potyvirus infection have been characterised. In common with eIF4E alleles from diverse plant species, the *sbm1* mutations were confirmed as residing proximal to key residues involved in cap binding. Further analysis of the crystallographic data also revealed regions of eIF4E able to undergo conformational changes and provides a structural explanation for the apparent cap-binding activity of the natural avirulence *sbm1* protein and related site-specific mutants. We are using our structural understanding of pea eIF4E to perform a mutagenic survey of residues functionally relevant to virulence, virus-host interactions and endogenous translation initiation. Together with complementary crystallography approaches, these findings will allow us to compile a functional map of eIF4E for future use in both predicted and engineered potyvirus resistance strategies.

(L13) Common and distinct features of *eIFiso4G*-mediated resistance to *Rice yellow mottle virus* compared to *eIF4E*-mediated resistance to potyviruses

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While most recessive resistant genes against phytoviruses encode eukaryotic translation initiation factors 4E (eIF4E or eIFiso4E), the resistance gene against *Rice yellow mottle virus* (RYMV) encode eIFiso4G, another factor involved in the same protein complex (Albar et al, 2006, *Plant Journal* 47: 417-426). Mutations of eIF4Es induce resistance against potyviruses and mutations in potyviral VPgs (viral protein genome-linked) are involved in the breakdown of *eIF4E*-mediated resistances. Similarly, resistant alleles of eIFiso4G contain mutations, mutations in the VPg of RYMV induce the resistance-breakdown of rice resistances (Pinel-Galzi et al, 2007, *PLoS Pathogens* 3: e180). Potyviral VPgs and non-mutated (susceptible) eIF4Es directly interact in yeast double hybrid system; resistance mutations of eIF4Es disrupt the interaction with VPgs; resistance-breaking (RB) mutations of VPgs restore the interaction with eIF4Es. The middle domain of rice eIFiso4G and the RYMV VPg were cloned in yeast double-hybrid vectors. By contrast to potyviral VPgs, RYMV VPg directly interacted with rice eIFiso4G. Thus, the eIF4 partners of VPgs are different depending of the viral taxon. The diversity in the interaction strategy would be explained by the diversity in the VPg sequences. Although the virulence factor is common between potyviruses and RYMV, no sequence identity is detected in VPgs. *Lettuce mosaic virus* (LMV) and RYMV VPgs were expressed in bacteria and analysed by several biophysical methods. They shared common structural properties that provide explanations for their ability to interact and to rapidly adapt to resistance alleles. Otherwise, RB mutations in VPgs sometimes induce fitness losses for potyviruses in susceptible plants. The fitness losses induced by RB mutations in RYMV VPg were quantified by Q-RT-PCR. The impact of three virulence mutations on the fitness of two isolates was quantified in one susceptible and two resistant hosts. The mutation effects on the *in vitro* eIFiso4G interaction were correlated to the fitness gains or losses in susceptible and resistant plants. Taken together, these results reveal common and distinct features between viral strategies to interact with host proteins.

(L14) Mechanism of plant eIF4E-mediated virus resistance: Cap-independent translation of a viral RNA controlled *in cis* by an (a)virulence determinant

Veronica Truniger, Cristina Nieto, Ana M. Rodríguez-Hernández, Daniel González-Ibeas, and Miguel. A. Aranda

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Translation initiation factors are universal determinants of plant susceptibility to RNA viruses but the underlying mechanisms are poorly understood. We have been able to show for the first time that a sequence in the 3´-untranslated region (3´-UTR) of a viral genome responsible for overcoming a plant eIF4E-mediated resistance (virulence determinant) functions as a cap-independent translational enhancer (3´-CITE). The virus/plant pair studied was *Melon necrotic spot virus* (MNSV; genus *Carmovirus*, family Tombusviridae) and melon, for which a recessive resistance controlled by melon eIF4E has been described (Nieto *et al.*, 2006, *Plant J.* 48:452-462). Chimeric viruses between virulent (Díaz *et al.*, 2004, *Mol. Plant-Microbe Interact.* 17:668-675) and avirulent isolates enabled us to map the virulence and avirulence determinants to 49 and 26 nucleotides, respectively. The translational efficiency of a *luc* reporter gene flanked by 5´- and 3´-UTRs from virulent, avirulent and chimeric viruses was analysed *in vitro* in wheat germ extract and *in vivo* in melon protoplasts, showing that (i) the virulence determinant mediates efficient cap-independent translation *in vitro* and *in vivo*, (ii) the avirulence determinant was able to promote efficient cap-independent translation *in vitro* but only when eIF4E from susceptible melon was added *in trans* and, coherently, only in protoplasts of susceptible melon, but not of resistant melon, (iii) these activities required *in cis* the 5´-UTR of MNSV. Therefore the virulence and avirulence determinants function as 3´-CITEs and a long-distance interaction between the 5´- and 3´-UTR is required for the increase in cap-independent translational activity. Translational efficiency experiments with partial deletions of the 5´- and 3´-UTRs allowed us to propose that a short sequence track of the 3´-UTR base-pairs to the corresponding sequence in the 5´-UTR. The activity of the 3´-CITEs of MNSV was host specific, suggesting that an inefficient interaction between the viral 3´-CITE of the avirulent isolate and eIF4E of resistant melon impedes the correct formation of the translation initiation complex at the viral RNA ends, leading to resistance. Direct interactions between eIF4E of resistant and susceptible melon and the 3´-CITEs of MNSV are currently under study.

(L15) Sobemoviruses possess a common genome organization and a highly modified VPg protein

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Based on structural differences in the ORF2 region, the sobemoviruses have been subdivided earlier into southern cowpea mosaic virus (SCPMV)-like and cocksfoot mottle virus (CfMV)-like types of genome organization. CfMV-type of viruses were believed to express their full-length polyprotein via -1 ribosomal frameshifting mechanism, whereas SCPMV-type of viruses seemed to encode the whole polyprotein from one continuous reading frame. The region of ORF2 just upstream from the frameshifting signal encodes for the virus VPg. This opened up the possibility that CfMV-type of viruses may express two different types of VPg-s – one completely from ORF2a and the other from ORF2a and 2b due to the frameshifting. This has potential impact also for the resistance against sobemoviruses, as VPg protein is the sobemoviral avirulence factor.

Recently, we have resequenced the putative frameshifting regions of four SCPMV-like viruses: lucerne transient streak virus, ryegrass mottle virus, southern bean mosaic virus, and SCPMV. A comparison of nucleic acid composition of these sequences with previously published ones revealed crucial differences that established a common CfMV-like genomic organization for these sobemoviruses. Thus, all sobemoviruses display CfMV-type of genome organization and also the possible existence of two different VPg-s was therefore common for all sequenced sobemoviruses. To test the latter hypothesis experimentally, we have taken the mass spectrometry approach in order to map the exact C-terminus of the CfMV VPg protein. MS analysis has revealed that the C-terminal processing site of VPg is located upstream from the frameshifting signal. Therefore, we conclude that sobemoviruses still encode one type of VPg-s. However, MS analysis also showed that CfMV VPg is a highly modified protein with several amino acid residues being methylated or phosphorylated. The significance of these modifications for the binding of sobemovirus VPg to cellular components (translation initiation factors) remains to be elucidated.

Symposium III: Plant responses to viruses and/or their vectors

Chairperson: Michael Wasseneger

(L16) The role of RNA silencing in antiviral defence

David Baulcombe

Address + E-mail

Abstract missing

(L17) Is the silencing suppressor activity of P0 targetting ARGONAUTE enough to establish a successful infection of poleroviruses in Arabidopsis?

Diane Bortolamiol, Maghsoud Pazhouhandeh, Julia DeCillia and <u>Véronique Ziegler-Graff</u>

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Plants employ post-transcriptional gene silencing (PTGS) as an antiviral defense response. In this mechanism, viral-derived small RNAs are incorporated into the RNA-induced silencing complex (RISC) to guide degradation of the corresponding viral RNAs. ARGONAUTE1 (AGO1) is a key component of RISC as it carries the RNA slicer activity. As a counter-defense, viruses have evolved various proteins that suppress PTGS. These viral silencing suppressors exhibit remarkable structural diversity and their mode of action is still poorly understood. Recently, we showed that the polerovirus P0 protein carries an F-box motif required to form an SCF-like complex (a type of E3 ubiquitin ligase), F-box motif that is also essential for P0's silencing suppressor function. Using a transient expression assay in *N. benthmiana*, P0 was found to impair a step in the PTGS pathway downstream of Dicer-mediated siRNA production. Expression of P0 in Arabidopsis led to various developmental abnormalities reminiscent of mutants affected in miRNA pathways, that were accompanied by enhanced levels of many miRNA-target transcripts, suggesting that P0 acts at the level of RISC. Importantly, ectopic expression of P0 triggered AGO1 protein decay *in planta*. Finally, we provided evidence that P0 physically interacts with AGO1. Based on these results, we proposed that P0 hijacks the host SCF machinery to impair gene silencing by degrading AGO1.

As P0 does not impair the generation of siRNA, we investigated by a genetic approach in Arabidopsis the potential role of three of the four DCL (Dicer-like) proteins involved in the production of viral-derived siRNA. Preliminary results indicate that *dcl2* mutants are hypersensitive to Beet western yellows virus and *dcl2dcl4* mutants are even more susceptible to the virus. This suggests that DCL2 plays the major antiviral activity against poleroviruses and that DCL4 may be preferentially inhibited by these viruses to establish an infection. Identification of a putative second suppressor is under investigation and the latest results will be presented and discussed.

(L18) Cymbidium Ringspot Virus harnesses RNA silencing to control the accumulation of the virus parasite SatRNA

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Satellite RNA (SatRNA) of Cymbidium ringspot virus (CymRSV) is a sub-viral RNA replicon, which replicates and accumulates on the cost of helper virus. It consists of a 621nt long RNA species sharing only a 51 nt long region with high sequence similarity with the helper virus (termed Helper-Satellite Homology region, HSH). Here we show that the accumulation of SatRNA strongly depends on the temperature and the presence of p19 silencing suppressor protein of helper virus, suggesting that RNA silencing plays a crucial role in the accumulation of SatRNA. We have also demonstrated that Carnation Italian ringspot virus (CIRV), another Tombusvirus member, can support the SatRNA accumulation at higher level than CymRSV. Our results suggest that siRNAs derived from CymRSV can target SatRNA more efficiently than siRNAs from CIRV, because of the higher sequence similarity between the HSH regions of helper and SatRNA. Indeed, we have shown that sensor RNA carrying the putative target site of SaRNA in HSH region was efficiently cleaved when it was transiently expressed in CymRSV-infected plants but not in CIRV-infected plants. Strikingly, the replacement of CymRSV HSH box2 sequence with that of CIRV restores the accumulation of SatRNA both at 24°C or in lack of p19 suppressor. These findings demonstrate an extraordinary adaptation of the virus to its host by harnessing the antiviral silencing response of the plant to control the virus parasite SatRNA.

(L19) Viroid-induced symptoms are dependent of *rdr6* activity, a key component of the RNA silencing pathway

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Viroids are small self-replicating, single-stranded circular, non- encapsidated RNAs that infect plants. Viroids are classified into two families, *Pospiviroidae* whose replication takes place in the nucleus and *Avsunviroidae* that replicate in the chloroplast (Flores *et al.*, 2005 *Annu Rev Phytopathology* 43: 117-139). How these non-coding RNAs interact with hosts to induce disease symptoms is a long-standing unanswered question. Recently it has been proposed that the viroid pathogenesis could be mediated by the interference of specific host-mRNA expression via viroid-induced RNA silencing mechanism (Wang *et al.*, 2004 *PNAS* 101: 3275-3280). However, evidence of a direct relation between key components of the RNA silencing pathway and the symptom expression in infected plants remains elusive.

To address this issue, we used a symptomatic transgenic line of *Nicotiana benthamiana* that expresses and processes *Hop stunt viroid* (HSVd) (Gomez and Pallas, 2007 *Plant Journal* 51: 1041-1049). These plants were analyzed under different growing temperature conditions, and used as stocks in grafting assays with the *rdr6i-Nb* line, in which the RNA-dependent RNA polymerase 6 (*NbRDR6*) is constitutively silenced (Schwach *et al.,* 2005 *Plant Physiology* 138: 1842-1852).

The obtained results indicate that the symptom expression in *N. benthamiana* plants is independent of HSVd accumulation levels, but dependent on an active state of the viroid-specific RNA silencing pathway. The scion of *rdr6i-Nb* plants remained asymptomatic when grafted onto symptomatic plants, despite an accumulation of a high level of mature forms of HSVd indicating the requirement of RDR6 for the viroid-induced symptom production. In addition, the RDR6 requirement for symptom expression was also observed in *wild type* NB plants mechanically infected with HSVd, providing biological evidence of the involvement of the viroid-specific RNA silencing pathway in the symptoms expression associated with viroid pathogenesis

An intriguing question emergent from these findings is which of the RDR6-dependent pathways could be associated to the expression of viroid-induced symptoms. Several remarkable observations prompted us to propose a pathogenicity model (for members of the *Pospiviroidae* family) by which the viroid-induced symptoms would be consequence of the interference of their replication-intermediates in the regulatory pathway involving trans-acting small interfering RNAs (tasiRNAs) biogenesis. The potential involvement of other relevant players in this regulatory pathway would shed light on the role of the RDR6 in viroid pathogenesis.

(L20) ASYMMETRIC LEAVES1 is a receptor of betaC1, the pathogensis factor of TYLCCNV

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Viruses induce pathogenic symptoms on plants but the molecular basis is poorly understood. We found that transgenic *Arabidopsis* expressing the pathogenesis protein betaC1 of *Tomato yellow leaf curl China virus* (TYLCCNV) can phenocopy to a large extent disease symptoms of virus-infected tobacco plants in having upward curled leaves, radialized leaves with outgrowth tissues from abaxial surfaces, and sterile flowers. These morphological changes are paralleled by a reduction in miR165/166 levels and an increase in *PHB* and *PHV* transcript levels. Two factors, ASYMMETRIC LEAVES 1 (AS1) and ASYMMETRIC LEAVES 2 (AS2), are known to regulate leaf development as AS1/AS2 complex. Strikingly, betaC1 plants phenocopy plants overexpressing AS2 at the morphological and molecular level and betaC1 is able to partially complement *as2* mutation. BetaC1 binds directly to AS1, elicits morphological and gene expression changes dependent on AS1 but not AS2, and attenuates expression of selective jasmonic acid (JA)-responsive gene. Our results show that betaC1 forms a complex with AS1 to execute its pathogenic functions and to suppress a subset of JA responses.

Symposium III: Plant responses to viruses and/or their vectors

Chairperson: John Carr

(L21) Sap vs. spit: The role of aphid salivary proteins in manipulating host plant defences

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We have identified, mapped, and are now cloning dominant resistance genes of major and minor effect against a number of aphid species in the model legume *Medicago truncatula*. All appear to be members of the NBS-LRR class of "R-genes", which mediate resistance by eliciting a defence response after recognition of a pathogen-derived signal (*Avr* factor). By comparing the aphid-induced transcriptional response in near-isogenic resistant and susceptible plants, we have identified known defence-related genes, transcription factors and downstream biosynthetic pathway genes triggered by aphid feeding only in resistant plants. We are now investigating whether aphid resistance can be achieved by up-regulating these genes in otherwise susceptible lines.

Like pathogens, aphids can circumvent R-gene mediated resistance by modifying the secreted effector protein whose structure or function is recognised by the plant NBS-LRR protein. This adaptation leads to resistance-breaking virulent "biotypes", such as those observed in invasive populations of the Russian wheat aphid (*Diuraphis noxia*) in the United States and South Africa. In aphids, effector proteins must be localised to the saliva because only salivary proteins make contact with the interior of the plant. We are investigating the evolution of virulence in Russian wheat aphid by comparing salivary gland protein profiles of virulent and non-virulent strains. We have discovered an unusually high level of amino acid diversity in many salivary gland proteins, and we are now looking for mutations that correlate with the resistance-breaking phenotype. Once we understand how R-gene proteins interact with aphid-derived effectors, we should have the capacity to engineer R-genes for crops in which aphid resistance does not naturally occur.

(L22) Aphid and virus resistance triggered by the CC-NBS-LRR *Vat* melon gene

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Dominant plant resistance genes are widely assumed to be involved in specific recognition of pests and pathogens and subsequent activation of plant defense responses. The melon *Vat* allele confers resistance to the aphid *Aphis gossypii*; it has also the unique feature to confer resistance to transmission of unrelated non–persistent viruses (Cucumber mosaic virus, Potyviruses) when transmitted by the vector species *A. gossypii* specifically. Using a map-based cloning strategy and functional analysis using transgenic melon plants, we demonstrated that a single gene which encodes a CC-NBS-LRR protein confers both functions. The recognition between the Vat protein and an aphid gene product likely triggers plants responses preventing aphid feeding, fecundity and survival as well as antiviral responses, non specific to the virus species. Using the same map-based cloning strategy, the *Pm-W* gene, which confers resistance to the fungus *Podosphaera xanthii,* was shown to be a true allele of the *Vat* gene.

Our objective was to study the molecular diversity at the *Vat/Pm-W* locus and to characterize allele recognition specificity. Four aphid clones were used to assess aphid and virus transmission resistance in order to determine the specific relationship between plant resistance alleles and aphid clones; the chosen clones belonged to the two major multilocus genotypes identified in the host race Cucurbitaceae of *A. gossypii*.

Twenty accessions from Korea, China, Japan and India amplified the *Vat* allele (or a gene almost identical to *Vat*); they all exhibited aphid and virus transmission using aphid clones of both genotypes NM1 and C9. In contrast, 11 accessions from Spain, Africa and India, did not amplify the *Vat* allele and likely have another allele or a distinct genetic basis for aphid resistance. They exhibited a phenotype slightly different from the *Vat*-mediated resistance. Among them, the Indian accession 90625 was resistant to aphids and to virus transmission but only using NM1 aphid clones. C9 aphid clones overcome both resistances. Using Long Range PCR, we identified in this accession the full length sequence of a *Vat* analog, which have 92.3 % protein identity with the *Vat*-encoded protein and may be a novel allele for aphid resistance. Two other accessions have resistance to aphids but not to virus transmission. Their molecular characterization may allow identifying new alleles with novel specificities.

Further studies of the variability at the locus will elucidate the molecular evolution of the locus and the molecular determinants of recognition specificity.

(L23) Plant-virus-insect interactions: a new role for the 2b protein of cucumber mosaic virus?

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The multifunctional 2b protein of cucumber mosaic virus (CMV) is well known for its ability to interfere with plant defence responses to pathogens such as RNA silencing and salicylic acid (SA)-induced resistance. However, recent work in our lab has indicated that there may be a novel role for the 2b protein in overcoming plant defence responses to aphid infestation. We compared aphid mortality rates on wild type *Arabidopsis thaliana* and *Nicotiana tabacum* plants infected with CMV or CMV-Δ2b (a mutant strain of CMV that is unable to express the 2b protein). We found that mortality rates were increased on those infected with CMV-Δ2b. Furthermore, we found that when transgenic *A. thaliana* plants that constitutively express the 2b protein were infected with CMV-Δ2b, this effect was ameliorated and mortality rates were similar to plants infected with CMV. Aphid mortality rates on *coi1* mutants (deficient in jasmonate signalling) infected with CMV-Δ2b were also similar to plants infected with CMV. The jasmonic acid (JA)-defence pathway is involved predominantly in plant-insect defence. We undertook a microarray study investigating the role of the 2b protein in suppressing JA-induced gene expression in *A. thaliana*. Constitutive expression of the 2b protein in *A. thaliana* strongly inhibited JA-induced gene expression. Consistent with this, infection of plants with CMV but not with CMV-Δ2b, inhibited several known insect defence related genes. We propose that the 2b protein interferes with JA-induced anti-insect defence responses in order to favour virus transmission.

(L24) Differential effects of 2b protein from two Cucumber mosaic virus strains and satRNA co-infection in the alteration of microRNA-regulated gene expression in tomato

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Cucumber mosaic virus (CMV) encodes for a multifunctional protein, the 2b protein, that operates as the viral suppressor of RNA silencing in host plants. It has been demonstrated that in Arabidopsis the 2b protein can interfere with the microRNA (miRNA) -mediated regulation of gene expression of endogenous factors controlling plant development and leaf architecture, and that 2b protein encoded by CMV strains belonging to two different subgroups was differently able to display such interference. We synthesized a chimeric infectious clone of CMV RNA2, pFny(LS2b), in which a 3'-terminal portion of RNA2 including the 2b coding sequence of CMV-Fny (an aggressive subgroup IA strain) was replaced by the analogous fragment of CMV-LS (a mild subgroup II strain). The clone was used to obtain a recombinant CMV strain, denoted CMV-Fny(LS2b), in which the original RNA2 of CMV-Fny was replaced by the chimeric construct and co-inoculated with CMV-Fny RNAs 1 and 3.

CMV-Fny-induced symptoms in tomato consisted in severe mosaic, leaf shoestring and growth reduction, whereas those induced by either CMV-LS or CMV-Fny(LS2b), were limited to mild leaf narrowing, suggesting that most virus-induced developmental defects are due to the activity of CMV-Fny 2b protein.

Gene expression profiling by quantitative RT-PCR analysis of some transcription factors and other genes involved in the short RNA processing, which are known to be regulated by miRNA-guided mRNA cleavage, showed that CMV-Fny infections induced the modulation of most transcript levels, whereas there was no evidence for this in the case of both CMV-LS and CMV-Fny(LS2b) infections.

In another set of mRNA quantification assays, the same group of genes resulted not modulated when CMV-Fny was inoculated on transgenic tomato plants expressing an ameliorative satellite-RNA (satRNA) variant, codetermining an asymptomatic phenotype.

Altogether, these results suggest that the pathogenic properties of CMV-Fny on tomato are due to the ability of its 2b protein to interfering with developmental processes regulated by miRNA, that CMV-LS 2b protein has limited effects on such regulation, and that the suppression of symptoms mediated by ameliorative satRNAs may be driven by the substantial down-regulation of CMV RNA and gene products, including the 2b protein, in infected cells.

(L25) Genetic and functional characterization of the RTM-mediated resistance

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The RTM-mediated resistance was first identified to restrict long distance movement of the potyvirus, *Tobacco etch virus* (TEV), in the *Arabidopsis thaliana* accession Col-0 (Whitham et al., 1999, *Proc Natl Acad Sci U S A* 96(2), 772-7). Recently we have shown that this resistance is also active against to two other potyviruses, *Lettuce mosaic virus* (LMV) and *Plum pox virus* (PPV; Decroocq et al., 2006, *Mol. Plant-Microbe Interact,* 19, 541-549). At least three dominant genes named *RTM1*, *RTM2* and *RTM3* are involved in this resistance process. Remarkably, a mutation in anyone of these three genes is sufficient to abolish the restriction of the potyvirus long distance movement, suggesting that these genes act in an interdependent way to block the generalized invasion of the plant. *RTM1* encodes a lectin-like protein (Chisholm et al., 2000, Proc Natl Acad Sci U S A 97(1), 489-94) and *RTM2* encodes a protein with similarities to small heat shock proteins (Whitham et al., 2000, *Plant Cell* 12(4), 569-82). Both of these genes are expressed in phloem-associated tissues and the corresponding proteins localize to sieve elements (Chisholm et al., 2001, Plant Physiol 127, 1667-1675).

In order to elucidate the RTM-mediated resistance mechanism, we have undertaken several kinds of experiments to address several important questions: which kind of protein does *RTM3* encode? What is the viral determinant involved in overcoming the RTM resistance? How to explain why some *Arabidopsis* accessions are susceptible to potyvirus strains which are restricted to inoculated leaves in Col-0 because of the RTM resistance?

Using a F2 population produced between the susceptible mutant *rtm3* and the RTM-mediated resistant accession Ws-2, *RTM3* was mapped and identified in a genomic interval including 22 genes in chromosome 3. Using PPV recombinants produced between a strain able to overcome the RTM resistance and another one unable to overcome this resistance, and after sequencing of LMV variants able to overcome the RTM resistance, we could show that the N-terminal part of the viral coat protein contains the genetic determinant responsible for overcoming the RTM resistance.

Finally, in order to understand why the RTM-mediated resistance is not active in some *Arabidopsis* accessions, we sequenced the RTM genes from a set of LMV susceptible accessions and we carried out allelism tests by crossing susceptible accessions with the three different *rtm* mutants. Several distinct alleles were identified for each RTM gene and we could show that the loss of the RTM resistance in some susceptible accessions was related to some of these RTM alleles.

All together these data showed that amino acid variations in either the RTM proteins or the potyvirus coat protein govern the RTM-mediated resistance in Arabidopsis. Whether the coat protein of potyvirus interacts with the RTM proteins during the resistance process is now a new question to address.

Symposium IV: Innovative approaches, including transgenic resistance, to control plant viruses and vectors

Chairperson: Margit Laimer da Camara Machado

(L26) Engineering virus resistance using artificial miRNAs

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Plant microRNAs (miRNAs) regulate the abundance of target mRNAs by guiding their cleavage at the sequence complementary region. We have modified an *Arabidopsis thaliana* miR159 precursor to express artificial miRNAs (amiRNAs) targeting viral mRNA sequences encoding two gene silencing suppressors, P69 of turnip yellow mosaic virus (TYMV) and HC-Pro of turnip mosaic virus (TuMV). Production of these amiRNAs requires *A. thaliana* DICER-like protein 1. Transgenic Arabidopsis plants expressing amiR-P69-159 and amiR-HC-Pro-159 are specifically resistant to TYMV and TuMV, respectively (Niu et al, 2006). Expression of amiR-TuCP159 targeting TuMV coat protein sequences also confers specific TuMV resistance. However, transgenic plants that express both amiR-P69-159 and amiR-HC-Pro-159 from a dimeric pre-amiR-P69-159/amiR-HC-Pro-159 transgene are resistant to both viruses. The virus resistance trait is displayed at the cell level and is hereditable. More important, the resistance trait is maintained at 15 ºC, a temperature that compromises small interfering RNA-mediated gene silencing.

Using simlar approach we have generated transgenic plants with resistance to CMV, gemini viruses and tospoviruses. The amiRNA-mediated approach should have broad applicability for engineering multiple virus resistance in crop plants.

Reference:

Niu QW, Lin SS, Reyes JL, Chen KC, Wu HW, Yeh SD, Chua NH. 2006 Expression of artificial microRNAs in transgenic Arabidopsis thaliana confers virus resistance. Nat Biotechnol. 24:1420-8.

(L27) RNAi-mediated resistance to *Potato spindle tuber viroid* in transgenic tomato expressing the viroid hairpin DNA construct

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Because of their highly ordered structure, mature viroid RNA molecules were assumed to be resistant to degradation by RNA interference (RNAi). Here we report that a transgenic tomato plant line expressing a hairpin construct derived from *Potato spindle tuber viroid* (PSTVd) sequences exhibited resistance to PSTVd infection. This was correlated with the high level accumulation of hairpin-derived short interfering RNAs (siRNAs) in the plant. Thus, in contrast to small RNAs produced in PSTVd-infected non-transgenic plants (srPSTVds), these hairpin-derived siRNAs (hp-siRNAs) appeared to efficiently target the mature viroid RNA. Genomic mapping of the hp-siRNAs revealed an unequal distribution of 21 and 24 nt siRNAs of both (+)- and (-)-strand polarities along the PSTVd genome. These data suggest that RNAi can be employed to engineer plants for viroid resistance, as has been well established for viruses.

(L28) Molecular breeding for virus resistance in cereals

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In Europe virus diseases of cereals, i.e. soil-borne *Barley mild mosaic virus* (BaMMV), *Barley yellow mosaic virus* (BaYMV) and *Soil-borne cereal mosaic virus* (SBCMV) as well as the insect transmitted *Barley yellow dwarf virus* (BYDV) and *Wheat dwarf virus* (WDV) are continuously gaining importance. Molecular mapping of resistance genes and QTL has provided a basis to genetically armour new cultivars by marker assisted breeding efforts.

With respect to barley yellow mosaic virus disease, many resistance genes being effective against single or all strains of BaMMV/BaYMV known in Europe have been mapped to distinct chromosome regions. Closely linked markers, or in case of the gene *Rym4*, markers derived from the gene itself facilitate efficient marker based selection procedures, including marker assisted backcrossing, and pyramiding of different resistance genes, which on the one hand help broadening of the genetic base of resistance and on the other hand prolong the shelf live of partly overcome resistance genes. As it turned out that the resistance locus *Rym4* comprises the translation initiation factor 4E (*Hv-eIF4E*) screening of gene bank accessions and TILLING populations for allelic diversity at this locus resulted in the identification of novel alleles which, after verification of their efficiency, will be available for further introgression into elite material. Besides this, genes of the translation machinery are currently mapped in barley to identify additional candidate genes for resistance to BaMMV/BaYMV.

In contrast to barley, only one resistance gene being effective against SBCMV has been mapped in wheat on chromosome 5DL. For this gene a closely linked marker has been developed recently, facilitating efficient marker based selection. This is of paramount importance in countries like Germany, where non uniform infestation of fields frequently impedes phenotypic selection. Using a functional genomics approach, a series of candidate genes was identified based on their differential expression between resistant and susceptible genotypes.

Besides soil-borne viruses, insect transmitted viruses will gain importance due to increasingly mild temperatures during fall and winter resulting in a longer vector activity. With respect to BYDV-PAV present studies aim at pyramiding of QTL using molecular markers and DH-technology. With respect to WDV which is transmitted by the leafhopper *Psammottetix alienus* only one tolerant barley accession has been detected up to now and genetic analyses are being carried out to get information on the mode of inheritance and to identify QTL.

(L29) Subcellular visualisation of homologous and heterologous protein-protein interactions of the 2b protein of CMV, and their significance to its suppressor of gene silencing function

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The 2b suppressor of gene silencing from *Cucumber mosaic virus* (CMV) accumulates in the plant cell nucleus (Mayers et al., 2000. J. Gen. Virol. 81, 219), and its functional activity has been associated to this subcellular targetting (Lucy et al., 2000. EMBO J. 19, 1672). More recently, the way the protein performs its suppressor role has been associated with its interaction with and inhibition of AGO1, a component of the RISC complex which is part of the plant antiviral silencing machinery (Zhang et al., 2006. Genes & Dev. 20, 3255). However, it also seems that the 2b can bind directly to small RNAs (Goto et al., 2007. Plant Cell Physiol. 48, 1050).

Using bimolecular fluorescence complementation (BiFC) we show here that wild type (wt) 2b protein of CMV (strain Fny) forms aggregates inside the plant cell, predominantly within the nucleus. With the same technique we have also visualised the interaction of 2b with AGO proteins. BiFC studies using six 2b mutants affecting the protein nuclear localisation signals and putative phosphorylation sites showed that fluorescence indicating formation of homologous aggregates was absent for each of the mutants. However, some combinations between mutant and wt 2bs allowed the formation of heterologous 2b aggregates, which either maintained the nuclear fluorescence pattern, or showed instead a cytoplasmic and perinuclear fluorescence phenotype. Interactions between 2b and AGO were also affected by the mutations.

The ability of these 2b constructs to aggregate, to show nuclear vs. cytoplasmic localisation, and to bind to AGO was determined in vivo, and their functional significance was assessed in relation to the capacity of these 2b constructs to suppress local RNA silencing in agroinfiltration assays.

(L30) Analysis of the effects of high selection pressure on recombination between RNA3 of *Cucumber mosaic virus* and transgene mRNAs

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It has been shown that mixed infections (with two or more viruses) occur frequently in nature, and that when two related viruses replicate simultaneously in the same cells, genetic recombination between them can take place. There is also evidence that recombination can occur between cellular and viral RNAs. Thus, virus-resistant transgenic plants (VRTPs) that express viral sequences could be a source of novel recombinant viral genomes. From a biosafety point of view, it is therefore important to understand if the recombinants that are generated in VRTPs are novel, and if they could contribute to an increased risk of emergence of recombinant viruses. Since it is impossible to test all possible recombinants, the best way to evaluate this risk is to compare the recombinants that appear in infected transgenic plants with those that appear in doubly infected non-transgenic plants under conditions of low selection pressure.

Cucumber mosaic virus (CMV) is the most important member of the genus *Cucumovirus*, family *Bromoviridae*. As with all *Bromoviridae*, the CMV genome consists of three plus-sense, single-stranded RNA molecules (RNA1, RNA2, and RNA3). CMV isolates are divided in two main subgroups (subgroup I and subgroup II), distinguished by serological and nucleic acid properties. Infecting over 800 plant species, CMV is considered one of the most important plant viruses, causing serious economically diseases in many parts of the world.

Using a low selection pressure approach, colleagues in the lab had previously shown that in tobacco plants infected with two strains of CMV, a subgroup I strain (I17F-CMV) and subgroup II strain (R-CMV), the populations of recombinant viral RNAs found were equivalent to those found in tobacco transgenic for the entire coat protein (CP) open reading frame and the 3' non-coding region (3'NCR) of R-CMV infected with I17F-CMV (Turturo et al. 2008). These results suggest that in this case novel viral recombinants are not expected to appear. But, since the detection of host-messenger/viral RNA recombinants under conditions of low selection pressure is difficult because of the low number of recombinant molecules in relation to parental molecules, it was decided to analyze the recombinants appearing under conditions of high selection pressure in favor of recombinants. To do this, we inoculated 44 plants of the same line expressing the R-CMV CP+3'NCR transgene with *in vitro* transcripts of a mutant I17F-CMV that had been attenuated by a 6-nt deletion in the 3'NCR of RNA3. Samples were taken from symptomatic plants 8, 10, and 15 days post-inoculation. As for the studies under low selection pressure, plants were screened for recombinant RNA3 by RT-PCR, and the recombinant nature of the amplicons was verified by cloning and sequencing. Many of the recombinants were different from those observed in the same plant line when inoculated with wild-type I17F-CMV in the absence of selection pressure. Further studies will determine the abundance of the different recombinants, and will aim to clarify why they were not previously detected.

Technology Transfer Round Table

Chairperson: Yolanda Hernando

(L31) A company perspective on knowledge and technology transfer from academia to industry

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The classic division between fundamental research and application in everyday life still exists in places. The archetypical academic researcher, though often not blind for applicability of their research to raise funding, is by enlarge interested in fact finding due to fundamental research, which is best carried out in model organisms. Typically, companies are not interested in the details of this research, particularly not if this is carried out in models systems that have no commercial value. Nonetheless, the industry relies heavily on publicly available data to be able to improve their products. It is the mission of company R&D units to mediate transfer of fundamental knowledge into digestible products for their production units or clients.

Of course, publicly funded research does not only involve basic research. Strategic research with high applicability is carried out in both universities and research institutes, and also commercial awareness is quite high in many of those places. The spectrum from fundamental to research to application in the market is well covered and collaborations take place on all levels, thereby crating transfer of knowledge and technology.

KeyGene is a medium sized research company with a strong focus on molecular genetics and biotechnology for the plant breeding industry. It was funded in the late 80s by a number of Dutch seed breeding companies to combine their strategic research efforts. KeyGene forms a connection between fundamental and strategic public research and application in breeding, by perform own research as well as contributing to research consortia such as those funded by the EU and national governments, or industry itself. Through collaborations with academic partners all over the world companies like KeyGene gain access to basic knowledge while in return they offer financial compensation or access to proprietary technology or their high cost high tech machine park.

In this presentation I will address how a medium sized research company views collaboration with academic partners, what we would expect from them in collaboration and what we can give in return to boost academic research and in turn implement this knowledge and technology in our products.

Symposium IV (cont.): Innovative approaches, including transgenic resistance, to control plant viruses and vectors

Chairperson: Jari Valkonen

(L32) Host targets of viral proteins and their manipulation in transgenic plants

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Plant pathogens have evolved a wide range of sophisticated strategies to allow their systemic spread, suppression of plant defence and induction of sink function to support their metabolism with nutrients. Viruses exploit the cell-to-cell and long distance transport system of plants to facilitate their systemic spread. Systemic invasion of plants by viruses depends on compatible interactions between host and virus-encoded factors to facilitate genome replication, cell-to-cell movement via plasmodesmata and long-distance transport through the vascular tissue. The nature of host factors involved in the various steps of potyvirus infection, in particular during intra- and intercellular trafficking of virus material is largely unknown. Only recently, the eukaryotic translation initiation factor eIF4E, previously implicated mainly in genome replication, and a cysteine-rich plant protein of unknown function have been identified as susceptibility factors supporting potyvirus movement through interaction with the virus genome-linked protein VPg. Similarly, a limited number of host proteins have been demonstrated to interact with MPs of other virus groups. For instance, cell wall-associated pectin methylesterase (PME), ER-localized proteins calreticulin and NtCAPP1 involved in the plasmodesmal transport pathway have been isolated as host proteins contributing to cell-to-cell transport of tobacco mosaic virus (TMV) through interaction with the MP. The biological role for most of these unrelated host proteins during virus spread has yet to be elucidated. In case of TMV MP interaction with the microtubule-associated plant factor 2C (MPB2C) has been demonstrated to be essential for microtubular accumulation of the movement protein, but not for viral spreading. This suggests a role for microtubule association in MP degradation rather than PD targeting. This could be confirmed by analysing plasmodesmal targeting of the PLRV MP17.

The capsid protein (CP) of potyviruses has multifunctional properties and is required for various steps during plant infection. This suggests a series of compatible interactions between potyviral CPs and putative host factors which, however, are largely unknown. Performing a yeast two-hybrid screen with potato virus Y (PVY) CP as bait, we identified a novel subset of DnaJ-like proteins from tobacco which were shown to be essential host factors in transgenic plants. Over-expression of J-domain deficient variants of PVY-CP interacting DnaJ-like proteins strongly increased virus resistance of transgenic tobacco plants indicating a crucial role of functional DnaJ-proteins during PVY infection. The negative effect of impaired chaperone function on viral pathogenicity seemed to be the consequence of strongly delayed cell-to-cell movement, as visualized by particle bombardment of transgenic plants with GFP-tagged PVY. Therefore, we propose that DnaJ-like proteins act as important susceptibility factors during PVY infection, possibly by recruiting heat shock protein 70 (HSP70) chaperones for viral assembly and/or cellular spread.

(L33) "SharCo FP7 European initiative: Testing new strategies of resistance to *Plum pox virus*"

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Within the 7th Research Framework, the concept of the 4-year European small collaborative project "SharCo" is to combine prophylactic and genetic solutions to prevent or limit the spread of the sharka disease caused by a virus, the *Plum pox virus* (PPV). PPV is infecting, in orchards and nurseries, stone fruit trees, e.g. most species of the *Prunoideae* subfamily (peach, apricot, plum as well as *Prunus* ornamentals and rootstocks).

Breeding for resistance to PPV encounters the usual problems of breeding perennial plants together with the difficult procedure of screening for PPV resistance that hinder the programmes. In SharCo, we will achieve the development of molecular tools linked to the trait, and they will be used to accelerate the selection of progenies in the first steps of breeding programmes, on seedlings. In apricots, implementation of marker assisted selection for resistance to PPV will be achieved in EU-member and associated states, by transferring tools and knowledge to Eastern European breeding stations.

However, although natural sources of resistance have been identified in some *Prunus* species, they are limited in number. In order to unravel new mechanisms of resistance and use them to diversify sources of resistance to PPV, we will identify *Prunus* plant proteins necessary to the virus and test strategies (natural variants, RNAi constructs) which might interfere with PPV infection. Complementary approaches will be evaluated, from the expression of recombinant antibodies or virus-derived constructs to the interference in glycosylation or phosphorylation of PPV proteins. The achievement of the SharCo genetic pillar will thus be the development of traditional and innovative approaches for a durable resistance to sharka disease.

(L34) Geminiviruses: when VIGS defeats PDR

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Geminiviruses are ssDNA viruses that infect a range of economically important crop species in tropical and subtropical regions of the world, and development of geminivirus resistant plants still remains, after two decades from the discovery of pathogen-derived resistances (PDR), a major challenge. In fact, the first attempts at using pathogen-derived sequences to confer resistance to geminiviruses were not as successful as those against plant RNA viruses suggesting that this class of pathogens should be able to evade PDR to some extent. In this context, some years ago we showed that carboxy-terminal truncated forms (*e.g.* Rep_{210} and Rep_{130}) of the replication associated protein (Rep_{359}) of the phloem-limited *Tomato yellow leaf curl Sardinia virus* (TYLCSV) strongly interfere with TYLCSV transcription and replication (Lucioli *et al.*, 2003, *Journal of Virology* 77:6785-6798). However, in transgenic plants expressing Rep_{210} the duration of resistance appeared to be inversely related to the ability of the challenging TYLCSV to shut off transgene expression by virus-induced gene silencing (VIGS). As we have an interest in clarifying the molecular bases of previous failures, we tested a general anti virus-induced transgene silencing strategy building a synthetic Rep130 transgene (*Rep130syn*) in which silent point mutations were introduced in such a way that the continuous homology between the *Rep130syn* sequence and the corresponding wild-type viral transgene sequence (*Rep130wt*) was mostly below or equal to 5 nucleotides. Both *Rep130wt* and *Rep130syn* transgenes encode the same protein, the Rep_{130}. The progeny of Rep_{130} transgenic *Nicotiana benthamiana* lines derived from both Rep130wt and Rep130syn constructs were challenged by agroinoculation with TYLCSV. Based on the molecular analysis of challenged plants, a simplified protein-mediated PDR model can be envisaged for geminiviruses in which the progression of viral accumulation in transgenic plants occurs in two distinct phases: the first independent and the second dependent on VIGS. The VIGS-dependent phase well explains why, using this technology, the most common "resistant" phenotype is delayed infection. Indeed, if the transgenic protein does not stop viral expression/replication in the initial infected cells then the virus will shut off transgene expression leading to a late susceptible phenotype.

Our data have clarified that VIGS is an Achilles' heel for practical applications of protein-mediated PDR to geminiviruses and that it can be overcome, in some instances, using opportunely modified synthetic transgenes.

(L35) The high and wide-ranging action of PPV 5'UTR/P1 hairpin construct to confer resistance to *Plum pox virus*

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Plum pox virus (PPV) is the causal agent of Sharka, one of the most important diseases of stone fruits worldwide. Six PPV strains have been characterized, of which PPV-D, -M and -Rec are the most important from an agro-economical point of view. The best agriculturally sustainable approach to prevent Sharka disease consists in developing PPV-resistant plants. In this context we have shown that *Nicotiana benthamiana* plants transformed with PPV-M sequences, covering the P1 and HC-Pro genes and arranged to express self-complementary "hairpin" RNA, are immune to the homologous PPV-M isolate (Di Nicola-Negri et al., 2005, *Transgenic Res.* 14:489-94). This data suggests that the RNA silencing constructs we developed could be potentially used to effectively control Sharka disease. To test this hypothesis we firstly analyzed the PPV resistance spectrum conferred by the four transgenic PPV-M hairpin constructs: 5'UTR/P1, P1/HCPro, HCPro and HCPro/P3. R1 transgenic *N.benthamiana* plants for each construct were challenged with PPV isolates belonging to M, D, Rec, C and El Amar strains. All the transgenic plant lines were resistant to the PPV-D, -M and -Rec. Moreover, the transgenic plant line harboring the 5' UTR/P1 sequence was also resistant to the distantly related PPV-C and PPV-El Amar strains. Since it's known that some abiotic - low temperature - and biotic stresses - mixed viral infection - could have a detrimental impact on RNA silencing-mediated viral resistances, 5' UTR/P1 R2 homozygous *N.benthamiana* plants were challenged with PPV-M under different conditions. Results show that transgenic plants harboring the PPV 5' UTR/P1 construct were resistant to PPV infection both at high (30°C) and low temperature (15°C). Furthermore, no susceptibility to PPV was observed in 5' UTR/P1 R2 plants previously inoculated with *Potato virus Y* (PVY) or *Cucumber mosaic virus* (CMV), suggesting that prior virus-mediated expression of HCPro (PVY) and 2b (CMV) RNA silencing suppressors was not able to defeat PPV resistance.

The total data suggests that the 5' UTR/P1 hairpin construct can be profitably used to confer resistance to the Sharka disease in *Prunus* species.

(L36) Evaluation of resistance conferred to Italian isolates of TYLCSV and TYLCV by *Ty-1* and *Ty-2* resistance genes and application of new CAPS markers for introgression in a traditional Italian tomato variety

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Tomato yellow leaf curl disease (TYLCD) is a well-known devastating viral disease worldwide. In Italy the disease is present in Southern regions, essentially in protected crops of Sicily and Sardinia. Two related viral species have been identified some years ago and are still present, *Tomato yellow leaf curl Sardinia virus* (TYLCSV, since 1989) and *Tomato yellow leaf curl virus* (TYLCV, since 2002). Although cultural practices can lead to a reduction in the incidence of TYLCD, genetic resistance is the best long-term strategy to control the damage caused by this viral disease. Some genes conferring partial resistance or tolerance to TYLCD have been found in wild *Solanum* species, such as *Ty-1* from *S. chilense* and *Ty-2* from *S. habrochaites*. However, in several cases different levels of resistance have been attributed to the same genetic sources, mainly because of variability in assay conditions and in virus strains. In a geographic region, it is therefore important to use the local virus species and strains for the assays.

We have performed resistance assays under laboratory conditions using two viral isolates from Italy, TYLCSV type-strain and TYLCV-IL-[IT:Sic:04]. Different types of tomato plants were used: a susceptible fresh tomato traditional Italian cultivar, "Cuore di Bue di Albenga"; a breeding line carrying the *Ty-1* resistance gene, LA3473; another line with the *Ty-2* resistance gene, H24; and several F3 families obtained by crossing the susceptible cultivar with the two breeding lines. These F3 families were selected using two already reported and two new CAPS markers, linked to either *Ty-1* or *Ty-2*. Plant infection was evaluated by tissue print hybridization with virus-specific probes. *Ty-1* gene provided partial resistance to TYLCSV whereas *Ty-2* proved effective against TYLCV. In the F3 plants, as well as in the original breeding lines, the two new CAPS markers, TG178 linked to *Ty-1* and C2_At5 g25760 linked to *Ty-2*, were effective in selecting progeny carrying the resistance genes and will be further used in marker-assisted breeding programs.

Symposium III: Plant responses to viruses and/or their vectors

Chairperson: Alan Schulman

(L37) Anti-viral responses induced by plant NB-LRR proteins involve Argonaute-dependent control of viral transcript translation

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Active resistance to viruses is afforded by the products of plant disease resistance (*R*) genes encoding proteins with nucleotide-binding site (NB) and leucine-rich repeat (LRR) domains. Upon recognition of pathogen-derived elicitors, these NB-LRR proteins are thought to initiate a number of signalling pathways that lead to the containment of the pathogen. However, little is known about what molecular mechanisms ultimately curtail virus accumulation. We have developed a novel experimental system to study how viruses are curtailed during an NB-LRR-induced anti-viral response. Using the potato virus X (PVX) as a reporter construct**,** we show that targeting of PVX during the anti-viral response depends on a relatively small *cis* element present in the PVX genome. Furthermore, we find that both genomic and subgenomic PVX RNAs accumulate normally during the anti-viral response. However, virus-encoded proteins do not accumulate and viral RNAs do not associate with ribosomes, suggesting an induced translational control mechanism. We have further dissected this response using virus-derived suppressors of RNAi. Whereas most suppressors have no effect on NB-LRR-induced translational control, the P0 protein inhibits this response completely. P0 is known to target Argonaute proteins, which play a role in both RNAi-mediated RNA degradation and in degradation-independent translation repression. The induced control of virus translation and NB-LRR-mediated virus resistance were compromised by the down-regulation of *Argonaute4*-like genes. Members of the Argonaute protein family have been implicated in RNAi-mediated RNA degradation and in degradation-independent translational control. Our results suggest that the engagement of Argonaute proteins in specific translational control of viral transcripts is a key factor in virus resistance mediated by NB-LRR proteins.

(L38) Characterization of the interaction between Rx and *Potexvirus* coat protein: what can we learn from the elicitor side?

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Disease resistance is often an induced response to the interaction of a resistant host and an avirulent pathogen. The Rx-mediated resistance against potato virus X (PVX), like the one controlled by many disease resistance genes, can be described in terms of an elicitor-receptor model which operates on the basis on a "gene-for-gene" hypothesis (Flor, 1971). In the Rx-PVX system, Rx encodes a protein with a nucleotide binding site (NBS) and leucine-rich repeats (LRR) that confers resistance against PVX (Bendahmane et al., 1999). The trigger of this resistance is the recognition of PVX coat protein (CP) by the Rx gene (Bendahmane et al., 1995).

In this project, we investigate the characterization of the interaction between the CP elicitor and the product of the Rx gene by two different approaches.

In the fisrt approach, a mutated serie at two critical positions in PVX CP (amino acids 121 and 127 which are different between the avirulent TK and the virulent KR strains) has been created. The subsequent viral strains have been used to inoculate wild type or Rx expressing *Nicotiana benthamiana*. The results showed a variety of phenotypes ranging from extreme resistance (as for TK and 7 other combinations) to systemic hypersentive response (for one mutant strain) and systemic necrosis (for the KR strain). The comparison with previous results, obtained in potato (Goulden et al., 1993; Kohm et al., 1993; Bendahmane et al., 1995), have revealed that the genetic background (potato versus *Nicotiana*) could modulate the affinity between CP and Rx and also the intensity of the Rx-mediated response (Baures et al., 2008).

In the second approach we investigated the effect of natural sequence variations in the CP of viruses related to PVX (*Potex-* and *Carlavirus* genera). In this analysis, we demonstrated that 3 *Potexviruses* other than PVX were able to induce Rx-mediated response and that their coat protein was the elicitor of the response. Moreover for three of them (NMV, WClMV and PVX), we have shown that the transient expression in *Nicotiana* of a CP fragment of 90 amino acids is sufficient for the elicitor activity (Baures et al., 2008). The primary amino acid sequence analysis revealed less than 40% of identity and in consequence the recognition of CP has to be based on a particular structure rather than on specific amino acids. The future of this project is now to characterize the biochemical features of these different minimal elicitors and compare the structure(s) of CP with or wothout an elicitor activity.

(L39) Plant virus infection induced persistent host gene down regulation in systemically infected leaves

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The dissection of virus infections induced alterations in the host plant gene expression and metabolism leading to the formation of virus disease symptoms is both scientifically and economically important. Here, we show that viruses belonging to different RNA virus families are able to induce efficient host gene mRNA down regulation (shut-off) in systemically infected leaves. We demonstrated that the host gene mRNA shut-off is spatially overlapped with virus occupied sectors indicating the direct role of virus accumulation in this phenomenon. The establishment of shut-off was not directly connected to the active viral replication or the RNA silencing machinery. Importantly, the induced shut-off phenomenon persisted for several weeks imposing severe mRNA deficiency of important housekeeping genes on the infected plants. Interestingly, we found that some other RNA viruses do not induce or only slightly induce the shut-off phenomenon of the same set of genes implying genetic determination in this process. Nuclear run-on experiments suggest that a plant virus, similarly to animal viruses, mediates the suppression of host mRNA synthesis in the nucleus. By investigating different host-virus interactions we revealed a correlation between the intensity of shut-off phenomenon and the severity of disease symptoms. Our data suggests that the efficient and persistent down regulation of host genes can be an important component of symptom development in certain host virus interactions.

(L40) RNase III encoded by a plant RNA virus suppresses antiviral defence in sweetpotato plants

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Sweetpotato (*Ipomoea batatas* L.) is the seventh most important food crop in the world and the third most important tuber and root crop after potato and cassava. In developing countries, it is consumed by the resource-poor rural populations for subsistence and serves as a famine reserve crop owing to its high tolerance to draught and productivity in poor soils. The only severe disease affecting sweetpotatoes and thereby food security is caused by virus complexes. They develop in plants infected with the phloem-limited *Sweet potato chlorotic stunt virus* (SPCSV; family *Closteroviridae*) and which also are infected with heterologous, non phloem-limited viruses, most notably *Sweet potato feathery mottle virus* (SPFMV; family *Potyviridae*). The yield losses of these sweetpotato virus disease (SPVD) affected plants can reach 100 %. The titers of SPFMV are increased up to 600-1000 fold, whereas the titers of SPCSV do not increase but may slightly decrease.

SPCSV encodes a double-stranded RNA (dsRNA) specific endonuclease (Rnase3) that enhances suppression of RNAi when co-expressed with another SPCSV protein, p22, a strong silencing suppressor. However, p22 is dispensable for development of SPVD since SPCSV isolates lacking the *p22* gene also caused SPVD in sweetpotato plants co-infected with SPFMV. Transgenic sweetpotato plants were generated to express the Rnase3 protein in cv. Huachano that is extremely resistant to SPFMV. Expression of Rnase3 alone was sufficient to render Huachano fully susceptible to SPFMV. The transgenic plants accumulated high titers of SPFMV and developed the typical symptoms of SPVD. This knowledge offers means for planning novel strategies to engineer sweetpotato for resistance to SPVD and the heavy yield losses it causes.

(L41) Functional domains in Helper-Component of ZYMV involved in suppressor activity and symptom expression

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The potyvirus-encoded Helper-Component proteinase (HcPro) is a multifunctional protein that functions as a suppressor of gene silencing. To identify specific HcPro functional domains we made deletion and replacement mutant ZYMV viruses and transient over-expression constructs. By deletion of 105 amino acids from the N' terminus of ZYMV HcPro we demonstrated that the N' terminus is neither essential for suppressor activity nor for virus viability. In contrast, the 36 amino acids of the carboxy terminus are required for suppressor activity. In the central region of the HcPro, known to be involved with the suppression of gene silencing, we identified several boxes rich in aromatic and charged amino acids: FRNH (amino acids 164-167), FRNK (179-182) and KRLF (215-218). In several cases mutations in these boxes influenced virus viability. Notably, mutations that caused loss of virus viability did not always eliminate suppressor activity.

Deletions in the highly conserved FRNK box of the ZYMV HcPro abolish both suppressor activity and virus viability. However, replacement of R or K in this box with non-charged amino acids, creating FINK, FRNA and FRNL, maintained suppressor activity while dramatically reducing plant symptom expression.

We demonstrated that both the wild type FRNK and the mutant FKNK, which also elicited severe symptoms, specifically bound siRNA-like and miRNA/miRNA*-like duplexes in an *in vitro* assay with a much higher affinity than the asymtomatic FINK and FRNA. We compared miRNA/miRNA* expression levels *in vivo* in healthy and virus-infected squash, and show dramatic differences between the attenuated FINK mutant and the wild-type (FRNK) virus. These data demonstrate the importance of charged amino acids in the FRNK box for siRNA duplex sequestration. Our data suggests that the highly conserved FRNK motif as well as FRNH and KRLF motifs in the HcPro of potyviruses are in contact with these duplexes, and that symptom severity is linked to binding affinity to this site.

Thus, it is possible that the interaction of the FRNK motif with miRNA/miRNA* duplexes directly influences miRNA function, thereby unbalancing target-gene accumulation and by this means causes symptom appearance.

Symposium V: Resistance Durability

Chairperson: Hervé Lecoq

(L42) Factors involved in the durability of resistance: can it be predicted?

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The use of genetic resistance in cultivars is one of the most efficient strategies for the control of crop diseases and, for viral diseases, the only direct means of control. However, the long-term protection conferred by genetic resistance is compromised by the evolution of pathogen populations: extensive deployment of a successful resistance factor exerts a selective pressure on the pathogen population so that genotypes that overcome the resistance, i.e., resistance-breaking genotypes, may become prevalent in the pathogen population. A major goal of plant pathology has been to understand this process, and to predict the durability of genetic resistance. Resistance-breaking is the result of changes in pathogenicity (i.e., the capacity of parasites to infect hosts and cause disease), and/or virulence (i.e., the degree of harm caused by parasites to their hosts), two major properties of parasites that determine parasite evolution and host parasite coevolution. During the last 30 years theoretical analyses of pathogenicity and virulence evolution have been extensive, and predictions have been put forward under different assumptions on host-parasite interactions. Similarly, the molecular genetics of pathogenicity and resistance (but not of virulence and tolerance) have been extensively analysed in plant-parasite systems. However, there has been little connection between these two fields of research, and there is the need to test experimentally theoretical predictions of host-parasite co-evolution under different models of plant-parasite interactions.

Current evidence shows that on the average resistance to viruses has been more durable than resistance to cellular plant pathogens, but why this is so, and why different resistance factors vary in their durability, is poorly understood. Different, non-excluding, hypotheses have been put forward that consider the molecular mechanisms of resistance and pathogenicity, the costs of pathogenicity, or the evolutionary potential of the virus, based all of them in circumstantial evidence. We will review these hypotheses, and the supporting evidence, within the context of the theoretical framework of host-parasite coevolution, and considering different models of host-parasite interactions, such as the gene-for-gene and the matching allele models. We will also present results on the evolution of pathogenicity and virulence in viruses that infect wild hosts (e.g. Arabidopsis) or crops (e.g. pepper) as they may relate to resistance durability.

(L43) Constraints on evolution of virus avirulence factors predict the durability of corresponding plant resistances

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Understanding the factors driving pathogen emergence is a major challenge, particularly in agriculture where the use of resistant plant cultivars could impose strong selective pressures on plant pathogens and leads frequently to "resistance breakdown". Presently, durable resistances are only identified after a long period of large-scale cultivation of plant cultivars carrying them. Because resistance breakdown involves modifications in the avirulence factors of pathogens, we tested for correlations between the evolutionary constraint acting on avirulence factors or their diversity, and the durability of the corresponding resistance genes in the case of plant-virus interactions. An analysis of 20 virus species-resistance gene combinations revealed a significant relationship between the durability of plant resistance genes and the selective constraint on amino acid substitutions in the corresponding avirulence factors. Based on this result, a model predicting the durability of resistance genes as a function of the selective constraint applied on the corresponding avirulence factors is proposed. Using that model upstream into breeding programs could help select the most durable resistance genes.

(L44) African rice cultivated species, *Oryza glaberrima*, opens new perspectives for analysis and breeding of resistance against *Rice yellow mottle virus*

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Rice yellow mottle virus (RYMV) is a major pathogen of rice in Africa and Madagascar. A unique resistance gene (*Rymv*1) has been identified so far in cultivated rice, and encodes the translation initiation factor eIF(iso)4G (Albar et al., 2006, *Plant Journal* 47:417-426). This gene is involved in a recessive resistance characterized by a complete absence of symptoms and a very low virus content. A resistance allele (*Rymv*1-2) has been identified in the only two known resistant varieties from Asian cultivated rice, *Oryza sativa,* but the African cultivated rice, *O. glaberrima,* may constitute an interesting source of resistance alleles or genes against RYMV.

A large collection of *O. glaberrima* accessions was checked with two RYMV isolates representative of the virus diversity in West Africa. High resistance, detected in 25 accessions, appeared to be far more frequent than in *O. sativa* species. The central domain of *Rymv*1, characterised by point mutations or deletion in resistant alleles, was sequenced and, on most interesting accessions, genetic analysis were performed. The *Rymv*1-3 allele was prevalent in resistance varieties, but a new resistance allele (*Rymv*1-5), characterised by a deletion of three aminoacids, was identified. In addition, the *Rymv*1 central domain of several resistant accessions did not differ from that of susceptible control ones, suggesting that, in these accessions, resistance may rely on an independent genetic determinism. Indeed, an additional resistance gene, *Rymv*2, was identified and controlled a recessive resistance in at least two of them. Preliminary mapping results indicated that *Rymv*2 is distinct from genes encoding translation initiation factors from eIF4E and eIF4G families, which are largely involved in recessive resistance of plants against viruses. Fine mapping and characterization of *Rymv*2 is under progress.

Different combinations of alleles on both *Rymv*1 and *Rymv*2 are currently developed in homogeneous genetic backgrounds in order to estimate and compare their durability. Altogether, these results will have major implications for RYMV resistance breeding and characterisation of rice/RYMV interactions.

(L45) Base-by-base analysis of siRNA production by a plant inverted-repeat transgene giving viral resistance

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Tobacco plants (*Nicotiana tabacum* L.) were transformed with a construct containing a "hairpin" inverted repeat of 598 nucleotides of the *Potato Virus Y* (PVY; family Potyviridae) replicase gene. Such constructs efficiently produce dsRNA and thereby confer virus resistance by a post translational gene silencing mechanism. Homozygous plants were challenged with a range of PVY strains and resistance was measured by symptom expression, ELISA titer, and back-inoculation of controls with extracts from resistant plants. The nucleotide homology of PVY strains to the transgene was: WP (99.5%) PVY-NTN (96.3), PVY-H (95.6%), PVY-O (88.9%), strain 52 (88.3%), and local field isolates from tomato (86.8%), and pepper (86.3%). A transgenic tobacco line was immune to the five potato PVY strains with which the transgene had the greatest homology. Infection with the tomato and pepper PVY isolates, which had the lowest degree of homology with the transgene, caused delayed symptom appearance in the transgenic tobacco compared with control non-transgenic plants.

siRNA production was observed by northern blot. The production of siRNA by the transgene was evaluated in non-inoculated plants using a custom-designed chip for the detection of small RNA molecules. Probes of 25 bp were printed on the chip covering the sense and antisense PVY sequences. Each probe was moved 1 bp along the transgene from the previous probe so that siRNA production by the whole transgene could be mapped in both sense and anti-sense directions. Small RNA populations from transgenic and non-transgenic controls were differentially labeled and applied to the chip. siRNA was observed only in the samples from the transgenic plant line. Resistance-breaking viral mutations against specific plant-produced siRNA peaks were identified. siRNA peak intensities were 20-30 fold lower than that of major miRNA species (e.g. miRs 156, 157, 159, 166, 168, 319, 403 etc). siRNA peaks were noted throughout the inverted repeat transgene. The transgene dsRNA was apparently cut at random: no rhythm in siRNA production (i.e. on a basis of 21, 22 or 24 bps) was observed. Generally, siRNA signal intensity increased with GC content, although this relationship was stronger for the siRNA against sense probes than against antisense probes. Effective siRNA molecules were GC-enriched, and greatly preferred G or C in the extreme 3` position. Strand selection preference was found to be disagreement with the "animal" model of Schwarz et al (*Cell* 115, 199, 2003). A model for strand selection in plants has yet to be determined.

(L46) Broad-spectrum resistance to *Turnip mosaic virus* in *Brassica rapa* (Chinese cabbage)

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Turnip mosaic virus (TuMV) is a member of the *Potyvirus* genus, infects a wide range of cultivated plant species and causes significant economic losses in *Brassica* crops (Shattuck, 1992, *Plant Breeding Reviews* 14:199-238). It is a positive strand RNA virus (genome comprises 9830-9835 nucleotides) and is the subject of advanced molecular characterisation in terms of its interaction with brassicas (Walsh & Jenner, 2002, *Molecular Plant Pathology* 3:289-300). Plant genes for resistance to TuMV have been mapped in lettuce (*Tu*, Robbins *et al.*, 1994, *Theoretical and Applied Genetics* 89:583-589), *B. napus* (*TuRB01*, Walsh *et al.*, 1999, *Theoretical and Applied Genetics* 99:1149-1154; *TuRB03*; Hughes *et al.*, 2003, *Theoretical and Applied Genetics* 107:1169-1173; *TuRB04* and *TuRB05*, Walsh & Lydiate, unpublished) and *B. rapa* (*TuRB01b*, Rusholme *et al.*, unpublished). All these brassica genes are dominant *R* genes that control resistance to narrow spectra of TuMV isolates; the viral avirulence determinants for these genes have been identified (Jenner *et al.*, 2000, *Mol. Plant-Microbe Interact* 13:1102-1108; Walsh *et al.*, 2002, *European Journal of Plant Pathology* 108:15-20; Jenner *et al.*, 2002, *Virology* 300:50-59; Jenner *et al.*, 2003, *Mol. Plant-Microbe Interact* 16:777-784).

The *B. rapa* line RLR22 is resistant to a diverse range of TuMV isolates from different regions of the world, different pathotypes, different serotypes and different genetic groups (Walsh *et al.*, 2002, *European Journal of Plant Pathology* 108:15-20; Rusholme *et al.*, 2007, *Journal of General Virology* 88:3177-3186). A *B. rapa* genetic map based on 213 marker loci segregating in 120 first backcross (B$_1$) individuals was established. B$_1$ individuals were self-pollinated to produce B$_1$S$_1$ families. The existence of two loci controlling resistance to TuMV isolate CDN 1 was established from contrasting patterns of segregation for resistance and susceptibility in the B$_1$S$_1$ families. The first gene recessive TuMV resistance 01 (*retr01*) had a recessive allele for resistance, was located on the upper portion of chromosome R4 and was epistatic to the second gene. The second gene Conditional TuMV Resistance 01 (*ConTR01*) possessed a dominant allele for resistance and was located on the upper portion of chromosome R8. These genes also controlled resistance to TuMV isolate CZE 1 and might be sufficient to explain the broad-spectrum resistance of RLR22. The dominant resistance gene, *ConTR01*, was coincident with one of the three eukaryotic initiation factor 4E (*eIF4E*) loci of *B. rapa* and the recessive resistance gene *retr01* was apparently co-incident with one of the three loci of *eIF(iso)4E* in the A-genome of *B. napus* and therefore, by inference, in the *B. rapa* genome. This suggested a mode of action for the resistance that is based on denying the viral RNA access to the translation initiation complex of the plant host. *retr01* is the first example of a recessive resistance gene mapped in a brassica.

Abstracts of posters

Symposium I: Important/emergent plant virus diseases

(P1-1) *Tomato Yellow Leaf Curl Sardinia Virus* (TYLCSV): first report on pepper in Italy

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Tomato yellow leaf curl (TYLC) disease is primary known as one of the most damaging diseases of tomato (*Lycopersicon esculentum*) worldwide, often with yield losses of 100%, particularly when plants are infected early in development.

Two TYLC virus species are known to cause TYLC in Italy: *Tomato yellow curl virus* (TYLCV) and *Tomato yellow leaf curl Sardinia virus* (TYLCSV). Both species cause severe disease in tomato, however TYLCV - currently the most prevalent species in Europe - also affects pepper (*Capsicum annuum*) and bean (*Phaseolus vulgaris*), often with a severe impact on bean production, and TYLCSV-*Sicily* has recently been reported to be spread on pepper and bean in Tunisia.

During a survey in summer 2007, a disease of pepper under plastic tunnels was observed in Policoro (Matera), on the Ionic coast of Basilicata Region, with a disease incidence in some cases of more than 50%. The diseased plants exhibited light mosaic or mottling, leaf distortion, interveinal and marginal leaf chlorosis, upward curling of leaf margins of older leaves. The causal pathogen was suspected to be a *Begomovirus* due to the large population of the whitefly *Bemisia tabaci* observed on the crop and the high pressure of TYLCSV in the surveyed area.

Instrumental analyses (DAS-ELISA and PCR-RFLP) allowed the association of the observed symptoms to a *Tomato yellow leaf curl Sardinia virus* (TYLCSV) infection.

Phylogenetic analyses of the sequenced coat protein gene revealed that the pepper isolate clusters specifically in the *Sicilian* strain of the TYLCSV. Moreover, it has similarity values over 97% with other TYLCSV isolates of the same group, isolated from tomato, suggesting no significant nucleotide changes reflecting any biological property associated to the host.

TYLCSV was not reported before on pepper in Italy, but it was recorded with severe outbreaks on tomato, both in protected and in open field crops. This species was probably the primary source of infection from which subsequent diffusion by way of the vector *B. tabaci* followed on pepper.

To our knowledge this is the first report of the natural occurrence of TYLCSV in our country, with serious implications for its epidemiology.

(P1-2) Genetic diversity and evolution of *Pepino mosaic virus* in Southeastern Spain

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Pepino mosaic virus (PepMV; genus *Potexvirus*, family Flexiviridae) is an emerging pathogen that is causing severe economic losses in tomato (*Solanum lycopersicum*) crops in Europe and other continents. The analysis of its genetic variability and evolution has clear practical implications as well as a significant basic interest to understand the phenomenon of emergence of viral diseases. A recent report showed that Spanish PepMV populations sampled between 2000 and 2004 were genetically very homogeneous, being most isolates highly similar to the so called European tomato strain (PepMV-EU in this work). However, a few isolates sampled in 2004 in the Murcia province (Southeastern Spain) were distinct and highly similar to a strain reported from America (PepMV-US in this work) (Pagán *et al.*, 2006, *Phytopathology* 96:274-279). In order to study the outcome of this new introduction, we analyzed PepMV populations sampled during 2005, 2006, 2007 and 2008 in tomato crops in Southeastern Spain. A collection of 334 isolates were characterized by molecular hybridization in dot-blots using probes able to discriminate PepMV-EU and -US. During 2005, 97% of the isolates corresponded to the PepMV-US type; no double infections were detected. In contrast, during 2006, 2007 and 2008, double infections were detected in the 76, 27 and the 50% of the samples, respectively; during these years, PepMV-EU was only detected in mixed infections, whereas PepMV-US was detected in single and mixed infections. The variability and genetic structure of PepMV populations were analyzed by sequencing the triple gene block and the coat protein gene (1,914 nt) of a random sample of 50 isolates. Nucleotide diversity values were low; a strong purifying selection seemed to operate for open reading frames (ORFs) 2, 3 and CP, as ratios d_{ns}/d_s were well below the unity. In contrast, the ratio d_{ns}/d_s was above one for ORF 4, providing evidence that this ORF might be under positive selection. However, when considering the yearly subpopulations, within- and between-subpopulation diversity values showed no differentiation with time. On the other hand, our preliminary observations on the biological fitness of PepMV-EU and PepMV-US showed that PepMV-US was able to accumulate at much higher levels in tomato than PepMV-EU. Additional biological experiments, including host range and competition experiments are under way; the corresponding results will be presented at the meeting.

(P1-3) Genetic diversity of two important melon viruses in Southeastern Spain

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The Southeastern area of Spain is one of the main vegetable-growing areas of the country. Intensive cultivation of melon and other cucurbits, such as cucumber, squash and watermelon, is very important in this area. However, crop yield and fruit quality are often threatened by cucurbit viruses, among them *Melon necrotic spot virus* (MNSV; genus *Carmovirus*, family Tombusviridae) and *Cucurbit aphid-borne yellows virus* (CABYV; genus *Polerovirus*, family Luteoviridae) (Kassem *et al.*, 2006, *Plant Disease* 91:232-238). We have studied the variability and genetic structure of populations of MNSV and CABYV sampled from this area during 7 and 3 growing seasons, respectively. Both viruses have different factors determining their epidemiology, including survival, mode of transmission, host range, tissue localization and strategies used to control them. The population structure and genetic diversity of a collection of 33 isolates of MNSV sampled during 1999-2006 was estimated by sequence analysis of 2,216 nucleotides (nt) of four genomic regions coding for proteins p89, p7A, p7B and p42 and also for the 3'-untranslated region (3´-UTR). The data obtained showed mean nucleotide diversity values in synonymous positions of 0.055, 0.089, 0.152 and 0.038 for each coding gene, respectively, and a mean nucleotide diversity of 0.239 for the 3'-UTR. Phylogenies estimated for each of the coding regions showed that MNSV isolates could be clustered into two genetic groups, with seven isolates corresponding to group 1 and 26 corresponding to group 2. For the 3´-UTR, in contrast, two isolates were clustered in an additional and unrelated group, correlating with their ability to overcome the resistance conferred by the *nsv* gene. On the other hand, a total of 50 CABYV isolates were collected from melon and squash crops from commercial fields of the same region during 3 successive years (2003, 2004 and 2005). About 600 nt were sequenced for each isolate corresponding to the coat protein (CP) and open reading frame 5 (ORF5) genes. Mean nucleotide diversities for the whole population in synonymous positions were 0.022 and 0.021 for CP and ORF5, respectively. These values are of a similar order of magnitude than values estimated for MNSV. The deduced phylogenies suggested that the CABYV isolates analyzed could be clustered, like MNSV, into two genetic groups, with seven isolates belonging to group 1 and 42 isolates belonging to group 2. Within- and between-subpopulation diversity values showed that both, the MNSV and CABYV populations, are not subdivided by host, year or area of isolate recollection; therefore, other selection pressures may be acting to maintain the population structures observed.

(P1-4) Investigation of furovirus virulence in winter barley

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In contrast to the furovirus *Soil-borne cereal mosaic virus* (SBCMV), which is widespread in several European countries, *Soil-borne wheat mosaic virus* (SBWMV) has been detected so far only at a single location (Heddesheim) in Southwest Germany. Since the latter is known to naturally infect not only wheat, rye, and triticale but also barley, an assortment of fifty genebank accessions and barley cultivars was tested for resistance under field condition between March and May 2006. All sixteen currently known genes conferring resistance to the bymoviruses *Barley mild mosaic virus* and/or *Barley yellow mosaic virus* were present in this material. As visual scoring, electron microscopy and virus detection by DAS-ELISA and IC-RT-PCR revealed, genotypes varied considerably in their reaction to SBWMV. Resistant forms could be selected. In a next step the susceptible genotypes were planted in SBCMV-infested soil. Applying the tissue print immunoassay, IC-RT-PCR, and DAS-ELISA the virus could be readily detected in roots, shoots, and leaves of field grown plants during the winter period (November till March). With raising temperature and intensive plant development in spring SBCMV disappeared and was no longer detectable, which is in clear contrast to SBWMV. Therefore, this virus has to be considered as a potential threat for production of winter barley in Germany.

(P1-5) Tomato torrado virus, an emerging pathogen causing significant problems in tomato crops of Southeastern Spain

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The torrado disease was first identified in spring 2001 in protected tomato crops of the Murcia province. Initially, it seemed to be confined to a small geographical area but it has spread progressively northward and southward threatening the economic viability of tomato crops in Southeastern Spain. Recently, a new picorna-like plant virus, tentatively named Tomato torrado virus (ToTV), has been isolated from tomato plants showing torrado symptoms and characterised (Verbeek *et al.*, 2007, *Arch. Virol.* 152:881-890). By using trap tomato plants in a greenhouse affected by torrado, we have also isolated the torrado causal agent from other viruses frequently infecting tomato plants in Southeastern Spain. To characterise the transcriptome of torrado diseased plants, we sequenced over 1,400 expressed sequence tags (ESTs) from a subtracted cDNA library made from diseased and healthy tomato plants. A proportion of ESTs (14.7%) showed high nucleotide similarity (99%) with the ToTV published sequence, and covered the 53% of the ToTV genome. ToTV sequences also showed a 70% nucleotide similarity with those of Tomato apex necrosis virus (ToANV) (Turina *et al.*, 2007, *Plant Dis.* 91:932-941), suggesting that both are members of the same, newly identified, group of viruses. No other EST showed similarity with any other viral sequence in databases. Additionally, our analyses have shown that pepper and aubergine are also hosts of ToTV, and that *Bemisia tabaci* and *Trialeurodes vaporariorum* can both transmit this virus. Furthermore, field surveys showed the presence of ToTV in 80.5% of samples from tomato plants showing torrado-like symptoms; interestingly, ToTV was also present in 4.9% of the samples with no torrado symptoms. Coinfection with *Pepino mosaic virus* (PepMV) was very frequent: 48.9% of the plants positive for ToTV were also positive for PepMV; however, not all plants infected by ToTV (and showing torrado-like symptoms) were also infected by PepMV. Finally, a histological (*in situ* hybridization), and ultrastructural study is being carried out to get insights on the ToTV and PepMV distribution *in planta*. Preliminary results on ToTV localization indicate that phloem is the translocation route used by ToTV in tomato.

(P1-6) Assessment the resistance of *Ribes* cultivars to the *Blackurrant reversion virus*

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Blackcurrant reversion virus (BRV; genus *Nepovirus*, family *Comoviridae*) is the causal agent of the economically most important Blackcurrant Reversion Disease (BRD) in black currant cultivars. It is also associated with the Full Blossom Disease (FBD) in red and white curants. In the field BRV is transmitted by the mite *Cecidophyopsis ribis*. Experimental assessment of the BRV resistance in *Ribes* cultivars is very difficult. BRV transmission by mechanical inoculation to *Ribes* is extremely difficult, similarly as field experiments with BRV mite transmission from infected shrubs to the cultivars tested for resistance. Those are influenced by the affinity of mites to a particular cultivar and biotic and abiotic factors. In both cases the evaluation can be done first 2-3 years after BRV transmision. We conducted graft transmission experiments for the assessment the BRV resistance in currants. Scions from FBD infected red currants "Vitan" and "Heinemann" and white currant "Blanka" were grafted on red currant cultivars "Rondom", Jonkheer" and "Vitan", white currant "Blanka" and black currants "Viola" and "Öjebyn". Fifty to sixty plants of each cultivar were grafted and kept in an insect-free screenhouse at the Biology Centre. The number of symptomatic plants and symptom appearance were observed for 9 years. The presence of BRV was detected by repeated RT-PCR testing according to Přibylová *et al.,* 2008, Eur. J. Plant Pathology 121: 67-75. The first symptoms appeared in the 2nd year after grafting. We observed high correlation between the symptoms and positive RT-PCR results. Among red cultivars the highest resistance was observed in "Rondom" with no BRV infection, whereas 10% and 23% of "Jonkheer" and "Vitan" plants were positive in RT-PCR, respectively. White currant cultivar "Blanka" was infected in 23%, black currant "Viola" in 10% and "Öjebyn" only in 3%, the latter without any symptoms of virus infection. Although the transmission success rate may be influenced by the compatibility between the rootstock and scion, the method allows evaluation of the resistance to BRV in individual cultivars in 3 years.

(P1-7) Diagnostic multiplex RT-PCR analysis for the detection of soil-borne mosaic viruses and their natural vector *Polymyxa graminis*

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In Europe soil-borne mosaic viruses are of great economic importance for the production of the dominating cereal crops wheat and barley. They are transmitted by the obligate root-infecting plasmodiophorid *Polymyxa graminis* and retain their infectivity for more than 10 years in the thick walled resting spores of their vector. *P. graminis* is ubiquitous in soils and cannot be controlled by pesticides. Once a field is infested growing of resistant cultivars is the only chance to prevent severe yield losses. To investigate epidemiological aspects of occurrence and distribution of several soil-borne cereal viruses, to understand their evolution, to characterize the relationship between the viruses and their natural vector and to find new sources of resistance, reliable and rapid diagnostic systems must be available. Therefore, a simple, sensitive and costs reducing method for simultaneous detection of both the different soil-borne viruses and *P. graminis* was developed. Moreover, the two separate reaction mixtures proposed allow to discriminate between the bymoviruses (*Barley mild mosaic virus*, *Barley yellow mosaic virus*) as well as between the furoviruses (*Soil-borne cereal mosaic virus* and *Soil-borne wheat mosaic virus*), respectively. A universal poly dT-primer was applied to synthesize the cDNAs of all four virus genomes. Specific primers for PCR were developed based on sequence differences between the viruses of the same genus. For the detection of *P. graminis* new primers specific to the region of the nuclear ribosomal DNA, containing ITS1, 5,8S DNA and ITS2, were designed. Total RNA preparations followed by mRT-PCR were performed to generate the virus specific amplicons, which were easily distinguished from each other by size in agarose gels. Multiplex assays were validated on artificial mixtures of root plant material, harbouring viruses and *P. graminis* and then successfully applied for the study of infection processes in field plants along with ELISA and the tissue print immunoassay. The method described is efficient and more sensitive than immunological procedures and does allow the clear and simultaneous early-season/early stage detection of both the viruses (single or mixed infections) and their vector.

Symposium II: Factors required for virus multiplication and spread

(P2-1) Identification of plant partners of polerovirus structural proteins

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Polerovirus are icosaedric plant viruses with a positive RNA genome, localized in phloem cells and obligatory transmitted by aphids in a circulative and non propagative mode. In order to look for plant proteins potentially involved in virus transmission, we developed two different screens (*in vitro* and *in vivo*) to identify partners of virus particles or structural viral proteins.

Using Far-Western blot on protein extracts from non-infected cucurbit sap, we identified 9 proteins able to bind *in vitro* purified particles of polerovirus. Most of the proteins were defence proteins but we also found among the candidates the major phloem protein 2 of cucurbits, a mobile phloem lectin able to bind viroids *in vitro* and *in vivo*. This protein could potentially be involved in virus transport in the plant or in virus acquisition by aphids.

The second method is based on yeast two hybrid system to screen *Arabidopsis thaliana* cDNA libraries using structural viral baits. Several candidates were identified among them cytoskeleton related proteins, a kinase, and a protease. The cytoskeleton proteins could be involved in intracellular virus transport or in cell to cell movement of the virus. The identified protease could be responsible for the cleavage of the minor capsid protein (the readthrough protein) observed in purified virions and in sieve tubes of infected plants. As this viral protein is absolutely required for aphid transmission and involved in virus movement in the plant, we can hypothesize that the protease might either control virus dispersion by aphid or virus transport in the plant. This protease has been previously reported to be a plant defence protein which may also suggest that its function in the virus cycle could be related to the perception of the pathogen by the plant.

Work is in progress to confirm some of the interactions observed between viral proteins and plant proteins. *A. thaliana* knock-out mutants of the candidate gene are being tested for viral accumulation to assess the importance of these genes in the viral cycle. If infected, these mutants will be used as virus source in aphid transmission experiments to evaluate the role of the candidates in virus acquisition by aphids. The results will be presented and discussed.

(P2-2) Complexity of *Tomato chlorosis virus* counter defense strategy to the antiviral RNA silencing plant response

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To ensure successful invasion, many viruses express proteins with silencing suppression activity to counteract the RNA silencing-mediated response of the host. The existing relationship between viral pathogenesis and RNA silencing suppression highlights the importance of the studies directed to the identification of new RNA silencing suppressors and their modes of action to better understand this interesting mechanism of plant-virus confrontation. *Tomato chlorosis virus* (ToCV, genus *Crinivirus*) is an emerging plant virus that belongs to the complex family *Closteroviridae*, in which examples of multiple-component RNA silencing suppression systems have been reported. To ascertain if this is a general strategy in this group of viruses, we have explored the bipartite genome of ToCV. We have identified the RNA1-encoded p22 protein as an effective silencing suppressor by using an *Agrobacterium* co-infiltration assay. This protein suppressed very efficiently local RNA silencing induced either by sense RNA or dsRNA, but did not interfere with short or long-distance systemic spread of silencing. We have also demonstrated by using a PVX-based heterologous vector, the silencing suppression activity of the RNA-2 encoded coat (CP) and minor coat (CPm) proteins. Taken together, these results indicate an even greater complexity of silencing suppressor activity for a member of the *Closteroviridae* family, and for the first time we show the presence of RNA silencing suppressor genes encoded by more than one RNA molecule of a multipartite genome for a plant virus.

(P2-3) A proteomic approach to characterize aphid vector proteins capable to interact with the HCPro transmission factor of Tobacco etch potyvirus (TEV)

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Many plant viruses are transmitted by insect vectors from plant to plant, and there is a considerable interest to understand in detail the molecular mechanisms of the transmission process as a way to find new resistance strategies based in interference with the virus spread.

The members of the large *Potyvirus* genus are transmitted by aphids in a very efficient non-circulative non-persistent manner. The transmission process of potyviruses is highly specific and involves the reversible retention of virions to unknown components of the cuticle lining the vector stylets, an association mediated by a virus encoded helper component known as HCPro protein. This multifunctional product plays a key role in transmission, presumably acting as a molecular bridge between virions and the vector. While the predicted interaction between virion and HCPro has been confirmed experimentally, the search for putative HCPro specific receptors in the aphid is still in progress. A recent work performed with caulimoviruses, another non-circulative transmitted virus, has provided evidence for the presence, near the tip of aphid stylets, of a non-glycosylated protein capable to specifically bind to the viral factor mediating transmission (Uzest *et al.*, 2007, *Proc. Natl. Acad. Sci. USA* 104:17959-17964). In the case of potyviruses, the most relevant recent achievement in this area is the description of the specific association between the HCPro of Zucchini yellow mosaic virus (ZYMV) and several aphid cuticle proteins (Dombrovsky *et al.*, 2007, *J. Gen. Virol.* 88:1602-1610).

We have tried to characterize in aphids the possible specific receptors of Tobacco etch potyvirus (TEV), using overlay assays with transmission-active purified HCPro protein (Ruiz-Ferrer *et al.*, 2005, *J. Virol.* 79:3758-3765). Proteins extracted from heads of *Myzus persicae,* a highly efficient vector of TEV, were separated on 2D gels and transferred to nitrocellulose membranes. The analysis using overlayed TEV HCPro allowed to detect nine candidate protein spots, apparently different from the cuticle proteins found with ZYMV. A similar procedure performed with proteins extracted from heads of *Lipaphis erysimi,* a non-vector of TEV, showed a different protein interaction pattern with only five equivalent spots binding to HCPro. The TEV HCPro-interacting insect proteins were submitted to mass spectrometry or *de novo* sequencing analysis, and seven of them were identified after searches with the obtained peptides in the available databases of aphid sequences (Gauthier *et al.*, 2007, *Bioinformatics* 23:783-784). The possible involvement of each candidate in vectoring capacity remains to be determined.

(P2-4) Arabidopsis genetics reveals an unexpected role for a plasmodesmal protein in regulating *Grapevine fanleaf virus* movement protein tubule formation

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Arabidopsis has provided the source for the proteomic and cell biological characterisation of several new plasmodesmal proteins. One of these, named PDLP1 (Plasmodesmata Located Protein 1), comprises a family of type-I membrane proteins specifically located on the plasma-membrane at plasmodesmata (Thomas *et al.*, 2008, *PLoS Biol* 6**,** e7. doi:10.1371/journal.pbio.0060007). The eight members of the PDLP1 family are expressed at widely different and partially overlapping tissue locations. To investigate PDLP1 functions in the context of macromolecular trafficking of fluorescent reporter molecules, endogenous non-cell-autonomous molecules and viruses, insertional knock-out mutant lines were identified for six of the eight genes and mutants combined to address potential functional redundancy within the PDLP1 family. As one candidate plasmodesmally-related protein we tested the effect of the combinatorial knock-out lines on the function of the grapevine fanleaf virus movement protein.

Grapevine fanleaf virus (GFLV, genus *Nepovirus*, family *Comoviridae*), a major viral pathogen of grapes worldwide, possesses a bipartite genome. The RNA1-encoded proteins are involved in viral replication, whereas the movement protein (MP) and the capsid protein (CP) encoded by the RNA2 are both required for cell-to-cell movement. During infection, the MP forms tubules in plasmodesmata (Pd) through which GFLV virions pass into non-infected cells according to the tubule-guided mechanism. It had been shown previously, that ectopic expression of MP was sufficient for Pd-targeting and tubule formation. However the manner by which this protein is transported intracellularly and later assembles into tubules remains unknown. Since the MP is an intrinsic membrane protein and that a functional secretory pathway might be required for specific MP-targeting to plasmodesmata we tested whether the MP could depend upon PDLP1 for secretion to plasmodesmata (see abstract: Khalid Amari). These proteins also possess the necessary features required for GFLV receptor-like function at plasmodesmata. In support of this idea, it was demonstrated that PDLP1 was present at the base of the tubules within modified plasmodesmata and, using fluorescence lifetime imaging microscopy-based FRET, that 2B but not TMV MP (30K), interact with specific PDLP1 members *in vivo*. Using the combinatorial PDLP1 insertional knockout mutants to validate these interactions, we showed that in PDLP1 triple knockout mutant lines, tubule formation was significantly reduced, providing genetic evidence in favor of the function of these host proteins as receptors for 2B. The potential for PDLP1 proteins to act as receptors for other viruses using tubule-guided movement, and their role in virus movement, are under investigation.

(P2-5) Involvement of the cytoplasmic inclusion (CI) protein in the overcoming of an eIF4E-mediated resistance against *Lettuce mosaic virus*

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The recessive allelic genes *mo1*[1] and *mo1*² in lettuce, corresponding to mutant alleles of the gene encoding the eukaryotic translation initiation factor 4E (eIF4E) (Nicaise *et al.*, 2003, *Plant Physiol.* 132: 1272-1282), are currently used to protect lettuce crops against the potyvirus *Lettuce mosaic virus* (LMV).

LMV-E is a resistance-breaking isolate that induces symptoms in *mo1*[1] or *mo1*² carrying cultivars, while LMV-0 is unable to induce symptoms on cultivars carrying the *mo1*² allele (tolerance) and generally does not systemically invade cultivars carrying the *mo1*[1] allele (resistance). A reverse genetic analysis using full-length infectious clones of LMV-0 and LMV-E has previously shown that the resistance-breaking determinant(s) map to the 3' half of the LMV-E genome *(*Redondo *et al.,* 2001*, Mol. Plant-Microbe Interact.* 14: 804-810), including the region encoding the VPg. So far, the VPg has been identified as the single potyvirus virulence determinant for the overcoming of eIF4E-mediated recessive resistances. The aim of the present report was (i) to narrow down the region carrying the LMV virulence and (ii) by the use of site-directed mutagenesis, to allow the identification of a key amino acid for the ability of LMV isolates to overcome eIF4E-mediated resistance in lettuce.

Results showed that exchange of the VPg from a virulent isolate into an avirulent one is sufficient to restore a full compatibility with lettuce varieties carrying the *mo1*[1] allele but not the *mo1*² one, while the region coding for the C-terminal portion of the CI and 6K2 allows the overcoming of both eIF4E alleles. Site directed mutagenesis of the CI at position 1946 was sufficient to affect, in a reciprocal manner, the infection phenotypes of the parental viruses, in plants carrying both resistance alleles, further demonstrating that the C-terminus of the CI is directly involved in the breaking of eIF4E-mediated resistance in lettuce.

This is the first example of a potyvirus CI gene acting as a determinant for eIF4E-mediated recessive resistance breaking.

(P2-6) TMV movement protein interacts with GFP-tagged microtubule *end-binding protein 1* (EB1)

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The ability of the *Tobacco mosaic virus* (TMV) movement protein (MP) to facilitate the cell-to-cell spread of infection is tightly correlated with interactions of the protein with microtubules, indicating that the microtubule system is involved in the transport of viral RNA (Boyko, V., Ferralli, J., Ashby, J., Schellenbaum, P., and Heinlein, M. (2000) Function of microtubules in intercellular transport of plant virus RNA. *Nat Cell Biol* 2: 826-832). While the MP acts like a microtubule-associated protein able to stabilize microtubules during late infection stages, the protein was also shown to cause the inactivation of the centrosome upon expression in mammalian cells, thus suggesting that MP may interact with factors involved in microtubule attachment, nucleation, or polymerization (Ferralli, J., Ashby, J., Fasler, M., Boyko, V., and Heinlein, M. (2006) Disruption of microtubule organization and centrosome function by expression of *Tobacco mosaic virus* movement protein. *J Virol* 80: 5807-5821). To further investigate the interactions of MP with the microtubule system *in planta*, we expressed the MP in the presence of GFP-fused microtubule end-binding protein EB1a of Arabidopsis (AtEB1a:GFP). The two proteins co-localize and interact *in vivo* as well as *in vitro*, and exhibit mutual functional interference. These findings suggest that MP interacts with EB1 and that this interaction may play a role in the associations of MP with the microtubule system during infection (Brandner, K., Sambade, A., Boutant, E., Didier, P., Mely, Y., Ritzenthaler, C., and Heinlein, M. (2008). TMV movement protein interacts with GFP-tagged microtubule *end-binding protein 1* (EB1). *Plant Physiol.* in press).

(P2-7) Towards the identification of Arabidopsis proteins involved in TYMV replication: a proteomic analysis of replication complexes

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A number of recent results indicate that positive-strand RNA virus genome replication depends on a wide range of host factors that play important roles in the assembly of the viral replication complex, in the selection and recruitment of the viral RNA template, or in the regulation of the RNA synthesis process. Those host factors play a critical role in the development of the viral infection but also represent a potential target for virus control.

To gain some understanding of the composition and functioning of viral replication complexes, we aimed to identify the cellular proteins involved in the replication of TYMV (*Turnip yellow mosaic virus*), using a proteomic approach. TYMV is a model virus belonging to the alphavirus-like supergroup of viruses, that is able to infect *Arabidopsis thaliana*.

As TYMV replication occurs at the periphery of the chloroplast envelope, both chloroplast membranes and soluble and active TYMV replication complexes were purified from infected plants. To facilitate the obtention of such fractions, we also generated an Arabidopsis cell line stably infected by TYMV, from which viral replication complexes could be successfully purified (Camborde *et al.*, 2007, *FEBS Letters* 581:337-341). Those fractions were then analyzed by 1D and 2D-blue native gel electrophoresis and a number of proteins recognized by an antibody raised against a highly purified TYMV replicase fraction were selected for further mass spectrometry analyses.

(P2-8) Effect of the absence of the proteins C2, C4 and V2 on the infection by *Tomato Yellow Leaf Curl* Sardinia Virus

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Tomato yellow leaf curl Sardinia virus (TYLCSV) is one of the most devastating diseases of cultivated tomato, affecting crops in tropical and subtropical regions, specially in the Mediterranean basin, and causing economic losses up to 100%. The important impacts caused by this virus make the study of the infection a priority, since understanding the process is a requisite for fighting the crop disease properly.

In our laboratory, transgenic plants of *Nicotiana benthamiana* containing a green fluorescent protein (GFP) expression cassette among two direct repetitions of the intergenic region of TYLCSV have been constructed (2IRGFP plants) (Morilla *et al.*, 2006, *J Virol.* 2006 Apr;80(7):3624-33). When these plants are infected with TYLCSV, the cassette is mobilized and replicated as an episomal replicon and, as a result, an increase of the expression of the reporter gene is observed. The release of episomal trans-replicons from the transgene and the subsequent increase in fluorescence is dependent on the geminiviral replication-associated protein, Rep, and requires interaction between Rep and the intergenic region (IR) located at both sides of the cassette; thus, this expression system is able to monitor the replication status of TYLCSV in plants, providing a mean to follow the progression of the infection in a quick and easy way.

Gene silencing has been described as a major defence mechanism against viruses in plants. As a counterattack, viruses have evolved silencing suppressor proteins. In order to study the relative importance of the roles of three putative silencing suppressors encoded by TYLCSV (C2, C4 and V2) during the infection, we have constructed mutants in the start codon of each gene and monitored the subsequent infection in 2IR *N. benthamiana* plants and tomato.

The C2 mutant virus is able to infect *N. benthamiana* but not tomato, which is the virus natural host. In *N. benthamiana* the infection is much milder, being the replication rate considerably lower. This suggests an important role of C2 in the development of the infection, and might be related to its ability to act as a transcriptional activator.

The V2 mutant is not able to infect neither *N. benthamiana* nor tomato, which might imply that V2 is essential for the infection.

The C4 mutant is able to infect both hosts and the infection is not hindered in any case, even though the symptoms are remarkably milder. Moreover, the mutant infection in *N. benthamiana* seems to be stronger, and the replication lasts longer than in the wild type infection. These data suggest C4 might act as an avirulence factor, being recognized by the host plant.

(P2-9) Genome activation of *Raspberry bushy dwarf virus*: requirement for coat protein for RNA replication

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Raspberry dwarf virus (RBDV) is the only member of the *Idaeovirus* genus of plant viruses and has 33nm-diameter isometric particles that contain three species of positive-strand RNA. RNA1 is about 5.5kb and encodes the probable viral RNA-dependent RNA polymerase. RNA2 is 2.2kb and encodes a probable movement protein together with the coat protein (CP). RNA3 is about 1kb and is a subgenomic RNA derived from the 3' part of RNA2 that encodes the CP. RBDV is perhaps the most important viral pathogen of cultivated raspberry worldwide, causing yield loss and malformation of fruit, and producing very severe disease symptoms as a result of synergism with various other viruses. RBDV is transmitted by infected pollen, which makes control of this virus very difficult to achieve. In order to assess raspberry germplasm for potential resistance to the virus we have constructed infectious cDNA clones of RBDV. Using these constructs in *Nicotiana benthamiana* we have found that productive infection occurs only when a source of CP is included in the inoculum. This situation resembles the CP-mediated genome activation process that occurs with *Alfalfa mosaic virus* (AMV) and ilarviruses such as *Tobacco streak virus*. Fusion of the N-proximal 145 amino acids (aa) of RBDV CP to red fluorescent protein (mRFP) allowed this protein to activate virus infection. Fusion of smaller CP peptides to mRFP, and deletion of defined parts of the complete CP, identified a region near the N-terminus of the CP that is required for genome activation of RBDV.

(P2-10) The *dotcom* mutant series: ORMV-MP transgenic *Arabidopsis* mutants impaired in their ability to complement the movement of MP-defective *oilseed rape mosaic tobamovirus* (ORMV)

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Intercellular movement of macromolecules has become a central issue in many aspects of plant molecular physiology. Numerous processes have been described that involve the movement of protein or nucleic acids at short and long distances within the plant body. These include important developmental transitions, systemic spread of gene silencing, and viral movement among others. Intracellular dynamic macromolecular distribution, symplasmic macromolecular movement through plasmodesmata, and long distance vascular movement reside in the mechanistic bases of this intercellular exchange of biological information. However, information about genes and gene products mediating these processes is scarce and fragmented, largely due to the lack of good genetic approaches to identify and interrogate the plant genes involved.

We have developed a genetic approach specifically designed for the identification of *Arabidopsis* genes involved in cell-to-cell movement of a tobamovirus, used as a representative of macromolecular movement. The system is based on complementation of a movement protein (MP)-defective tobamovirus with transgenic MP protein produced by *A. thaliana*. The defective virus contains a GFP gene, so the infection could be followed by fluorescence detection. Arabidopsis mutants that DOn´t Transgenically Complement ORMV Movement (*dotcom* mutants, *dcm*) have been selected and preliminary characterized. The *dotcom* mutants did not support systemic viral movement, and they showed different complementation phenotypes in the inoculated leaves. Different phenotypes could reflect mutants specifically affected in different stages of cell-to-cell viral movement.

(2-11) Dissection of the oligogenic resistance to C*ucumber Mosaic virus* in the melon accession PI 161375

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Resistance to *Cucumber mosaic virus* (CMV) in the exotic melon accession PI 161375, cultivar "Sonwang Charmi" (SC), had previously been described as oligogenic, recessive (Karchi et al, 1975, Phytopathology 65:479-481) and quantitative (Dogimont et al, 2000, Acta Hortic. 510:391-398), with a major QTL residing in linkage group XII (LGXII). Traditionally, these characteristics have made more difficult the introgression of resistances to CMV into commercial cultivars.

We have used a collection of Near Isogenic Lines (NILs) with introgressions of SC into the genome of the susceptible accession Piel de Sapo (PS) (Eduardo et al, 2005, Theor Appl Genet 112:139-48) to further characterize this resistance.

Infection of NILs carrying introgressions on LGXII showed that only NIL SC12-1 was resistant to CMV strains P9 and P104.82, but not to strains M6 and TL. Further mapping of this region showed that the resistance, named *rcm1* (resistance to CMV 1), maps in an area of 2 cM, between markers CMN61_44 and CMN21_55. Moreover, *rcm1* confers total resistance to strains P9 and P104.82, indicating that in these cases it is not quantitative and that *rcm1* is sufficient to confer full resistance to these CMV strains. Candidate gene mapping of 10 translation initiation factors in the melon genome failed to locate any of them in the interval between markers CMN61_44 and CMN21_55.

Altogether, these results suggest that the resistance to CMV present in SC is oligogenic, where different loci confer resistance to different CMV strains, but not necessarily quantitative, since at least one of these genes (*rcm1*) confers total resistance, similar to that of the parental SC, and does not need the contribution of other loci.

(P2-12) Expression or extinction of some eukaryotic translation initiation factor alleles in transgenic tomato and lettuce: effect on virus infection

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Eukaryotic translation initiation factors (eIF) play a key role during virus infection in plants (Robaglia and Caranta, 2006, Trends Plant Sci. 11:40-45). Because viruses rely upon host factors for their own survival during infection process, modification or disruption of such host factor(s) lead to plant resistance. Among plant translation initiation factors, eIF4E or its isoform, eIF(iso)4E, were shown to be involved in recessive resistance to several RNA viruses, in numerous plant families. More recently, other initiation factors like eIF4G and eIF(iso)4G have been also shown to play a role in plant resistance against viruses. Both eIF4E and eIF4G belong to small multigenic families, and a selective recruitment of these proteins was observed depending on the potyvirus and the host plant. To investigate the specificity of use of these factors, we tested the capacity of susceptible eIF4E alleles isolated from tomato, lettuce and pepper to complement for *Potato virus Y* (PVY) and *Lettuce mosaic virus* (LMV) infection in tomato and lettuce. Overall, a contrasted behaviour of transgenic plants was observed depending on the homologous or heterologous nature of the transgene and also on the potyvirus. Transgenic tomatoes suppressed for eIF4E or eIF(iso)4E expression were also challenged against a set of 13 viruses including 8 potyviruses. While eIF4(iso)E suppression showed no obvious phenotypic effect, suppression of eIF4E gave marked phenotypic effect on plant growth and also a reduced susceptibility to several potyviruses.

(P2-13) Experimental transmission and segregation of D and M types of *Plum pox virus* by aphids and their persistency in the vector

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Plum pox virus (PPV, *Potyvirus*), the causal agent of the sharka disease causing important economic losses in the stone fruit industry, is transmitted by grafting and by aphids in a nonpersistent manner following the "helper strategy". The transmission rate depends on the aphid species vector, the PPV isolate and the plant host, being its main vectors, *Aphis spiraecola* Pagenstecher and *Myzus persicae* Sulzer. Among the six main PPV strains described (D, M, EA, C, W and Rec) showing different biological, serological, molecular and epidemiological features, D and M types are the most prevalent. D and M strains differ in their ability to infect peach. M isolates appear to cause, in general, faster epidemics and more severe symptoms than D isolates.

The transmission of PPV-D and M isolates by aphids (*M. persicae*) from single and mixed infections, the segregation of both viral type populations, and the interference between them in the transmission process have been analysed. Moreover, the vector capability of acquire the virus and the persistency of the virus in the aphid have been studied. In the transmission experiments, "Sun Gold" Japanese plum trees (*Prunus salicina* L.) infected with PPV-D (RB3.30 isolate), M (5M-IVIA isolate) or D+M isolates, were used as viral sources, and young GF305 peach seedlings were the receptor plants. All experiments were conducted according to a nonpersistent protocol. The higher transmission rate was obtained with PPV-M (74.3%) followed by PPV-D (66.6%). When the donor plant was infected with both D and M isolates, the transmission rate obtained (40.0%) was lower than in the case of single infections and only PPV-M was detected by real-time RT-PCR in the receptor plants.

The persistency of PPV in the aphid during the transmission process was estimated in nonpersistent sequential experiments in which the PPV detection in single aphids and the PPV transmission were evaluated. Plants of *Nicotiana benthamiana* infected with PPV-M (3M Gladys/Nb IVIA isolate) or PPV-D (PPV-D-GR, Larissa isolate) were used as virus sources. *N. benthamiana* was used as receptor plant. The inoculation of PPV-D and PPV-M occurred mainly during the two first hours of access period on the receptor plants. The transmission rates by a single aphid after 2 hours of inoculation period were similar for both isolates: 5.2% for PPV-D and 5.3% for PPV-M. Although the capability of PPV transmission was lost after the two first hours of inoculation, PPV detection in a single aphid after each inoculation period by squash real-time RT-PCR was possible with similar percentages for both isolates.

(P2-14) Characterisation of non-transmissible coat-protein mutants of *Tomato yellow leaf curl Sardinia virus* in the whitefly vector *Bemisia tabaci*

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The capsid protein (CP) of the monopartite *Begomovirus* (*Geminiviridae*) *Tomato yellow leaf curl Sardinia virus* (TYLCSV) is indispensable for plant infection and vector transmission. A short region between amino acid (aa) Q129 and D152 is critical for insect transmissibility. In this study, two previously described non-transmissible (NT) mutants, one with a Q129P and a Q134H mutation (PNHD) and another with a further D152E change (PNHE), plus a new NT mutant with a single N130D change (QDQD) were characterized for their relationship with the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum*, compared to the wild-type virus (QNQD). Using quantitative dot-blot hybridization, the retention kinetics of the NT mutants was analysed in whiteflies following feeding on infected plants. Mutant QDQD, forming unstable virions of anomalous shape, was hardly detectable in the vector *B.tabaci* and the non-vector *T.vaporariorum*, even at the end of the acquisition. PNHD was acquired and circulated in both whitefly species for at least 7 days, similarly to the wt virus, while the PNHE mutant circulated in *B. tabaci* only and its DNA was undetectable in *T. vaporariorum*. To determine if NT mutants interfered with the transmission of the wt QNQD virus, competitive transmission experiments were performed. A significant inhibition of the transmission of the wt virus was observed by pre-feeding insects on the PNHE mutant, but not on the other mutants. Immunolocalisation analysis of the viral CP on *B. tabaci* previously exposed to plants infected by the different mutants will be presented, showing a different pattern of accumulation in the gut lumen and the salivary glands.

(P2-15) Analysis of the multiple silencing suppressors of geminivirus and their effect on infection

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Tomato yellow leaf curl disease (TYLCD) is one of the most important diseases affecting tomato crops in many tropical and subtropical regions of the world. It is well known that RNA silencing acts as an adaptative defense in plants. Viruses from different families have acquired a variety of unrelated suppressors that affect different, and perhaps multiple, steps in the silencing pathway. Geminivirus encode three distinct silencing suppressors: TrAP (AC2, C2, AL2...), C4 and V2, underscoring the importance of silencing as a host defense against DNA viruses. However, not all these suppressors are equally functional in different viruses or in different hosts. We tested the effect of putative silencing suppressors of geminivirus causing TYLCD in silencing and infection and also of curtovirus BCTV. To achieve this aim, geminiviral proteins TrAP, C4 and V2 of those species were cloned in binary vectors and used in a transient *Agrobacterium*-based system. Results from the transient expression experiments were confirmed by Northern and Western blot analysis. Besides we also monitored the accumulation of the GFP siRNAs. Some *Arabidopsis* transgenic lines were also transformed with construct to express TYLCD proteins showing PTGS suppression in the transient assays. We transformed *Arabidopsis* wild type plants and three different transgenic *Arabidopsis* lines: lines CHS-RNAi and suc-sul (in these lines the expression of an endogenous gene is silenced) and amplicon line (in this line the expression of an exogenous gene is silenced).

As a result of these experiments V2 protein was selected to perform a more detailed study including directed muthagenesis in some conserved domains, localization and interaction assays with the protein and its mutated versions and also the evaluation of the effect of these mutations on infection by constructing mutant viruses.

(P2-16) Is glycosylation of viral structural proteins involved in CABYV aphid transmission?

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Cucurbit aphid borne yellows virus (CABYV, genus *Polerovirus*, family *Luteoviridae*) is a plant virus, localized in phloem cells and obligatory transmitted by aphids. Previous studies have shown that post-translational modifications such as N-linked glycosylation can affect polerovirus transmission by aphids. We analyzed the glycosylation status of the 2 structural proteins of CABYV (Coat Protein-CP: major capsid protein, ReadThrough-RT: minor capsid protein) by using different approaches to determine more precisely how this modification may affect aphid transmission.

We first constructed four CABYV mutants modified in potential N-linked glycosylation sites (named Ngly-1, -2, -3, -4). Two of them (Ngly-1 and -2) contained a mutation in the major CP, whereas the two others were affected in the RT. When electroporated to plant protoplasts, the four mutants replicated as efficiently as the wild-type virus suggesting that the mutations introduced did not affect virus replication. When introduced in plants, the mutant Ngly-2 was almost totally impeded in long-distance movement whereas Ngly-1, -3, -4 showed a viral accumulation similar to the wild-type virus in systemic leaves. Analysis of the viral progeny in infected plants showed that mutations are maintained and no reverse nor compensatory mutation appeared. Aphid transmission of Ngly-1, -3, -4 mutants was assessed using infected plants as virus source. We observed a reduction in aphid transmission of the 3 mutants using either *Aphis gossypii* or *Myzus persicae*, two species known to be efficient vectors of CABYV. In order to conclude if the reduction in transmission efficiency of these viruses was due to a reduction in viral accumulation in plants or to a direct effect in vector interactions, aphid transmission experiments are being performed using purified virus particles as virus source. However, at this stage, we cannot correlate the effects on systemic movement or aphid transmission to modifications of the glycosylation status of the virion.

We then analyzed by Mass-Spectrometry if post-translational modifications are present on the two structural proteins of CABYV. So far, our results showed that no glycosylated peptide is present on the viral structural proteins. Glycosylation of virions was also assessed by immunodetection with antibodies specific to complex plants glycanes, and by using the lectin Concanavalin A. No N-glycane could be identified on the viral structural proteins. However, a 90kDa protein, an α-glucosidase, that co-purify with polerovirus was identified and shown to be modified by oligomannosidic and complex N-glycanes.The role in aphid transmission of this plant protein is currently being investigated.

(P2-17) Viral determinant implicated in the transmission of the *Grapevine fanleaf virus* by its nematode vector, *Xiphinema index*

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Grapevine fanleaf virus (GFLV; genus *Nepovirus*, family *Comoviridae*) is an icosahedral virus with a positive sense bipartite RNA. GFLV is specifically transmitted from grapevine to grapevine by the ectoparasitic nematode *Xiphinema index* (Andret-Link *et al.,* 2004, *J. Plant Path*. 86, 183-195). Previous experiments replacing the viral GFLV coat protein (CP) gene by the CP gene of *Arabis mosaic virus* (ArMV), a closely related nepovirus specifically transmitted by *Xiphinema diversicaudatum*, indicates that the specificity of transmission is solely determined by the CP (Andret *et al*, 2004, *Virology* 320, 12-22). The objective of our study is to identify the determinants of transmission specificity on CP. We hypothesized that amino acids involved in the specificity of transmission should be different between both viruses and located at the external surface of the capsids in order to interact with potential receptors in the food canal of nematodes.

To identify divergent external amino acids, a 3D-structural model of GFLV capsid has been deduced from the 3.5 Å resolution structure of *Tobacco ringspot virus* (Chandrasekar & Johnson 1998, *Structure* 6, 157-171). Based on this model, five potential external domains, from 6 to 12 amino acids, were selected. Sixteen mutants in which these five domains were substituted either in single or multiple combinations by their ArMV counterpart domains, have been engineered. Two mutants were able to induce a systemic infection in plants. Only one lead to the loss of transmission by *X. index*, indicating a potential function of this domain in GFLV transmission.

To fully validate our model, structural analyses (cryo-electron microscopy and X-ray crystallography) are performed in parallel. For cryo-electron microscopy, images of frozen-hydrated purified viral particles of GFLV and ArMV were recorded and are analysing using the polar Fourier transforms method developed by Baker. The crystallographic study is done with GFLV purified particles. The size homogeneity of GFLV particles have been analysed by dynamic light scattering (DLS) and revealed to be highly homogeneous. Several crystallization conditions have also been tested and have resulted in viral crystals which will be used for X-ray diffraction and modelling studies.

(P2-18) A plant virus movement protein forms ringlike complexes with the major nucleolar protein, fibrillarin, *in vitro*

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Fibrillarin, one of the major proteins of the nucleolus, has methyltransferase activity directing 2'-*O*-ribose methylation of rRNA and snRNAs and is required for rRNA processing. The ability of the plant umbravirus, groundnut rosette virus, to move long distances through the phloem, the specialised plant vascular system, has been shown to strictly depend on the interaction of one of its proteins, the ORF3 protein (protein encoded by open reading frame 3), with fibrillarin. Using atomic force microscopy, we have determined the architecture of these complexes as single-layered ringlike structures with a diameter of 18-22 nm and a height of 2.0±0.4 nm, which consist of several (n=6-8) distinct protein granules. We also estimated the molar ratio of fibrillarin to ORF3 protein in the complexes as approximately 1:1. Based on these data, we will provide a model of the structural organisation of fibrillarin-ORF3 protein complexes.

(P2-19) The exploitation of wild species *Solanum cardiophyllum* and *S. tarnii* as new sources for resistance to *potato virus Y*

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Solanum cardiophyllum Lindl and *S. tarnii* Hawkes et Hjerting are primitive potato species that grow in the warm and semi-arid highlands of Central Mexico. *S. cardiophyllum* is a diploid or triploid annual plant with a highly variable morphology, has a short flowering period and produces small edible tubers that contain low quantities of a few glycoalkaloids. *S. tarnii* is a diploid wild tuber-bearing species with lanceolate leaflets.

Evaluation of accessions of *S. cardiophyllum* and *S. tarnii* for resistance to pathogens and pests were made. The accession GLKS 108 and 2870, provided by the Genebank External Branch 'North', Gross Luesewitz, Germany, were selected for resistance to different strains of *Potato virus* Y (PVY).

Using mesophyll protoplast electrofusion between the wild species *S. cardiophyllum* and *S. tarnii* combined with commercial potato cvs., more than 160 somatic hybrids were produced (THIEME et al. 2004, 2008). Resistance to PVY was the primary trait investigated in the somatic hybrids. Additionally ploidy level, morphology, and yield were evaluated. The majority of the somatic hybrids of both genotype combinations showed no symptoms of virus infection after mechanical inoculation in green-house trials using PVY^N (CH605), PVY^O (205), PVY^C (Q3), PVY^{NTN} (Linda)**,** PVY^{N}W (Wilga O), PVY-NA^{NTN} (Nicola) and PVY^N (Amigo-N150/1), or when grown in the field.

The somatic hybrids with *S. tarnii* were backcrossed with the susceptible cv. Delikat and all tested BC$_1$ clones expressed no PVY incidence when exposed to different strains of this virus. For BC$_2$ clones resistant and susceptible genotypes were identified.

Hybrids with *S. cardiophyllum* displayed a wide range of variation with respect to morphology.

For hybrids of the combination *S. tarnii* + cv. Delikat only slight differences in habitus were observed. In general, the hybrids were more like the *S. tuberosum* parent. Most of the hybrids were fertile and the production of BC progenies was easily accomplished. Somatic hybrids and BC clones cultivated in the field produced acceptable tubers.For combination *S. tarnii* + cv. Delikat PVY resistance was transferred into hybrids, BC$_1$ clones and a number of BC$_2$ clones.

(P2-20) The avirulence determinant of a resistance found in the tomato relative *Solanum habrochaites* resides in a small region of the *Tomato yellow leaf curl virus* genome

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Tomato yellow leaf curl disease (TYLCD) causes severe yield losses in tomato crops woldwide, being the main limiting factor to its production in the south of Spain. This disease is caused by a complex of virus species of the genus *Begomovirus* (family *Geminiviridae*) that are transmitted by the whitefly *Bemisia tabaci*. The most efficient and simplest way to control this disease is the use of genetic resistance. Because few sources of resistance to TYLCD are available commercially, the search for new sources is crucial to get effective control. Also, understanding of the resistance mechanisms involved, can provide clues to develop more durable control strategies. Therefore, we screened germplasm collections of tomato (*Solanum lycopersicum* L.) and wild relative species for resistance to TYLCD. As a result, the accessions EELM-388 and EELM-889 of *S. habrochaites* were found resistant to the IL strain of *Tomato yellow leaf curl virus* (TYLCV-IL) either inoculated using infectious clones via *Agrobacterium tumefaciens* (agroinoculation), or using the natural insect vector *Bemisia tabaci* Gennadius. Resistance was also effective under natural infection conditions. Similar to TYLCV-IL, four other TYLCD-associated virus variants reported in the Mediterranean basin, the strain Mld of TYLCV (TYLCV-Mld), the strain ES of *Tomato yellow leaf curl Sardinia virus* (TYLCSV-ES), *Tomato yellow leaf curl Málaga* virus (TYLCMalV), and *Tomato yellow leaf curl Axarquia virus* (TYLCAxV), were tested by agroinoculation. Interestingly, both *S. habrochaites* accessions exhibited consistent resistance (neither virus accumulation nor symptoms were observed) to TYLCV-IL and TYLCAxV whereas they were tolerant (virus accumulated efficiently but no symptoms were observed) to TYLCSV, TYLCV-Mld and TYLCMalV. The comparison of the nucleotide sequences of the TYLCD-associated viruses used suggested that a small genomic region of TYLCV is candidate to contain the avirulence determinant of the resistance trait present in these two *S. habrochaites* accessions. This region corresponds to the 5' half of the intergenic region (containing the replicase binding site), the 5' end of the replication protein (C1) open reading frame and the whole C4 protein open reading frame, associated with long and short distance movement and gene silencing suppression. Studies are being conducted to understand the role of the avirulence determinant in the resistance.

Symposium III: Plant responses to viruses and/or their vectors

(P3-1) Analysis of the resistance mechanism to *Tomato yellow leaf curl virus* (TYLCV) in the Brazilian line TX-468 and its effect on TYLCV spread by *Bemisia tabaci*

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Tomato Yellow Leaf Curl Disease (TYLCD) is one of the most important pests affecting tomatoes (*Solanum lycopersicum*) that induces severe damages in crops from tropical and subtropical regions. It is caused by several distinct single-stranded-DNA-containing virus species of the genus *Begomovirus* (Family *Geminiviridae*) that are transmitted by the whitefly *Bemisia tabaci*. Among these virus species, *Tomato yellow leaf curl virus* (TYLCV) is one of the most widespread and economically important. Control of TYLCD is difficult and it is mainly based on the use of insecticides to reduce *B. tabaci* populations, although with limited success. Moreover, frequent pesticide applications have a detrimental environmental impact and repeated insecticide use has resulted in the development of resistant *B. tabaci* populations and treatments have become less effective. Therefore, genetic resistance in the host plant is the most desirable alternative. Recently, we identified an effective recessive resistance against TYLCV in the Brazilian line of *S. lycopersicum* named TX-468. This resistance results in a complete absence of TYLCD symptoms and reduction of virus accumulation. Characterization of the resistance mechanism operating in TX-468 and the effect of the resistance on TYLCD spread will be discussed. Our studies provide evidence that the resistance found in TX-468 is effective in reducing the primary spread of TYLCD. Using viruliferous whiteflies for a 48-h inoculation access period on TX-468 and Moneymaker (susceptible control) plants, we observed that TX-468 plants exhibited a lower propensity to be infected by TYLCV. The effect of TX-468 infected plants as virus sources for secondary virus spread is being evaluated.

(P3-2) Reaction of tomato hybrids and/or varieties to *Tomato Yellow Leaf Curl Sardinia Virus* (TYLCSV) under natural infection

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20 different hybrids and/or varieties of tomato (*Lycopersicon esculentum*) were evaluated for their reaction to *Tomato Yellow Leaf Curl Sardinia Virus* (TYLCSV) during 2006. The experiment was realised in Castrovillari (CS), Calabria region, southern Italy, in a 10 ha greenhouse where tomato is grown hydroponically in a NFT (Nutrient Film Technique) closed circulating culturing system.

In the area TYLC disease induced by TYLCSV is endemic since 2003-2004 and the viral pressure in the farm has been monitered costantly during the last 4 years.

For the experimental purpose, 100 plants for each hybrid and/or variety were transplanted in January 2006 in a greenhouse with a high TYLCSV pressure, in co-presence with 5 months old tomato plants, 100% affected by TYLC disease.

Plants were monitored by visual inspection for symptoms appearance and every 15 days post transplanting (d.p.t.). They were also evaluated by ELISA for virus presence respectively 30 and 60 d.p.t. All symptomatic plants resulted positive in ELISA. Symptomless and ELISA - negative plants were tested by PCR, to ascertain eventual false – negatives, revealing that all of them were infected.

After the phase of acute infection, 30 d.p.t. a gradual recovery could be observed in the hybrids and/or varieties that presented a resistance gene against TYLCV. The recovery was characterised by symptoms disappearance and complete decline of virus concentration. Results are reported and discussed.

(P3-3) Characterization of virulence/resistance determinants in the *Cucumber mosaic virus* and *Arabidopsis thaliana* interaction

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In the last 25 years, *Arabidopsis thaliana* has been widely used as a model organism for plant molecular biology studies. The genetic variation among *Arabidopsis thaliana* populations have received much attention recently as an alternative for the screening of laboratory-induced mutants, and this led to mapping various plant virus resistance genes. We used this approach to analyse virulence-resistance components in the interaction *Cucumber mosaic virus* (CMV)-*A. thaliana*. For this purpose, 21 *A. thaliana* accessions were challenged with three CMV isolates belonging to subgroups I and II, and resistance responses were monitored. One accession (Co-1) developed a systemic veinal necrosis upon infection with CMV isolates of subgroup I but not with isolates of subgroup II, what can be considered as a resistance response operating at the population level. The host and virus genetic determinants of this phenotype were analysed. The viral determinants for the systemic necrosis reaction were analysed in reassortants and recombinants between CMV isolates from subgroups I and II, and map to the coat protein. The inheritance of the plant determinants for this syndrome was analysed in the F2 population from a cross between Co-1 and L*er*, an accession that did not develop necrosis. The systemic veinal necrosis cosegregated with the *RCY-1* gene, which was described in accession C-24 as the determinant for a hypersensitive response against Y-CMV, but not against other subgroup I isolates. The sequence of *RCY-1* from Co-1 was determined, and its comparison with that of C-24, reveals two deletions and one insertion in the Leucine Rich Repeat region that could account for the different phenotype displayed by Co-1. Thus, we provide evidences that identify both the plant and viral determinants associated with the systemic veinal necrosis. Analyses of the expression pattern of *RCY-1* during viral infection and its relationship with viral spread allow a better understanding of this *Arabidopsis* defence response.

(P3-4) Involvement of P1 and HCPro proteins of Sweet potato mild mottle ipomovirus (SPMMV) in suppression of gene silencing

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Postranscriptional gene silencing (PTGS) is a key component of the antiviral responses in plants, and as a consequence viruses have developed potent suppression mechanisms, mainly through the action of viral gene products, to overcome this host defense. One of the first characterized viral suppressors was the HCPro of potyviruses. Although the different viral suppressors are unrelated proteins that can act at different levels in the silencing pathway, a direct interaction with ds siRNAs has been shown to be essential for their activity in many cases, including the HCPro of potyviruses (Lakatos *et al.*, 2006, *EMBO J* 25:2768-2780).

The large *Potyvirus* genus belongs to the family *Potyviridae*, along with other genera like the ipomoviruses. The type member of the genus *Ipomovirus* is *Sweet potato mild mottle virus* (SPMMV). Its single stranded positive sense genomic RNA encodes at least ten mature proteins, among them P1 and HCPro. Interestingly, *Cucumber vein yellowing virus* (CVYV), another virus within the genus, lacks the corresponding HCPro region, and presents a duplicated form of P1, with two P1a and P1b products, the second one being the suppressor of gene silencing (Valli *et al.*, 2006, *J. Virol.* 80:10055-10063). As in the case of the HCPro of potyviruses, the mode of action of CVYV P1b involves interaction with ds siRNA (Valli *et al.*, 2008, *J. Virol.* 82:974-986).

To gain insights towards understanding silencing suppression in SPMMV, we have initiated studies with this virus to analyze both *in vitro* and *in vivo* the functions of P1 and HCPro proteins. Constructs designed to express transiently these products in *Nicotiana benthamiana* leaf tissues were obtained and tested in agroinfiltration experiments performed with a GFP reporter gene. Our results indicated that SPMMV P1 was capable to suppress gene silencing, while HCPro alone did not exhibit that activity, although its presence in *cis* as part of a P1-HCPro construct contributed to extend the duration of the suppression effect. Visual observations were corroborated by Northern blot analysis of both mRNAs and siRNAs. However, and in contrast with the cases of potyvirus HCPro or CVYV P1b suppressors, attempts to find siRNA-binding activity with SPMMV proteins were unsuccessful, suggesting that a different mechanism could be operating. Assays performed with GFP sensor constructs that incorporated CymRSV or miRNA171 sequences indicated that the SPMMV P1 suppressor might be interfering with loaded RISC complexes, a mechanism not described previously in members of the *Potyviridae* family.

(P3-5) Transcriptomic responses of melon to virus infection

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Plant gene expression can be notoriously altered by viral infection. DNA microarrays have been described as powerful tools for analyzing expression of thousand of genes at the same time, in particular for analyzing the responses of plants to virus infection. The Spanish Consortium for Melon (*Cucumis melo* L.) Genomics has sequenced a large collection of melon ESTs (MELOGEN database; http://www.melogen.upv.es/) which have been used to design an oligo-based DNA microarray. We have performed microarray analyses of RNA samples derived from melon plants inoculated with several viruses, including *Cucumber mosaic virus* (CMV; genus *Cucumovirus*, family Bromoviridae), *Melon necrotic spot virus* (MNSV; genus *Carmovirus*, family Tombusviridae) and *Watermelon mosaic virus* (WMV; genus *Potyvirus*, family Potyviridae). Melon genotypes analysed included susceptible and resistant accessions. Results were in agreement with data generated by other authors and provided information on the potential mechanisms controlling the resistances considered. We were also interested in analysing the expression of small RNAs (sRNAs) ranging from 21 to 30 nucleotides under the same conditions. Cloning and sequencing sRNAs is interesting for understanding plant responses through virus induced RNA silencing, and also the biogenesis of small viral RNAs (viRNAs) and their hypothetical impact in host gene expression. Eight small RNA libraries were constructed from cotyledons of melon plants (of susceptible and resistant genotypes) inoculated with WMV and MNSV. These libraries were used in large scale sequencing of viRNAs and other small RNA molecules by pyrosequencing (454 Life Sciences). Using both microarray data and small RNA sequences we expect to get an overview of transcriptomic alterations and processes underlying the plant response to virus infection at the RNA level.

(P3-6) Suppression of RNA silencing in *Grapevine virus A* infected plants

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Grapevine virus A (GVA) is a member of the genus *Vitivirus,* family *Flexiviridae*. The ca. 800nm filamentous particle of GVA is considered to be a phloem-associated and contains a single-stranded RNA genome of about 7.4 kb, which consists of five open reading frames (ORFs). ORF1 encodes a 194-kDa polypeptide with conserved motifs of replication-related proteins. ORF2 encodes a protein of 19-kDa, with unknown function. ORF3 encodes a 31-kDa movement protein. The protein encoded by ORF4 is the coat protein. The ORF5 encodes a small 10-kDa protein (p10) that contains a basic, arginine-rich motif and a zinc-finger domain, and that interacts with nucleic acids. Additionally, GVA p10 is thought to possess a weak activity as a suppressor of RNA silencing. The aim of our work was to further explore the characteristics of this viral product. We found that p10 is dispensable for viral RNA replication but is essential for infection of plants. We obtained molecular and biological data that indicate that p10 affects the appearance of symptoms on *Nicotiana benthamiana* plants, and also found that the N-terminus of p10, especially its 8th amino acid, affects symptom development. In addition, we developed an assay whereby RNA silencing in leaves was induced by agroinfiltration of a mini-GVA-GFP replicon that possessed genes encoding viral RNA replicase and GFP fused to ORF2 product. The assay proved to be very sensitive for analyses of various RNAi suppressors. Using this system we found that p10 together with additional GVA-coded protein(s) or RNA(s) are involved in enhancement of RNA silencing suppression by the replicating virus. We also showed that p10s of the severe GR5 and of the mild GTR1-1 isolates exhibit essentially similar activities as RNA silencing suppressors, but affect symptom expression on *N. benthamiana plants* differently. These findings indicate that the ability of p10 to affect symptom development and to suppress the plant RNA-silencing machinery, are two functions that are not closely linked.

(P3-7) Disturbance of *Arabidopsis thaliana* development by a potyviral infection maps to the P3/p6k1 viral genomic region

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Infections of plants by viruses induce plant disease and associated symptoms result in economic losses in crops. The study of viral infections has led to the discovery of RNA silencing as a plant defence mechanism against plant pathogens and of viral suppressors of gene silencing as the viral mechanism to counter such plant defence. In addition, it has led to the unravelling of the role of small RNAs (sRNAs) in plant development. Developmental symptoms associated with plant disease have been attributed in some systems to the effects of the viral suppressors of gene silencing on the normal performance of the plant sRNA machinery.

In the model system *Arabidopsis thaliana - Turnip mosaic virus*, a potyvirus two different strains of which induce very different disturbances of the plant development, research was conducted to identify viral determinants of developmental symptoms. Chimeric constructs between the genomic cDNAs of both viral strains indicated that the viral determinant of developmental symptoms is in the P3/p6k1 region, different from the described viral suppressor of gene silencing (HC-Pro). Transgenic Arabidopsis plants were generated for the viral regions P1/HC-Pro or P3/p6k1. The presence of the P1/HC-Pro region from any of the strains induced indistinguishable phenotypes of altered development, whereas the presence of the P3/p6k1 region did not induce apparent phenotypic changes in the plants. This result emphasises the role of the different viral proteins in disease induction, opens the way to deepen our knowledge of the potyviral proteins in the viral cycle and also to better understand plant growth regulation.

(P3-8) *Arabidopsis* **transcriptomics reveals conserved and divergent gene expression profiles between A***rabidopsis thaliana* **accessions challenged with three different potyviruses**

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We used transcriptomics approach to identify candidate genes involved in *Arabidopsis* response to potyviruses.

With the aim to identify common and diverse responses, we challenged four different *Arabidopsis* accessions with three different potyviruses, *Lettuce mosaic virus* strain AF199 (LMV), *Tobacco etch virus strain* S69 (TEV) and *Plum pox virus* strain R (PPV). The *Arabidopsis* accessions were chosen according to their susceptible phenotype (Landsberg erecta-Ler-, Bologna 1-Bl1) in response to the three viruses or to their resistance to virus long distance movement (Columbia-Col-0 and Cap verde island-Cvi) in response to LMV and TEV.

Microarrays slides from the CATMA project for *Arabidopsis* were respectively hybridised with total RNA extracted from TEV, LMV and PPV infected and mock inoculated leaves from *Arabidopsis* accessions 7 days post inoculation. Two to three biological repeats were performed. Transcription changes were evaluated in inoculated leaves.

A quantitative and a qualitative analysis were conducted on transcriptome results. The quantitative analysis revealed the accuracy of the biological repeats which underlines, especially in the TEV experiment, the validity of the results. This analysis also pointed out some specific genes significantly expressed in some accession/potyvirus combinations. The qualitative analysis showed about 2500 genes deregulated in the susceptible ecotypes in response to TEV and LMV. Less genes appeared to be deregulated in response to PPV as well as in the Cvi resistant genotype. Among these, 79 common genes were repressed in response to the three viruses, while 36 were overexpressed in Ler. This accession shared about 400 common genes also deregulated in Bl1. Functionnal analysis of this set of genes revealed very various molecular functions.

Validation of the changes observed on microarrays slides is ongoing by phenotype/transcriptome correlation analysis, Q-PCR and KO mutant analysis.

(P3-9) DNA methylation and post-transcriptional gene silencing in virus resistant transgenic plants

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In the last few years abundant information has been gathered on the interplay between DNA methylation and transcriptional gene silencing (TGS) in transgenic plants. DNA methylation has also been described during post-transcriptional gene silencing (PTGS), but in contrast with the methylation of the promoter region occurring in TGS, the methylated region in PTGS occurs in the transcribed region. However, we are still far from understanding the causal relationships between DNA methylation of transcribed sequences and PTGS.

In order to assess the relevance of DNA methylation of the transcribed region during PTGS, we have analyzed the methylation of the transgenes of *Nicotiana benthamiana* plants transformed with different fragments of Plum Pox virus (PPV) genome. Two type of transgenic plants have been obtained showing a different pattern of resistance, line NIaΔV with a constitutive silencing is completely resistant to PPV infection while in line NIbV the resistance occurs after PPV infection due to virus induced gene silencing by which the recovered tissue became immune to a second inoculation.

Our results show that although in both type of transgenic plants there is extensive methylation in the transcribed regions, differences can be found in the distribution and in the context preference of the methylated cytosines.

(P3-10) Analysis of TYLCSV-tomato interactions by long serial analysis of gene expression

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The geminivirus *Tomato Yellow Leaf Curl Sardinia Virus* (TYLCSV) is one of the agents of the tomato yellow leaf curl disease, which causes dramatic crop losses in tropical and sub-tropical regions of the world. The viral genome is a 2,7 kb single stranded circular DNA; two proteins are encoded by the viral strand and four by the complementary one. The replication-associated protein (Rep_{359}) is the only viral product absolutely required for viral replication.

To gain insight into TYLCSV-tomato interactions we used long Serial Analysis of Gene Expression (longSAGE). LongSAGE identifies from each mRNA a unique short tag sequence of 20 bases allowing quantitative analyses of transcripts without the need for any prior knowledge of their sequences. Four longSAGE *Solanum lycopersicon* libraries were constructed: TYLCSV-infected, transgenic expressing either the first 130 (Rep_{130}) or the first 210 (Rep_{210}) amino acids of Rep_{359} and control uninfected wild type. The analysis of libraries representing healthy and TYLCSV-infected tomato leaf transcripts identified a total of 66.178 tags, accounting for about 8.000 unique *bona fide* transcripts. 436 tags were significantly ($P \leq 0,05$) over- or under-represented at least 3-fold between the two libraries and 60% of them matched a tomato unigene in the Solanaceae Genome Network data bank. Functional classification of the differentially expressed genes shows that TYLCSV infection mainly stimulates the expression of genes involved in protein metabolism, the ubiquitin cycle, regulation of transcription, signal transduction and defence/stress, while it reduces the expression of genes implicated in photosynthesis and metabolism.

(P3-11) Molecular determinants controlling aphid transmissibility of *Turnip mosaic virus* (TuMV)

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TuMV, a potyvirus, is non-persistently transmitted by aphids with the support of two viral proteins, coat protein (CP) and helper component (HC-Pro). By analogy to other potyviruses, the transmission process is dependent on three conserved specific motifs: Asp-Ala-Gly (DAG) found in the CP amino terminal region, and Gly-Lys-Ile-Thr-Cys (GKITC) and Pro-Thr-Lys (PTK), respectively located at the amino terminal region and central third of HC-Pro. The 'bridge hypothesis', the most widely accepted view on the transmission mechanism, poses specific interactions of HC-Pro and virion CP with some aphid factor, thus retaining virions in the vector stylet and allowing a later inoculation in the plant.

The involvement of GKITC and PTK in aphid TuMV transmission was approached by the creation of point mutations in these motifs on a viral infectious clone. Transmissibility of the different mutants (P**A**K, G**E**ITC and **E**KITC) was assayed with *Myzus persicae*, the TuMV main vector. Tests were performed with a single mutant or through sequential acquisition of two mutants. The identification of the transmitted virus was done by SNP (single nucleotide polymorphism), and the analysis of the SNP product was done by detection of a fluorescent label in an automatic sequencer. The results obtained showed that K → E in GKITC, T → A in PTK, and G → E in GKITC completely abolished transmissibility in assays with individual mutants. However, partial transmissibility rescues were obtained through sequential acquisition of different mutants. We propose that our results can only be explained in the light of the proposed multimeric nature of functional potyviral HC-Pro.

(P3-12) Microarray analysis of gene expression in tomato plants infected by different combinations of Cucumber mosaic virus and its satellite RNAs

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An analysis of transcriptional changes in tomato plants, induced by the infection of Cucumber mosaic virus (CMV), alone or in combination with satellite RNA (satRNA) variants, has been undertaken by microarray analysis. The analysis was performed a new CombiMatrix platform, on a tomato chip carrying 20200 specific probes from assembly of Tentative Consensus of the last Tomato Gene Index, release 11.0 (June 21, 2006).

Solanum lycopersicum cv. UC82 plants were infected with CMV-Fny or with CMV-Fny co-inoculated with three different satRNAs (benign: CMV-Fny/Tfn-satRNA; stunting: CMV-Fny/TTS-satRNA; necrogenic: CMV-Fny/77-satRNA). Mock-inoculated plants were used as controls. Gene expression was examined at 2 and 9 days post-inoculation. Hybridization data were extracted with the Microarray Imager software (Combimatrix). After normalization, only genes with a coefficient of variation < 0.8 in the three biological replicates of each sample were included in further analysis, and then considered as differentially expressed when showing a fold change ≥ 2 in at least one inoculum combination in comparison to mock inoculated samples. A hierarchical cluster analysis was performed using the Mev 4.0 software (Saeed et al., 2003 *Biotechniques* 2003, **34:** 374-378).

1179 genes were modulated in at least one condition. CMV-Fny, without any satRNA, provoked wide transcriptional changes, affecting about 80% of modulated genes. Core sets of transcripts coherently modulated (either up-or down-regulated) by all infections or by the 3 CMV/satRNA combinations were identified.

To identify genes that might account for the different symptoms observed in the different CMV/satRNA co-infections, the transcriptional effect of each single CMV/satRNA combination was compared with the effect of CMV-Fny: all satellites determined a clear down-regulation of genes that are expressed in CMV-Fny-infected plants, especially at 2 days p.i., while interesting differences could be identified between gene expressions specifically associated to each CMV/satRNA infection.

Differentially expressed genes have been grouped in putative functional categories. "General metabolism" and the "unknown function" categories were among the more frequently represented in all infections. However, the model systems considered present some peculiarities, such as a very small percentage of photosynthesis-related genes and, conversely, a consistent modulation of resistance-related and signal transduction–related genes. "Lipid metabolism" and "Nucleic acid metabolism" were also well represented.

This work might serve as a basis to identify candidate genes with a functional role in susceptibility and symptom determination.

Symposium IV: Innovative approaches, including transgenic resistance, to control plant viruses and vectors

(P4-1) RNAi-mediated resistance to *Pepper golden mosaic virus* REP

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Toward developing RNAi-mediated resistance to New World tomato-infecting begomoviruses (genus, *Begomovirus*; family, *Geminiviridae*) we have designed several hairpin REP constructs for the begomovirus *Pepper golden mosaic virus* (PepGMV) and used them to transform 'Moneymaker' tomato plants, an open pollinated tomato variety. The control constructs were antisense and sense orientations of the same fragment. The hairpin sequence is a non-coding fragment from GUS. Transgene expression is under the control of the 3X 35S promoter. Between 94-100% of the resultant tomato seedlings were transformed with the respective transgene (PCR analysis), whereas, 67-100% of constructs expressed transgene mRNA (+RT-PCR). The lowest expresser was the anti-sense AC1 construct control. The constructs under 'proof of principle' scrutiny expressed mRNA in 89% and 85% of the T0 events, highly promising results. The challenge viral genome (PepGMV DNA-A and –B) has been subcloned into a binary vector and shown to be infectious to pepper by Agro-inoculation. Seeds from ten T1 lines were sown in the transgenic greenhouse and agro-inoculated with PepGMV. Plants were scored (0-3) over a range of resistant or susceptible phenotypes. Extracts from inoculated test plants were subjected to molecular analysis to quantify RNAi levels, virus accumulation (Southern analysis), and transgene expression (northern analysis) using a fragment of PepGMV-REP as a probe. Results of these and ongoing experiments will be presented.

(P4-2) A simple methodology for testing virus resistance, including virus quantification, in pathogen-resistant transgenic plants

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Transgenic plants engineered to resist against a particular pathogen might exhibit an altered behaviour in front of other unrelated pathogens, ranging from higher susceptibility to enhanced resistance. Contrary to most agronomically valuable traits that are easily observable, the potentially altered performance of these plants remains unnoticed until pathogen challenge. Furthermore, when resistance against a severe pathogen is obtained, growers might be tempted to adopt the new technology without a proper evaluation of susceptibility against unrelated agents. For instance, caution is needed to avoid scenarios where plants resistant to fungi or bacteria might be more susceptible to viruses or viceversa. Since gathering multidisciplinar teams with breeders, geneticists and several pathologists, including virologists, is often difficult, we propose the use of simple methods for testing virus resistance based in infectivity assays complemented by virus quantification.

The maize Pathogenesis-Related PRms gene has been previously shown to confer broad-spectrum resistance against different types of pathogens (fungal, bacterial and oomycete pathogens) when overexpressed in tobacco and rice plants (Murillo *et al.*, 2003, *Plant J.* 36: 330-341; Gómez-Ariza *et al.*, 2007, *Mol. Plant Microbe Interact.* 20: 832-842). Interestingly, the PRms protein was described to localize in plasmodesmata (Murillo *et al.*, 1997, *Plant Cell* 9:145-156), a fact which might well influence the susceptibility of these transgenic lines against viruses, although this aspect has never been tested. Therefore, we decided to assess the virus susceptibility of *PRms* tobacco plants using simple methods. The study involved the evaluation of 9 independently generated transgenic lines and control untransformed plants challenged for individual infection with 4 different viral pathogens (TMV, CMV, PVX or TEV). After inoculation, quantification of viral RNA was performed in each plant. Different procedures of RNA extraction were compared, selecting the simplest one compatible with easy virus detection. Digoxigenin-labelled probes, derived from cloned fragments of virus genomes, were used to detect viral RNAs. Serial dilutions of *in vitro* transcripts of virus sequences were used as internal standards for quantification. A dot-blot hybridysation was adopted to test simoultaneously numerous samples, allowing assays to be performed rapidly for the 4 viruses here assayed. Visual inspection of symptom development accompanied detection of viral RNA.

Our results indicated no significant differences between *PRms* lines and control untransformed tobacco plants, either in the timing of symptom appearance or in the levels of virus accumulation. This observation allowed us to conclude that no alteration of virus susceptibility might counterbalance the benefits associated to the enhanced resistance of *PRms* tobacco plants against other types of pathogen. This methodology could be rapidly implemented for a more detailed characterization of transgenic plants as well as in breeding programmes for selection of virus-resistant genotypes.

(P4-3) Rootstock genetic engineering for GFLV resistance in grapevine

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The grapevine fanleaf disease, the major virus disease for the viticulture worldwide, is caused mainly by nepoviruses transmitted in a semi-persistant manner by longidorid ectoparasite nematodes. The fanleaf disease is causing to the French grapevine industry $ 1.5 billion losses per year with a prevalence of 540 000 ha (60 % of the total acreage cultivated with grapevines). *Grapevine fanleaf virus* (GFLV) belonging to the genus *Nepovirus* in the family *Comoviridae* is the main aetiological agent. The control of fanleaf disease is currently based on prophylactic measures, cultural practices (fallows over ten years) and certification programs. Despite soil desinfection and nematicides with the use of environmental unfriendly agrochemicals (some of them being already prohibited in the EU), fanleaf disease remains an expanding pandemy with technical deadlock in control strategies.

To date, no neither dominant nor recessive resistance genes have been found in grapevine toward GFLV. The implementation of genetic engineering provides new strategies based on rootstock mediated resistance, to develop virus-resistant grapevines generating RNA silencing. Transgenic rootstocks expressing the coat protein (CP) of GFLV genes have been obtained in the early 90ies. Their resistance toward fanleaf disease in vineyard conditions, has been assessed in an open-field trial in Champagne between 1996 and 1999, where 3 lines out of 18 were selected to be included in a following open-field trial starting in 2006 at INRA Colmar. This unique non-confined experiment has been set up through debates and recommendations issued from a local steering committee, including members of the public, of associations, of the local viticulture, wine growers and researchers. Results generated in a next future from this field experiment, will enable us to assess the behavior of these first generation of CP expressing transgenic rootstocks (GFLV resistance). In addition, their environmental impacts on the wild-type GFLV populations, on their longidorid vectors and on the putative horizontal transfer of the NPTII gene to the soil inhabiting bacteria will be analyzed.

Recent knowledge on RNA silencing led us to develop new types of constructs called "stem-loop" in order to optimize the induction, robustness and durability of the RNA mediated resistance. Transgenic *Nicotiana benthamiana* have been produced to assess the effectiveness of these constructs against GFLV infection. In parallel, rootstock transformation with these new constructs is being initiated.

Symposium V: Resistance Durability

(P5-1) Development of tomato lines and hybrids F1 varieties with complex resistance to viruses

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Our work encompassed species of the peruvianum complex – L. peruvianum, L. peruvianum var. humifusum and L. Chilense that are resistant to a significant number of pathogens, e.g. ToMV, CMV, TSWV, Fusarium, Verticilium and Meloidogine incognita.

The research work started in 1998 and was finalized in 2005. In the period 2001 – 2005 we cultivated two generations annually – one greenhouse and one field.

Cv. Mercury of L. esculentum was used as mother plant. There were significant reproductive barriers between the species of peruvianum complex and the remaining species of this genus, which complicated hybridization, hence, the small number of seeds obtained:

Mercury x L. peruvianum – 1 hybrid seed

Mercury x L. peruvianum var humifusum – 0 seeds

Mercury x L. chilense – 3 hybrid seeds

The first four F1 seeds were grown in vitro and the plants were intercrossed. This method of reproduction was used up to F3, incl. F4 was crossed as male parent with cv. Mercury in order to obtain BC1P1. The latter was subject to complex tests on resistance to ToMV, CMV, TSWV, Fuzarium, Verticilium and Meloidogine incognita.

F1 (Mercury x L. chilense) x(Mercury x L. peruvianum)→ F2 → F3 → F4 → BC1P1

Different varieties were included in the hybridization during the period that followed in order to maintain and improve the economic performance characteristics. At the end of selection in 2005, we had 6 lines, resistant to ToMV, CMV, TSWV and Meloidogine incognita and 2 lines, resistant to the six pathogens ToMV, CMV, TSWV, Fuzarium, Verticilium and Meloidogine incognita. They comply with the economic requirements to L. esculentum in terms of yield, early ripening, dimensions, shape and fruit hardness, etc.

 One of the lines" Rossela 07" is encompassed in variety testing and patent.

(P5-2) Transfer of resistance to *Plum pox virus* from *Prunus* interspecific hybrids to peach

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No natural resistance to *Plum pox virus* (PPV) has been identified in peach and so we decided to test *Prunus* interspecific hybrids and transfer the resistance to peach from them. Seven interspecific hybrids were evaluated as candidate sources of resistance to PPV for peach. Two most resistant hybrids 'Cadaman' (*P. persica* x *P. davidiana*) and GF 677 (*P. persica* x *P. amygdalus*) were crossed with the peach cultivar 'Cresthaven' susceptible to PPV. Seeds resulting from the crosses were stratified and after that sown in a greenhouse. The subsequent seedlings were placed in an insect-proof screen house and inoculated with a chip-bud from the apricot infected with PPV (D strain). Symptoms of sharka infection were visually evaluated on peach leaves. At the same time ELISA was applied to the leaves to verify the presence or absence of the virus in seedlings. PPV infection was evaluated over two consecutive growth periods. Almost every hybrid plant showed sharka symptoms on the leaves and positive ELISA reactions. The intensity and extent of symptoms differed among plants in both progenies. There was a higher proportion of plants with severe symptoms (81%) in the 'Cresthaven' x GF 677 progeny compared with the other one, while substantially more plants with mild symptoms (41%) were found in the 'Cresthaven' x 'Cadaman' progeny. Only one plant in the progeny 'Cresthaven' x 'Cadaman' and two plants in the progeny 'Cresthaven' x GF 677 did not show symptoms and were negative in ELISA test. The plants without symptoms and negative ELISA test and plants with mild symptoms are candidates for exploiting in the breeding programme. These are preliminary results and the evaluations are being continued. This research was supported by the grant MZE0002700603 from the Ministry of Agriculture of the Czech Republic.

(P5-3) Resistance durability of *rymv-1* alleles against *Rice yellow mottle virus*

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Rice yellow mottle virus (RYMV; genus *Sobemovirus*) is a single stranded, uncapped, positive sense RNA virus. RYMV is present in all rice-growing African countries where it causes high yield losses. Two *Oryza sativa indica* cultivars Gigante and Bekarosaka and a few *O. glaberrima* cultivars Togs expressed high resistance towards RYMV. The recessive resistance gene *rymv*1 towards RYMV was identified and encodes eIF(iso)4G (Albar et al, 2006, *Plant Journal* 47: 417-426). Several alleles of resistance namely *rymv*1-2*, rymv*1-4 and *rymv*1-3 were characterized by single substitutions or a short deletion, respectively, in the middle domain of the protein. However, the high resistances could be overcome. The breakdown of the resistance allele *rymv*1-2 was due to punctual mutations restricted in VPg (viral protein genome-linked) (Pinel-Galzi et al, 2007, *PLoS Pathogens* 3: e180). The major residue involved in this resistance breakdown is located in the central -helix of VPg at position 48. This site is polymorphic in resistant-breaking variants. Several mutational pathways were revealed, the major mutation occurred after two successive substitutions at the same codon. The durability of the allele *rymv*1-3 was evaluated. Twenty isolates were inoculated to 10-50 *rymv*1-3 resistant plants. Plants detected as positive by ELISA with anti-RYMV polyclonal antibodies were submitted to a total RNA extraction. RT-PCR with specific primers were performed to amplify the VPg. Sequences of the resistant-breaking variants were compared to sequences of the wild-type isolates to identify the mutated sites. Infected resistant plants were submitted to sequencing at different time to follow the evolution of the RYMV population. The mutational pathways taken by RYMV to overcome the two resistant alleles were compared.

(P5-4) Application of RNA silencing technology for the generation of transgenic plants resistant to *Plum Pox Virus* (PPV)

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PPV is an insect-borne pathogen that affects *Prunus* species, reducing marketability and crop yields. To evaluate resistance persistence of successive generations of transgenic lines to PPV infection, the hairpin-mediated RNA silencing technology has been applied in the herbaceous model species *Nicotiana benthamiana*. A synthetic gene able to produce dsRNA derived from the virus genome and a viral sense RNA producing gene have been constructed and transferred to *Agrobacterium* binary vector pART27 for plant transformation. For the synthetic hairpin gene, a *ca.* 500 bp region, spanning the conserved 3' prime end of the RNA dependent RNA polymerase of a greek PPV isolate, was combined in antisense orientation with a 1444 bp spacer DNA and a *ca.* 1500 bp long PPV cDNA in sense orientation, under the transcriptional control of the *Cauliflower Mosaic Virus* 35S promoter. Following transcription, a double-stranded RNA molecule with regions specific to PPV of about 500 bp length is formed, that may trigger downregulation of the NIb gene *via* post transcriptional gene silencing. For the sense gene, the same region of *ca.* 1500 bp spanning the NIb gene was inserted also under the control of CaMV35S. *N. benthamiana* plants have been transformed with *Agrobacterium* harbouring the hairpin and the sense PPV genes. Several independent transformant lines for both plasmids were isolated and tested for resistance to PPV at the T0, T1 and T2 generation.For the sense PPV RNA gene, plants from 4 different transformant lines showed no or very mild symptoms at the T0 generation (verified by DAS-ELISA) but none line remained resistant at the T1 generation. From the 41 transformed lines with the hairpin construct, several lines (T0) contained plants which did not have symptoms two to six months post infection with PPV. Some of these lines were selected for analysis at the T1 generation (16 resistant, 1 susceptible as control). Segregation ratio of the transgenes in these lines was first evaluated after germination on kanamycin containing agar plates. From these, seven lines (6 multiple locus and one single locus), were randomly selected for further analysis of the T1 generation. Five plants of the T1 generation were tested from each line, which could possibly show segregation of the phenotype. Four out of these seven lines showed complete resistance at the T1 generation, as judged by symptoms appearance and DAS-ELISA assays. Symptoms on susceptible T1 plants appeared 8 dpi, like in wild type plants, while resistant plants remained symptomless. Evaluation of resistance persistence has been conducted on the T2 generation by challenging the plants with PPV-M and PPV-D virus strains. The majority of the lines analyzed were resistant to the virus. The resistance was strong and not strain-specific. Phenotypic data were also in accordance with the ELISA readings. Although plants were prior selected on kanamycin containing agar plates, in one of the resistant lines both resistant and susceptible T2 individuals were found, following mechanical inoculation with PPV-M at the 5-leaf stage. All plants in this line remained resistant when inoculated with PPV-D. No phenotypic abnormalities were observed in the transgenic plants. Symptoms on all plants of the susceptible lines tested at the T2 generation appeared 8 dpi, like in wild type plants, and this phenotype was verified by a DAS-ELISA assay. Resistant plants remained symptomless throughout the testing period (2 months). To conclude, application of the RNA silencing hairpin technology in *Prunus* species could provide a novel and agricultural sustainable approach to obtain PPV-resistant plants.

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Summary Programme

Time	Sunday Nov. 23	Monday Nov. 24	Tuesday Nov. 25	Wednesday Nov. 26	Thursday Nov. 27
8:00		Registration opening	Registration opening,	Registration opening	
9:00 - 11:00		Symposium I: **Important/emergent plant virus diseases**	Symposium III: **Plant responses to viruses and/or their vectors**	Symposium IV (cont.): **Innovative approaches, including transgenic resistance, to control plant viruses and vectors**	*Excursion*
11:00 – 11:30		Coffee break	Coffee break	Coffee break	
11:30 – 13:30		Symposium II: **Factors required for virus multiplication and spread**	Symposium III (cont): **Plant responses to viruses and/or their vectors**	Symposium III (cont): **Plant responses to viruses and/or their vectors**	
13:30 – 15:30		Lunch	Lunch	Lunch	Lunch
15:30 – 17:30	Registration opening	Symposium II (cont.): **Factors required for virus multiplication and spread**	Symposium IV: **Innovative approaches, including transgenic resistance, to control plant viruses and vectors**	Symposium V: **Resistance Durability**	
17:30 – 18:00		Coffee break	Coffee break	Coffee break	
18:00 – 19:30	Presentation of the ResistVir project Welcome Reception	Poster Session I	Technology Transfer Round Table	Poster Session II	
20:00	**Social Evening**	**Social Evening**	**Social Evening**	**Banquet dinner**	