



## ISVDOP 13

The 13<sup>th</sup> International Symposium  
on Virus Diseases of Ornamental Plants  
Norway, June 24 - 29<sup>th</sup>, 2012

# ISVDOP 13

The 13<sup>th</sup> International Symposium  
on Virus Diseases of Ornamental Plants  
Norway, June 24-29<sup>th</sup>, 2012

## Book of abstracts

Editors:

Dag-Ragnar Blystad and Carl Spetz

Our Sponsors:



AUST-AGDER  
FYLKESKOMMUNE



Mester Grønn



UGLAND  
HOLDING



LOEWE®



The publisher of Bioforsk FOKUS is:  
Bioforsk, Frederik A. Dahls vei 20, 1432 Ås, Norway  
post@bioforsk.no

Editor in charge: Director of Research Nils Vagstad  
Current issue: Bioforsk Plant Health and Plant Protection Division  
Local editor: Director Arne Hermansen  
Scientific editors: Carl Spetz and Dag-Ragnar Blystad

Bioforsk FOKUS  
Vol 7 nr 9 2012  
ISBN: 978-82-17-00944-3  
ISSN 0809-8662

Revised edition, page 48 introduced on june 25

Cover photo: Dag-Ragnar Blystad, ed. E. Fløistad  
Repro & print: Kursiv Media AS

This issue can be ordered from:  
Bioforsk Plantehelse, Høgskoleveien 7, 1432 Ås, Norway  
plantehelse@bioforsk.no  
Price: 150 NOK

[www.bioforsk.no](http://www.bioforsk.no)

# Preface

Dear friends,

It is my pleasure to welcome you to the 13th International Symposium on Virus Diseases of Ornamental Plants and to Norway. I can assure you that the organizing committee has worked very hard not only to make this meeting scientifically interesting, but also making it a social experience you will never forget!

During this meeting we will listen to many talks ranging from the applied virology, such as detection methods and diagnostics, to the more research oriented. In addition, we are honored to have a great selection of guest speakers who will enlighten us in many aspects of plant virology.

Our meeting will be opened by a plenary talk given by Dr. Robert Owens, who will take us through the journey on the discovery of one of the most amazing plant pathogens, viroids. Subsequently, as part of the "Virus-Host Interaction" section, Dr. Eugene Savenkov will give us an insight on how virus-encoded protein can interfere with plant development whereas Dr. Sek Man Wong will tackle the role of microRNAs in virus resistance.

On Tuesday morning, Dr. Qiaochun Wang will tell us about the latest advances in the use of "cryotherapy" to generate virus-free material, whereas on Thursday morning we will hear two interesting talks by Dr. Jan Kreuze and Dr. Holger Jeske on the use of deep sequencing as a method to detect and discover viruses.

Finally, on Friday morning Dr. Abed Gera will give us an overview on the detection and identification of viruses in ornamental production. I assure you that all these talks will be worth listening to.

As I mentioned previously, this meeting is not only about science, but also a social event in which we "virologists" can mingle. Therefore, we have included excursions so you can admire the wonderful Norwegian landscape, a conference dinner where you will taste the best of Norwegian cuisine and also a visit to Norway's best known beer microbrewery.

Finally, on behalf of the organizing committee (Kari Munthe, Sissel Haugslie, Erling Fløistad, Carl Spetz and myself), welcome to Norway!

Dag-Ragnar Blystad

# Table of Contents

## Abstracts of presentations

Severe diseases induced by viruses and phytoplasmas in <i>Hydrangea</i> in Italy .....	8
A. Bertaccini, S. Paltrinieri, N. Contaldo, N. Mori, L. Cavicchi, & M.G. Bellardi	
The effect of <i>Kalanchoë mosaic virus</i> on growth and development on four cultivars of <i>Kalanchoë blossfeldiana</i> .....	9
L. Bjørke, K. Willumsen, G. Skjeseth, S. Haugslie & D.-R. Blystad	
Certification scheme for mass propagation of specific virus free <i>Phalaenopsis</i> orchid plantlets in Taiwan .....	10
C.-A. Chang	
Occurrence of pospiviroid species in The Netherlands from 2006 up to 2011 .....	11
J.Th.J. Verhoeven, M. Botermans, E.T.M. Meekes, & J.W. Roenhorst	
Important serological and nucleic acid tools for prompt diagnosis and identification of diseases caused by thrips-borne tospoviruses.....	12
T.-C. Chen, Y.-C. Kang, Y.-C. Yeh, J.-T. Li, J.-S. Weng, W.-T. Tsai, Y.-P. Lin, & S.-D. Yeh	
Production of compact poinsettia without phytoplasma and chemical spray .....	13
J.L. Clarke, M.A. Islam, S. Haugslie, S.K. Rasmussen, H. Lütken, & D.-R. Blystad	
Identification and molecular characterization of multiple phytoplasma infection in <i>Spartium junceum</i> and <i>Cytisus scoparius</i> .....	14
N. Contaldo, S. Paltrinieri, S. Ardizzi, B. Duduk, A. Bertaccini, & M.G. Bellardi	
First report of <i>Alstroemeria virus X</i> from <i>Xerochrysum bracteatum</i> in Norway .....	15
M.W. Dees, C. Spetz, & D.-R. Blystad	
Variability in <i>Alternanthera mosaic virus</i> isolates from different hosts.....	16
J. Hammond & M.D. Reinsel	
Progress in the development of a Universal Plant Virus Microarray for the detection and identification of viruses.....	17
J. Hammond, D.C. Henderson, B. Bagewadi, R.L. Jordan, K.L. Perry, U. Melcher, D. Wang, K. Fischer, & C.M. Fauquet	
Circomics of geminiviruses and their satellite DNAs .....	18
H. Jeske, B. Krenz, T. Paprotka, S. Strohmeier, & P.S. Wyant	
Detection and molecular characterization of two distinct closteroviruses infecting carnation .....	19
R. Jordan, M.A. Guaragna, V. Dolja, & A. Karasev	
Witches' brooms of coniferous plants as a source of phytoplasma infection.....	20
M. Kamińska & H. Berniak	
siRNA sequencing and assembly: a universal tool for identification and diagnostics of viruses .....	21
J.F. Kreuze	
AmplifyRP™, a novel rapid isothermal nucleic acid amplification platform, brings the molecular lab to the greenhouse .....	22
J. Lamborn	
Detection of pospiviroids in the Czech Republic and their discrimination by restriction analysis .....	23
H. Orságová, G. Schlesingerová, & M. Dziaková	



Identification of <i>Dasheen mosaic virus</i> in <i>Anthurium andraeanum</i> by Immune Precipitation Polymerase Chain Reaction in Brazil .....	24
J.A.A. Lima & A.K.Q. Nascimento	
Use of an improved RT-PCR/RLFP method for <i>Pospiviroid</i> detection and differentiation in ornamental plants in Italy .....	25
M. Luigi, E. Costantini, D. Luison, P. Mangiaracina, L. Tomassoli, & F. Faggioli	
Facilitative and antagonistic interactions among pospiviroids infecting solanaceous ornamentals .....	26
D. Luison, P. Mangiaracina, E. Costantini, M. Luigi, F. Faggioli, & L. Tomassoli	
A carlavirus infecting ornamental plants encodes a zinc-finger protein that acts as a plant transcription factor .....	27
N.I. Lukhovitskaya, A.D. Solovieva, A.G. Solovyev, & E.I. Savenkov	
Investigation on the phytosanitary status of major ornamental hibiscus species in Italy to assess virus infection .....	28
A. Manglli, S. Adkins, A. Tiberini, & L. Tomassoli	
A sensitive real-time RT-PCR assay for generic detection of pospiviroids.....	29
E.T.M. Meekes, M. Hooftman, R. Dekter, M. Botermans, J.Th.J. Verhoeven, J.W. Roenhorst, & B.T.L.H. van de Vossenber	
Emerging and new viruses in ornamentals in Germany .....	30
W. Menzel, J. Hamacher, & S. Winter	
Preliminary survey and study of the CymMV variability in orchids of Sao Paulo state, Brazil .....	31
L.A. Moraes, M.A. Pavan, & R.K. Sakate	
Detecting pospiviroids in ornamentals: a new, non-degenerate and highly sensitive PCR approach .....	32
J. Lamborn & D. Groth-Helms	
Viroid discovery - past achievements, future prospects.....	33
Robert A. Owens	
Changes in tomato gene expression during <i>Potato spindle tuber viroid</i> infection reveal a complex array of changes affecting hormone signaling.....	34
R.A. Owens, K.B. Tech, J.Y. Shao, T. Sano, & C. J. Baker	
First report of phytoplasmas associated with <i>Erysimum linifolium</i> L. stunting.....	35
S. Paltrinieri, N. Contaldo, A. Bertaccini, L. Cavicchi, & M.G. Bellardi	
Carlavirus detection 2008 to 2011 in Germany using electron microscopy .....	36
K. Richert-Poeggeler, S. Schuhmann, C. Maass, K.C. Eastwell, R.R. Martin, & B. Lockhart	
Electron microscopic studies of vegetative and generative tissues from <i>Hosta virus X</i> infected hosta .....	37
K. Richert-Poeggeler, U. Commandeur, K. Kobayashi, C. Maass, & B. Lockhart	
Protective mechanism of gentian plants pre-inoculated with a satellite RNA-containing attenuated <i>Cucumber mosaic virus</i> (CMV) strain against virulent CMV .....	38
H. Sayama, N. Takayanagi, M. Yamada, M. Kominato, H. Atarashi, & T. Hikage	
A microarray system for multiplex virus detection in ornamentals.....	39
C.F. Tsai, Y.Y. Lin, S.-C. Lu, & C.A. Chang	
A membrane-binding conserved motif in the coat protein of PnMV seems to mediate chloroplast targeting .....	40
C. Spetz & D.-R. Blystad	

Preliminary description of a new viroid species from dahlia.....	41
J.Th.J. Verhoeven, J.W. Roenhorst, E.T.M. Meekes, R. Flores, & P. Serra	
Occurrence of <i>Capsicum chlorosis virus</i> on ornamental crops in Taiwan .....	42
C.-C. Chen, C.-A. Chang, S.-D. Yeh, & T.-C. Chen	
Virus eradication by cryotherapy of <i>in vitro</i> -grown shoot tips .....	43
R. Wang, W. Bi, B. Li, Z. Zhang, Z. Cui, C. Feng, B. Wang, Z. Yin, & Q. Wang	
The role of artificial microRNAs in virus resistance .....	44
S.M. Wong	

## Astracts of Posters

The application of ribavirin for elimination of viruses in lily plants growing <i>in vitro</i> .....	46
D. Sochacki, E. Gabryszewska, & M. Kamińska	
Detection of mixed virus population in freesia plants with necrotic disease .....	47
A.M. Vaira, M. Vallino, R. Lenzi, V. Masenga, D. Salvi, & J. Hammond	
Molecular evaluation of the principal <i>Ranunculus asiaticus</i> L. viruses	
E. Sacco, C. Borghi, M. Laura, V. Bobbio, M. Rabaglio, M. Kawka, A.M. Vaira, & B. Ruffoni .....	48

## Abstracts of presentations



# Severe diseases induced by viruses and phytoplasmas in *Hydrangea* in Italy

A. Bertaccini<sup>1</sup>, S. Paltrinieri<sup>1</sup>, N. Contaldo<sup>1</sup>, N. Mori<sup>2</sup>, L. Cavicchi<sup>3</sup>, & M.G. Bellardi<sup>1</sup>

<sup>1</sup>DiSTA - Plant Pathology, *Alma Mater Studiorum*, University of Bologna, Via G. Fanin, 42, 40127 Bologna, Italy

<sup>2</sup>Dipartimento di Agronomia ambientale e Produzioni vegetali, Università degli Studi di Padova, Viale dell'Università, 16, 35020 Legnaro (Padova), Italy, <sup>3</sup>Plesso Didattico G. Scarabelli (Imola), *Alma Mater Studiorum*, University of Bologna, Via Ascarì, 17, 40026 Imola (Bologna), Italy  
assunta.bertaccini@unibo.it

In the last decade the economic importance of hydrangea diseases associated with virus and phytoplasmas in Italy increased therefore epidemiological surveys were carried out in Liguria and Lazio regions in 2010 and 2011. To detect virus infections mechanical inoculations on herbaceous plants, electron microscope observations of “leaf-dip” preparations, serology (PAS-ELISA and ISEM techniques) were employed; to verify phytoplasmas presence and to determine their identity, samples were tested by nested-PCR, followed by RFLP analyses on 16S ribosomal gene. In 2011, hydrangea plants showings stunting, flower virescence and phyllody, yellowing, necrosis and redness of the leaf edge, were collected in two commercial greenhouses of “Piana di Albenga” (Liguria region). All samples (belonging to three hybrids) were infected by *Hydrangea ringspot virus* (HRSV). One of these hydrangea plants, showing also flower virescence and red edges of leaves, was infected by phytoplasmas belonging to group 16SrI-B (‘*Candidatus* Phytoplasma asteris’). Further RFLP characterization of this phytoplasma strain on the GroEL gene with *Tru11* and *Alu1* allow assigning it to GroELI subgroup III, reported so far on several European Countries. During inspections performed in the biennium 2010-2011, severe virus-like symptoms were observed in almost all hydrangea plants growing in Bolsena city (Lazio region) such as stunting, leaf and flower malformations, mosaic, chlorotic and necrotic mottle, colour-breaking on petals. Virological tests revealed the presence of *Cucumber mosaic virus* (CMV) in three plants characterized by stunting, leaf mosaic in malformed leaves and flower colour-breaking. The presence of *Elm mottle virus* (EMoV; syn. *Hydrangea mosaic virus*) in two plants with symptomatic leaves was also detected. Mechanical transmission of viruses from hydrangea was obtained in *Gomphrena globosa*

with all the identified viruses but EMoV; this latter induced systemic infection in *Chenopodium quinoa*, however only serological analyses allow to identify the diverse viruses.

In 2011 one plant showing growth reduction, flower virescence and phyllody, and with asymptomatic leaves was found; molecular analyses allow to identify the presence of phytoplasmas belonging to ribosomal subgroup 16SrXII-A “stolbur”. Further strain characterization carried out on *stamp* and *tuf* genes confirmed the presence of “stolbur” phytoplasmas. RFLP analyses with *Tru11* on *stamp* gene show that the strain infecting hydrangea belongs to one of the two groups differentiable in “stolbur” phytoplasmas in Southern Europe. Leafhoppers present on this latter phytoplasma-infected plant were caught and identified as *Laodelphax striatellus*, *Anaceratogallia* sp., *Empoasca decipiens*, *Empoasca* sp., *Asymmetrasca decedens*, *Zyginidia pullula*. DNA from identified specimens maintained in 100% ethanol was extracted and tested by nested-PCR/RFLP analyses on 16S ribosomal gene and *tuf* genes. ‘*Ca. P. asteris*’-related phytoplasmas were identified in *L. striatellus* while “stolbur” (16SrXII-A) phytoplasmas were present in *Anaceratogallia* spp.

CMV and EMoV have been detected in this species for the first time in Italy; in addition, until now, only phytoplasmas belonging to subgroup 16SrI-B (aster yellows) have been found infecting hydrangea in Italy and worldwide. However 16SrXII-A phytoplasmas were only reported in hydrangea in Bulgaria more than 15 years ago in mixed infection with aster yellows, this is therefore the first confirmation of stolbur phytoplasma presence associated with virescence of *H. macrophylla*.

# The effect of *Kalanchoë mosaic virus* on growth and development on four cultivars of *Kalanchoë blossfeldiana*

L. Bjørke<sup>1,2</sup>, K. Willumsen<sup>2</sup>, G. Skjeseth<sup>2</sup>, S. Haugslie<sup>1</sup>, & D.-R. Blystad<sup>1</sup>

<sup>1</sup>Bioforsk, Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, N-1432 Ås,

<sup>2</sup>Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, PO Box 5003, N-1432 Ås, Norway  
dag-ragnar.blystad@bioforsk.no

*Kalanchoë blossfeldiana* has increased in popularity in Norway the last 20 years and has become one of the most important flowering pot plants. One of the viruses causing harm to this plant is *Kalanchoë mosaic virus* (family *Potyvirus*, genus *Potyvirus*). This virus typically produces uneven green color in the leaves often describes as “green islands”, curling of the leaves and reduced growth. However, little is known about the quantitative effect of KMV on the growth and development of this ornamental plant.

An investigation was undertaken to determine the effect of KMV both on the production and quality of cuttings and on the effect of virus infection on the development of the whole plant.

Four cultivars were chosen: Charm, Goldstrike, Goldstrike dk and Debbie. Healthy, virus-free plant material was obtained from the Norwegian nuclear stock program. Half of the material was graft-inoculated with KMV. In this way we established both virus-free and virus-infected mother stocks of all four cultivars. All mother stocks were cultivated according to the grower's schemes for an optimal production of cuttings.

The results of two independent experiments showed that KMV infection gave on average 21% and 16% reduction in fresh weight of the cuttings for the four cultivars. The cultivar ‘Charm’ was clearly the most sensitive cultivar, having a loss of fresh weight of the cuttings of 31% and 41%. In addition, the number of cuttings was reduced by KMV-infection. On average there was a reduction of 9.1% in experiment I and 9.9% in experiment II.

The growth of plants intended for production of flowering pot plants was measured three times during the production period: when rooted cuttings were potted, at the start of short-day treatment and at the flowering stage. At the two first measurements early in the production period, there were differences parallel to what was observed in the production of cuttings. However, when the plants were at the flowering stage there was on average no statistical difference in fresh weight between KMV-infected and KMV-free, but the KMV-free plants flowered on average 4-5 days before the KMV-infected plants.

# Certification scheme for mass propagation of specific virus free *Phalaenopsis* orchid plantlets in Taiwan

C.-A. Chang

Institute of biochemical sciences and technology, Chaoyang University of Technology, Taichung, Taiwan, Republic of China  
cachang@cyut.edu.tw

*Phalaenopsis* orchids are the most important ornamental crops produced in Taiwan. More than one hundred million US dollars of output value has been created annually and consistently by the Taiwanese *Phalaenopsis* exportation industry since 2000. Young plantlets or those in tissue culture flasks are the major item for exportation. Virus incidence is therefore considered the vital criterion evaluating quality of orchid plantlets. In order to promote the competitiveness of *Phalaenopsis* business, Taiwan government has implemented a voluntary virus certification system for *Phalaenopsis* plantlets since 2006. The system encourages *Phalaenopsis* nurseries and companies to follow the certification schemes before their plantlets can be certified as virus free. The key requisition for the system is to establish a restricted area to maintain virus free mother stocks. Only those *Phalaenopsis* mother plants certified as non-infected by viruses are allowed to be maintained in the area and further propagated by tissue culture. Any detected infected plants should be removed immediately from the restricted area. Nurseries once confirmed to violate this regulation will be flunk from the further

certification process. Necessary hygiene procedures to prevent orchid propagating materials from virus re-infection are recommended and regulated by this certification system. Only those applicants confirmed to follow the hygiene regulations are further processed for virus indexing on their propagating materials. Check points are designed and implemented during tissue culture propagation stages. Propagating young plantlets are sampled by inspectors and sent to virus detection center to perform virus indexing using ELISA or RT-PCR. Whenever virus infection is confirmed on any stages during propagation the further certification process will be terminated. Propagated plantlets ready to sell will be confirmed finally by virus indexing process before authorizing the certification. As this certification is a voluntary system instead of being enforced by law, not all *Phalaenopsis* nurseries in Taiwan have applied for certification since its operation in 2006. However, the principles and protocols for producing virus free *Phalaenopsis* plantlets are accepted by most orchid nurseries and applied in their own production system.

# Occurrence of pospiviroid species in The Netherlands from 2006 up to 2011

J.Th.J. Verhoeven<sup>1</sup>, M. Botermans<sup>1</sup>, E.T.M. Meekes<sup>2</sup>, & J.W. Roenhorst<sup>1</sup>

<sup>1</sup>National Plant Protection Organization, P.O. Box 9102, 6700 HC Wageningen, The Netherlands, <sup>2</sup>Naktuinbouw, P.O. Box 40, 2370 AA Roelofarendsveen, The Netherlands

j.th.j.verhoeven@minlnv.nl

To find potential sources of infection for previous pospiviroid outbreaks in tomatoes, the occurrence of pospiviroids in ornamental plants was surveyed in the Netherlands from 2006 up to 2011. In the first year *Potato spindle tuber viroid* (PSTVd) was found in *Brugmansia* spp. and *Solanum jasminoides*. Subsequent tracing of infections revealed many PSTVd-infected lots for both crops. All infected lots were destroyed. New infections were prevented by an obligatory plant passport system put in place by the European Commission, which included the use of tested of mother plants. Consequently, PSTVd has almost been eradicated in these crops and was only found infrequently since 2007. Besides PSTVd *Tomato apical stunt viroid* (TASVd) and *Citrus exocortis viroid* (CEVd) were found in *Cestrum* sp. and *Verbena* sp. in 2006. During the following years, the number of infections by both viroids increased, probably because destruction of infected plants was not obligatory. TASVd was identified additionally in *Brugmansia* sp.,

*Lycianthes rantonnetii*, *S. jasminoides* and *Streptosolen jamesonii*. In 2011 this viroid was found in many lots of *S. jasminoides*, in which it has replaced PSTVd as the most prevalent viroid. In addition, TASVd also was found in ca. 200 tomato plants in a single greenhouse in the Netherlands. The genotype of this TASVd isolate was identical to that found most frequently in ornamentals. This indicates that the outbreak in tomatoes originated from an ornamental species, and - like for PSTVd - shows again that ornamentals indeed may be sources of infection for tomato crops (Verhoeven *et al.* 2010).

## Reference

Verhoeven, J.Th.J., Jansen, C.C.C., Botermans, M., & Roenhorst, J.W. 2010. Epidemiological evidence that vegetatively propagated, solanaceous plant species act as sources of *Potato spindle tuber viroid* inoculum for tomato. *Plant Pathology* 59:3-12.

# Important serological and nucleic acid tools for prompt diagnosis and identification of diseases caused by thrips-borne tospoviruses

T.-C. Chen<sup>1</sup>, Y.-C. Kang<sup>2</sup>, Y.-C. Yeh<sup>1</sup>, J.-T. Li<sup>1,3</sup>, J.-S. Weng<sup>1</sup>, W.-T. Tsai<sup>2</sup>, Y.-P. Lin<sup>2</sup>, & S.-D. Yeh<sup>2</sup>

<sup>1</sup>Department of Biotechnology, Asia University, Wufeng, Taichung, <sup>2</sup>Department of Plant Pathology, National Chung Hsing University, Taichung, <sup>3</sup>Division of Pesticide Application, Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Wufeng, Taichung, Taiwan  
sdyeh@nchu.edu.tw

The thrips-borne tospoviruses are distributed worldwide and imperil the production of many crops including ornamentals. The serological and phylogenetic relationships of the S RNA-encoded nucleocapsid proteins (NPs) are key criteria for classification of a tospovirus species, and 22 species have been identified so far. Based on the serological relationships of tospoviral NPs, current tospovirus species are divided into four serogroups, with *Tomato spotted wilt virus* (TSWV), *Watermelon silver mottle virus* (WSMoV), *Iris yellow spot virus* (IYSV) and *Peanut yellow spot virus* (PYSV) as respective type members, and two distinct serotypes with monospecies of *Impatiens necrotic spot virus* (INSV) and *Soybean vein necrosis associated virus* (SVNaV). NP-specific polyclonal and monoclonal antibodies are commonly used for diagnosis of tospoviruses. However, the cross reactivity of polyclonal NP antibodies limits their utilization in prompt identification of a specific tospovirus without ambiguity. Recently, a monoclonal antibody (MAB) to the NSs protein of WSMoV, another S RNA-encoded protein, broadly reacting with the whole members of the WSMoV- and IYSV-serogroups, was produced in our laboratory. The same approach has been applied to

produce a specific MAB against the common epitope of the NSs proteins of all TSWV serogroup members. The mixture of the two MABs can be used to conveniently detect most tospoviruses by ELISA and immunoblotting. Using these two NSs MABs coupled with species-specific antisera or NP MABs can promptly identify a specific tospovirus without ambiguity. On the other hand, through the analyses of all available genomic sequences of tospoviruses in data base, the universal primer pairs designed from the highly conserved domains of L RNA or M RNA can be used to detect tospoviruses in one-step reverse transcription-polymerase chain reaction (RT-PCR) at the genus level. Similarly, serogroup-specific or species-specific primers have also been developed for identification of a specific tospovirus. All amplified products can be cloned and sequenced for verification of a specific known or exploration of a new tospovirus. Taken together, the highly efficient and sensitive serological methods based on tospoviral NPs and NSs proteins and the accurate RT-PCR methods based on specific primers are important tools for prompt diagnosis and identification of tospoviruses.

# Production of compact poinsettia without phytoplasma and chemical spray

J.L. Clarke<sup>1</sup>, M.A. Islam<sup>1,2</sup>, S. Haugslien<sup>1</sup>, S.K. Rasmussen<sup>3</sup>, H. Lütken<sup>3</sup>, & D.-R. Blystad<sup>1</sup>

<sup>1</sup>Bioforsk, Norwegian Institute for Agricultural and Environmental Research, Ås 1432, Norway, <sup>2</sup>Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, Ås 1432, Norway, <sup>3</sup>Copenhagen University, Faculty of Life Sciences, Frederiksberg C, Denmark

jihong.liu-clarke@bioforsk.no

Ornamental industry is one of the fast growing industries worldwide, especially in Japan and China. Global production of ornamental potted plants and cut flowers comprises about 50 billion €, corresponding to an estimated global consumer consumption between 100 and 150 billion €. Appropriate plant height is one of the most important traits in many ornamental potted plants, e.g. poinsettia. Poinsettia (*Euphorbia pulcherrima* Willd. Ex Klotzsch), a non-food and non-feed ornamental plant, is a contemporary symbol of Christmas in most parts of the world. Poinsettia has become very popular and economically highly significant as a potted flower in North America, Europe, Asia, Australia and different parts of the world. As a potted ornamental plant, compact plant production will have more qualitative criteria and also for aesthetic value. The dwarf characteristics in poinsettia induced by the Poinsettia branch inducing phytoplasma can be obtained by using the free branching cultivars,

and enhanced by spraying growth retardants such as CCC (chlormequat) or Alar (daminozide) that inhibits the biosynthesis of the plant hormone gibberellin (GA). Growth retardants are expensive, time consuming and have negative impact on human health as well as the environment and in the near future, the growth regulators will be banned in EU countries. Breeding for compact growth of poinsettia is thus of importance. Traditional plant breeding approach is time consuming and inefficient due to the heterozygous genetic background of poinsettia. In this context, genetic engineering is an important alternative to conventional breeding. This is because the transgenic plants with new traits and genetic framework remain unchanged due to vegetative propagation. This is considered a more straightforward and effective approach; and is increasingly adopted. Production of compact poinsettia without phytoplasma and chemical spray by transgenic approach will be presented in this talk.



# Identification and molecular characterization of multiple phytoplasma infection in *Spartium junceum* and *Cytisus scoparius*

N. Contaldo<sup>1</sup>, S. Paltrinieri<sup>1</sup>, S. Ardizzi<sup>1</sup>, B. Duduk<sup>2</sup>, A. Bertaccini<sup>1</sup>, & M.G. Bellardi<sup>1</sup>

<sup>1</sup>DiSTA - Plant Pathology, *Alma Mater Studiorum*, University of Bologna, Via G. Fanin, 42, 40127 Bologna, Italy, <sup>2</sup>Institute of Pesticides and Environmental Protection, Banatska 31b, Belgrade, Serbia  
assunta.bertaccini@unibo.it

Two genera of the fabaceae family showed phytoplasma symptoms in different locations in Europe i.e. Germany and Italy. *Spartium junceum* L. (Spanish broom) is a deciduous shrub with dark green, round stems and alternate leaves; inflorescences are terminal clusters of several bright yellow somewhat fragrant flowers. This ornamental shrub is frequently spontaneously growing especially in southern Italy where it is affected by spartium witches' broom (SpaWB) disease, characterized by proliferation of axillary buds and stem fasciation. Two different phytoplasmas have been associated to SpaWB: '*Candidatus* Phytoplasma spartii' (group 16SrX-D) and a phytoplasma belonging to elm yellows group (16SrV-C). Both were reported associated with SpaWB in Italy while only '*Ca. P. spartii*' was reported in Spain. In the spring of 2011, typical SpaWB symptoms were observed in a plant up to 2 m tall growing in the city of Ercolano (Campania region, Italy). A similar symptomatology was observed in a group of shrubs of *Cytisus scoparius* (L) syn. *Sarothamnus scoparius*, better known as common broom or scotch broom growing in Dahlem botanical garden in Berlin (Germany). This is a perennial shrub native to western and central Europe, but it is considered invasive plant in areas such as North America and New Zealand.

Symptomatic and asymptomatic samples were collected in both cases: five samples of *C. scoparius* and

two of *S. junceum* were analysed for phytoplasma presence by nested-PCR assays employing primer pairs P1A/P7A followed by F1/B6 and R16F2n/R2, phytoplasma identification was achieved by RFLP analyses with *Tru1l* on the two latter amplicons. Further confirmation of phytoplasma identity was achieved by nested-PCR assays with primers specific for phytoplasma groups 16SrI, 16SrV and 16SrX. All symptomatic samples produced amplicons of the expected lengths and no product was amplified from asymptomatic plants and using 16SrV specific primers.

Identification and classification of phytoplasmas allow to detect '*Ca. P. spartii*' subgroup 16SrX-D and '*Ca. P. asteris*' subgroup 16SrI-B in both genera. In some of the samples of *C. scoparius* also stolbur phytoplasmas were identified. Further phytoplasma characterization was carried out on *tuf* gene using a cocktail primers mix that was able to amplify phytoplasmas identified as '*Ca. P. asteris*' in *S. junceum* and phytoplasmas showing two different *Tru1l* profiles in *C. scoparius* from Germany that are not present in any published RFLP profile on this gene. Direct amplicon sequencing is in progress in order to verify possible affiliation to '*Ca. P. spartii*' group since the only available sequences of this gene on phytoplasmas are deposited in Qbank since they were obtained from the Qbol EU project.

# First report of *Alstroemeria virus X* from *Xerochrysum bracteatum* in Norway

M.W. Dees<sup>1,2</sup>, C. Spetz<sup>1</sup>, & D.-R. Blystad<sup>1</sup>

<sup>1</sup>Bioforsk, Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, N-1432 Ås, Norway, <sup>2</sup>Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, PO Box 5003, N-1432 Ås, Norway

dag-ragnar.blystad@bioforsk.no

Strawflower (*Xerochrysum bracteatum*, syn. *Helichrysum bracteatum*, syn. *Bracteantha bracteata*) is grown as an ornamental and used as dried flower. A virus (isolate HeLE91-1) was isolated from leaves of the *Xerochrysum bracteatum* with mottle symptoms in Norway. Plant sap was examined by electron microscopy. Preliminary results revealed a flexuous virus with a dominant particle length of ~500 nm, identifying it as a putative member of the genus *Potexvirus*. HeLE91-1 infected the test plant *Chenopodium quinoa* systemically.

Molecular and biological methods were further used to determine the coat protein (CP) sequence and the experimental host range of isolate HeLE91-1. Analysis of the nucleotide sequence of the putative CP revealed that it consisted of 693 nucleotides including

the termination codon UAA. Results from BLAST and sequence alignments showed that isolate HeLE91-1 had considerable homology to the Japanese isolate of *Alstroemeria virus X* (AlsVX) (79.8% nt-level, 90.9% aa-level).

The experimental host range of isolate HeLE91-1 was determined. Isolate HeLE91-1 infected plants in six out of nine families tested. The isolate was tested for seed transmission. One thousand seeds from the plant HeLE91-1 originated from were sown. Seeds were sown in groups of ten. The emerging 836 seedlings, on average 8.4 seedling per group of ten sown seeds were grouped, grinded in inoculation buffer and inoculated to *C. quinoa*. No plants became infected indicating no seed transmission of the virus.

# Variability in *Alternanthera mosaic virus* isolates from different hosts

J. Hammond & M.D. Reinsel  
USDA-ARS, USNA, FNPRU, Beltsville, MD 20705, USA  
john.hammond@ars.usda.gov

We have determined the complete genome sequences of *Alternanthera mosaic virus* isolate PA (AltMV-PA) and four infectious clone variants derived from AltMV-SP, as well as partial sequences of other isolates from various types of phlox, from portulaca, nandina, crossandra, and cineraria. Comparison of the amino acid sequences of the various gene products of these AltMV isolates and other AltMV sequences from GenBank reveals that there are two main groups of AltMV isolates. 'Phlox-type' isolates are differentiated from 'portulaca-type' isolates, and both are clearly distinguished from both the serologically-related *Papaya mosaic virus* (PapMV) and from *Potato virus X* (PVX).

A US isolate from Nandina is 'phlox-type', whereas isolates from hybrid annual phlox and from cineraria are 'portulaca-type'. Partial RdRp sequences are available for several other isolates; within this partial RdRp region a New Zealand isolate from Nandina is identical to AltMV-PA, whereas Brazilian isolates from torenia, and a 'portulaca-type' Florida isolate share several variant residues, one of which is also present in a Russian portulaca isolate. Many other residues differ between AltMV and both PapMV and PVX. These comparisons suggest that portulaca may have been the source of infection for most ornamentals other than perennial phlox species.

# Progress in the development of a Universal Plant Virus Microarray for the detection and identification of viruses

J. Hammond<sup>1</sup>, D.C. Henderson<sup>1</sup>, B. Bagewadi<sup>2</sup>, R.L. Jordan<sup>1</sup>, K.L. Perry<sup>3</sup>, U. Melcher<sup>4</sup>, D. Wang<sup>5</sup>, K. Fischer<sup>6</sup>, & C.M. Fauquet<sup>2</sup>  
<sup>1</sup>USDA-ARS, USNA, FNPRU, Beltsville, MD 20705, USA, <sup>2</sup>Danforth Plant Science Center, St. Louis, MO 63132, USA, <sup>3</sup>Cornell University, Ithaca, NY 14853, USA, <sup>4</sup>Oklahoma State University, Stillwater, OK 74078, USA, <sup>5</sup>Washington University, St. Louis, MO 63110, USA, <sup>6</sup>University of Utah, Salt Lake City, Utah 84112, USA  
john.hammond@ars.usda.gov

Microarrays based on oligonucleotides representing sequences conserved at the level of viral species, genera, and families are able to detect and identify both characterized and previously uncharacterized viruses infecting mammals and birds. Software initially developed for these animal virus microarrays has been further refined for both design and analysis of a Universal Plant Virus Microarray (UPVM). The UPVM is based on 9600 60-mer oligonucleotides, including at least four genus-level and four family-level probes per taxonomic group, and 44 control probes for highly conserved plant genes. These probes together represent all characterized plant viruses for which significant genomic sequence was publically available

in GenBank as of December 2009, and additional sequences made available to us prior to public GenBank release. Associated methods have been developed for high quality total nucleic acid extraction, applicable to a broad range of plant tissues containing metabolites such as phenolics, polysaccharides, latex, and resins that can interfere with nucleic acid extraction or subsequent amplification. Validation of the UPVM with a broad range of DNA and RNA plant viruses is in progress. Many high-titer viruses can be detected by direct labeling of total RNA extracts. Amplification and subtractive hybridization protocols to increase the sensitivity of detection of low titer viruses are being examined.

# Circomics of geminiviruses and their satellite DNAs

H. Jeske, B. Krenz, T. Paprotka, S. Strohmeier, & P.S. Wyant

Universität Stuttgart, Molekular Biologie und Virologie der Pflanzen, Pfaffenwaldring 57, D-70550 Stuttgart, Germany  
holger.jeske@bio.uni-stuttgart.de

Although geminiviruses (Jeske 2009) are generally important pathogens in agriculture, several cases exist where they enhance the beauty of ornamental hosts, such as *Abutilon mosaic viruses* in *Abutilon* species. Global virus transport in ornamental and medical plants needs careful diagnosis to prevent new epidemics. Identifying unknown viruses in this context is a major challenge for phytosanitary measures. Because geminiviruses encapsidate circular single-stranded DNAs, their genomes can be amplified easily and without any *a priori* knowledge of sequence or coat protein properties by use of rolling circle amplification (RCA), and can be characterized by restriction fragment length polymorphism (RFLP) (Habile *et al.* 2006) or direct sequencing of RCA products (Wyant *et al.* 2012, Jeske *et al.* 2010, Schubert *et al.* 2007). These diagnostic tools have been applied for various hosts, herbaceous and woody plants, for gene bank material, field crops and wild plants from all over the world (Wyant *et al.* 2011, Horn *et al.* 2011, Paprotka *et al.* 2010 a,b,c, Homs *et al.* 2008) with equal quality. In addition, the technique has been proven to be an excellent construction and detection method for basic research on silencing and gene expression (Paprotka *et al.* 2011, Krenz *et al.* 2010, 2011). Circomics (circular DNA genomics) is a novel approach which combines RCA/RFLP with high-throughput (pyro-)sequencing, which allowed a world-wide survey of geminiviral DNA and their DNA satellites (Wyant *et al.* 2012). This strategy will be explained and its limits discussed with reference to Brazilian and Cuban begomoviruses. The discovery of the first alpha-satellite DNAs in South- and Central America underscores the usefulness of Circomics to identify unexpected and unprecedented viral genome components.

## References

- Haible, D., Kober, S., & Jeske, H. 2006. *J. Virol. Methods* 135:9-16.
- Horn, J., Lauster, S., Krenz, B., Kraus, J., Frischmuth, T., & Jeske, H. 2011. *Virus Res.* 158:169 -178.
- Homs, M., Kober, S., Kepp, G., & Jeske, H. 2008. *Virus Res.* 136:124-129.
- Jeske, H. 2009. Geminiviruses. In: Torque Teno Virus: The Still Elusive Human Pathogens, H. zur Hausen, de Villiers, E.-M., Ed., Vol. 331:185-226. Springer, Berlin.
- Jeske, H., Gotthardt, D., & Kober, S. 2010. *J. Virol. Methods* 163:301-308.
- Krenz, B., Wege, C., and Jeske, H. 2010. *J. Virol. Methods* 169:129-137
- Krenz, B., Neugart, F., Kleinow, T., & Jeske, H. 2011. *Virus Res.* 161:194-197.
- Paprotka, T., Boiteux, L. S., Fonseca, M. E. N., Resende, R. O., Jeske, H., Faria, J. C., & Ribeiro, S. G. 2010. *Virus Res.* 149:224-233.
- Paprotka, T., Metzler, V., & Jeske, H. 2010. *Arch. Virol.* 155:813-816.
- Paprotka, T., Metzler, V., & Jeske, H. 2010. *Virology* 404: 148-157.
- Paprotka, T., Deuschle, K., Metzler V., & Jeske, H. 2011. *J. Virol.* 85:12001-12012.
- Schubert, J., Habekuß, A., Kazmaier, K., & Jeske, H. 2007. *Virus Res.* 127:61 -70.
- Wyant, P. S., Gotthardt, D., Schäfer, B., Krenz, B., & Jeske, H. 2011. *Arch. Virol.* 156:347-352 .
- Wyant, P. S., Strohmeier, S., Schäfer, B., Krenz, B., Assunção, I. P., de Andrade Lima, G. A., & Jeske, H. 2012. *Virology* 427:151-157.
- Wyant, P. S., Kober, S., Schwierzok, A., Kocher, C., Schäfer, B., Jeske, H., & Wege, C. 2012. *Virus Res.* in press.

# Detection and molecular characterization of two distinct closteroviruses infecting carnation

R. Jordan<sup>1</sup>, M.A. Guaragna<sup>1</sup>, V. Dolja<sup>2</sup>, & A. Karasev<sup>3</sup>

<sup>1</sup>Floral & Nursery Plants Research Unit, US National Arboretum, USDA-ARS, Beltsville, MD, USA; <sup>2</sup>Department of Botany & Plant Pathology, Oregon State University, Corvallis, OR, USA; <sup>3</sup>Department of Plant Soil and Entomological Sciences, University of Idaho, Moscow, ID, USA

Ramon.Jordan@ars.usda.gov

Carnation necrotic fleck disease was first described in Japan in 1973. The isolated virus was named *Carnation necrotic fleck virus* (CNFV). The flexuous, filamentous virus particles had the appearance typical of plant closteroviruses. A similar disease, carnation yellow fleck, caused by a filamentous virus, Carnation yellow fleck virus (CYFV), was described in Israel in 1974. In 1976, CNFV and CYFV were demonstrated to be serologically related to each other. CYFV was reassessed as a synonym of CNFV and subsequently only the name CNFV has been in use. During our analysis of virus-specific RNAs from plants infected with CNFV, we found that infected plants appeared to contain two different virus species of the genus *Closterovirus*. The two genomes were cloned, sequenced in their entirety and in phylogenetic analyses demonstrated to represent two distinct closteroviruses. We propose to name one *Carnation necrotic fleck virus* (14,825nt

genome) and the second Carnation yellow fleck virus (15,602nt genome). Phylogenetic analyses of proteins that are conserved throughout the family *Closteroviridae* demonstrated that both viruses belong to the lineage of aphid-transmitted closteroviruses typified by *Beet yellows virus*. The two viruses can be detected and distinguished in RT-PCR using total RNA isolated from carnation tissues. The two viruses could also be distinguished using several CNFV-specific McAbs and a polyclonal antiserum produced to a bacterially-expressed CYFV capsid protein. Single and mixed infections were identified in a series of screened carnation plants. These tools and information will be useful to growers and nurseries in their screening assays to detect and control these viruses in the parent propagation stock lines and subsequently in the large scale plant production phases.



# Witches' brooms of coniferous plants as a source of phytoplasma infection

M. Kamińska & H. Berniak

Department of Plant Protection, Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland  
maria.kaminska@insad.pl

Diseases of forest trees of uncertain aetiology as shoot proliferation have been widely distributed throughout the world. These aberrations named also witches' brooms are potentially attractive and they are used for propagation to get new selections of dwarf and slow-grow types of coniferous trees. They are aesthetically pleasing, and functionally valuable. However, little is known on the nature of those growth abnormalities of coniferous plants in Europe. Few references reported that they have arisen in the absence of casual organism, presumably through bud sports or mutations. Very recently, on the basis of PCR amplification of 16S rDNA and sequence analysis was indicated that shoot proliferation symptoms in conifer trees have been associated with phytoplasma infection.

The presence of phytoplasmas in ten out of thirteen tested plant species - *Abies procera*, *Picea abies*, *P. glauca*, *P. pungens*, *Pinus banksiana*, *P. mugo*, *P. nigra*, *P. sylvestris*, *P. tabuliformis*, and *Tsuga canadensis*, was demonstrated using PCR with P1/P7 followed by R16F2n/R16R2 primer pairs. Phytoplasma infection was evidenced in about 12% of the tested trees by direct PCR and in 24% by nested PCR.

After enzymatic digestion, *Abies procera*, *Pinus banksiana*, *P. mugo*, *P. nigra*, *P. sylvestris*, *P. tabuliformis*, and *Tsuga canadensis* tested samples showed a restriction pattern similar to that of '*Candidatus phytoplasma pini*', computer-calculated on the basis of the sequence of the reference isolate Pin127S. Nested PCR-amplified products, obtained with primers R16F2n/R16R2, were sequenced. Sequences of phytoplasmas found in the tested plants (GenBank Acc. No. FJ409228, FJ409230, FJ409231, FJ409234, FJ409232, GQ290113, FJ409233, HM190300,

HM190301, HM190302, GQ290115, FJ409235) were nearly identical. They were also closely related to other isolates of '*Ca. Phytoplasma pini*', group XXI, found in pine trees previously.

After enzymatic digestion, samples from *P. abies* and *P. glauca* trees showed a restriction pattern similar to that of phytoplasmas belonging to an X-disease group, 16SrIII. Samples from *P. pungens* trees showed two different restriction patterns; phytoplasma isolated from spruce named K1 showed a profile similar to that of '*Ca. phytoplasma asteris*', whereas phytoplasma isolated from spruce cv. Tomek revealed a profile similar to that of '*Ca. phytoplasma pini*'. Based on the results of RFLP and sequence analyses (GenBank Acc. No. EU753605, GQ290114 and FJ409229), tested phytoplasma isolates were classified as members of the phylogenetic group: 16SrI, '*Ca. Phytoplasma asteris*'; 16SrIII, X-disease phytoplasma group; or 16SrXXI, '*Ca. Phytoplasma pini*'. Phytoplasmas were detected in about 27% of the witches' broom originated plants and in some naturally infected hybrids. The present study provide evidence of the new plant host species which had never been tested for the presence of phytoplasma. Identification of three phytoplasma species in coniferous plants is of importance because some of the growth aberrations are recommended for propagation to get new selections of conifers. Propagation and distribution of plants originating from phytoplasma affected witches' brooms and their potential threatening should be given under careful consideration.

## Acknowledgment

This work was conducted within the framework of COST Action FA 0807.

# siRNA sequencing and assembly: a universal tool for identification and diagnostics of viruses

J.F. Kreuze

International Potato Center, Lima, Peru

j.kreuze@cgiar.org

Novel and emerging plant viruses, arising from pathogen evolution, global trade, crop intensification and potentially climate change, pose a key threat to agriculture worldwide. Even apparently symptomless virus infections can cause considerable yield losses, which can be further exacerbated by synergistic interactions with other viruses. The advent of next generation sequencing has revolutionized the sequencing of plant viruses. When previously it could take months or even years to sequence the genome of a novel virus it is now possible to carry this out in a matter of days. To make this approach cost effective, methods must be developed avoid excessive sequencing of non viral nucleic acids in a sample, which may pose a problem, particularly for low titer viruses. To this end we have developed an approach based on the sequencing of siRNA produced in plants in response to

viral infections through the RNA silencing mechanism. RNA silencing constitutes a fundamental antiviral defence mechanism in plants in which host enzymes cut viral RNA into pieces of 20-24 nt. When isolated, sequenced en mass and properly aligned these virus-derived small RNA (sRNA) sequences can reconstitute genomic sequence information of the viruses being targeted in the plant. This approach is independent of the ability to culture or purify the virus and does not require any specific amplification or enrichment of viral nucleic acids as it automatically enriches for small RNAs of viral origin by tapping into a natural antiviral defence mechanism. To date the method has been used to identify dozens new viruses including single and double stranded RNA, DNA and reverse transcribing viruses and viroids from hosts as diverse as plants and invertebrate animals.

# AmplifyRP™, a novel rapid isothermal nucleic acid amplification platform, brings the molecular lab to the greenhouse

J. Lamborn

Ornamental and Field Crop Diagnostics, Agdia, Inc., USA

JLamborn@agdia.com

Fast and accurate, on-site pathogen identification is crucial for the ornamental greenhouse grower. During the last decade, several companies have developed quick, field-based, serological tests to detect viruses, bacteria and fungi, giving the grower a large choice for early pathogen detection. In order to continue making advancements in early plant pathogen detection, we must move forward technologically. AmplifyRP now puts PCR-level sensitivity at the greenhouse bench. This technology not only offers greater sensitivity and specificity, but offers the ability to detect some pathogens (including viroids and Phytoplasmas) that cannot be screened using serological methods.

AmplifyRP uses a Recombinase-polymerase methodology for DNA and RNA amplification at a single temperature. In contrast to conventional or Real-time PCR, AmplifyRP has no DNA/RNA purification requirements, requires no thermocycling, and results can be read using small and user-friendly devices. A portable fluorescence reader or a lateral flow device (similar to Agdia's ImmunoStrip®) can be used to visualize results in as little as 30 minutes, compared to several hours for conventional PCR. AmplifyRP eliminates the need for expensive PCR equipment, a large number of reagent chemicals, and the need for technically trained staff.

# Detection of pospiviroids in the Czech Republic and their discrimination by restriction analysis

H. Orságová, G. Schlesingerová, & M. Dziaková

State Phytosanitary Administration, Division of Diagnostics, Olomouc, Šlechtitelů 23, Czech Republic  
gabriela.schlesingerova@srs.cz

*Potato spindle tuber viroid* (PSTVd), the member of the genus *Pospiviroid*, is a quarantine pest in European Union and its presence in ornamental solanaceous crops represents serious threat to susceptible vegetable and potato crops. Since the year 2007 the Czech Plant Protection Service (PPS) carries out a survey for PSTVd on ornamental solanaceous species. Within the first year of the survey, several new hosts of PSTVd and one another pospiviroid were identified. At the beginning of the survey, a method for detection of PSTVd recommended in the EPPO diagnostic protocol was adopted. Reverse transcription-polymerase chain reaction with semi-specific primer pair was carried out in the diagnostic laboratory of PPS while the sequencing of PCR product was performed in an external laboratory. Due to this time consuming process, which proved inefficient for the needs of rapid application of phytosanitary measures, the laboratory developed a faster procedure. The whole genomic

sequences of all viroids which contained primer identical segments were downloaded from the GenBank database and aligned to search for viroid-specific restriction sites. A combination of four enzymes was chosen to distinguish the detected viroids. Restriction analysis was successfully tested and applied for the PSTVd identification. Later on, after confirmation of other pospiviroids detected for the first time in the Czech Republic (*Tomato apical stunt viroid* and *Chrysanthemum stunt viroid* on *Solanum jasminoides* and *Citrus exocortis viroid* on *Solanum jasminoides* and *Verbena x hybrida*), the diagnostic method was improved to detect a wide range of pospiviroid species with the introduction of primers detecting the five most common pospiviroids in the ornamental solanaceous plants in Europe. Similarly, for identification of the amplified PCR products a new set of restriction enzymes was identified.

# Identification of *Dasheen mosaic virus* in *Anthurium andraeanum* by Immune Precipitation Polymerase Chain Reaction in Brazil

J.A.A. Lima & A.K.Q. Nascimento

Federal University of Ceará, Laboratory of Plant Virology, P.O.Box: 6046, Fortaleza, CE - 60.451.97, Brazil  
albersio@ufc.br

Correct identification of ornamental plant diseases caused by viruses can be accomplished by several methods involving their morphological, physical, biological, cytological, serological and molecular properties. Although serology constitutes the most used method for plant virus identification on a large scale, the use of molecular techniques for plant virus identification and characterization is increasing all over the world. Several molecular techniques have been developed for diagnosis and characterization of plant viruses and the reverse transcription polymerase chain reaction (RT-PCR) has been demonstrated to be a suitable method for research with RNA plant viruses. In the present study a variation of RT-PCR involving previous virus immune precipitation (IP-RT-PCR) was used for identification of *Dasheen mosaic virus* (DMV), genus *Potyvirus*, in anthurium (*Anthurium andraeanum*), a promising ornamental produced in the State of Ceará, Brazil. Leaves and flowers from symptomatic plants were collected in a commercial anthurium plantation in Baturité Mountain in the State of Ceará and taken to the Plant Virus Laboratory at the Federal University of Ceará. The plants were exhibiting mosaic, chlorotic stripes along the foliar veins and reduced development. The IP-RT-PCR method involved addition of DMV polyclonal antiserum to extracts of plant samples, concentration of immunoprecipitated virus by centrifugation, extraction of nucleic acid from the precipitate, and analysis of the nucleic acid by RT-PCR, using the universal primers Nib2F and Nib3R for detection of virus from the genus *Potyvirus*. According to the results, the samples from anthurium plants with virus symptoms presented a band corresponding to a DNA fragment of

350 bp which was expected for those primers, confirming the presence of DMV in the symptomatic plants. The DMV has a wide host range and its occurrence has been demonstrated in several ornamental and edible plants from the family *Araceae*. The virus could be transmitted by aphids in a non-persistent manner, by vegetative plant propagation and by mechanical inoculation. The development of virus infection in anthurium causes color degradation in the flowers and bractless deformations, with serious consequences in the appearance and quality of the flowers. Considering the importance of DMV for anthurium production in Ceará, control strategies are being taken with the goal of producing virus free nurseries to avoid virus dissemination by plant tissue propagation.

The new technique was also efficient for detecting the presence of five virus species in different infected plant tissues: a) cowpea (*Vigna unguiculata*): *Cowpea severe mosaic virus* (CPSMV) *Comoviridae*, genus *Comovirus* and *Cucumber mosaic virus* (CMV) *Bromoviridae*, genus *Cucumovirus*; b) melon (*Cucumis melo*): *Squash mosaic virus* (SqMV) *Comoviridae* genus *Comovirus*; c) watermelon (*Citrullus lanatus*): *Zucchini yellow mosaic virus* (ZYMV) *Potyviridae* genus *Potyvirus* and d) papaya (*Carica papaya*): *Papaya lethal yellowing virus* (PLYV), family *Sobemoviridae*, possible genus *Sobemovirus*. These additional results demonstrate that IP-RT-PCR should be considered a specific and sensitive method for molecular identification of RNA plant viruses, minimizing problems with total RNA extraction from virus infected plants to be used in the conventional RT-PCR.

# Use of an improved RT-PCR/RLFP method for *Pospiviroid* detection and differentiation in ornamental plants in Italy

M. Luigi, E. Costantini, D. Luison, P. Mangiaracina, L. Tomassoli, & F. Faggioli  
CRA - Centro di Ricerca per la Patologia Vegetale, Rome, Italy  
francesco.faggioli@entecra.it

Viroids have the lowest biological complexity of known pathogens; they consist of a single unit of single-stranded, covalently-closed, circular RNA of 246-401 nucleotides. According to biochemical, biological and structural properties, viroids are classified into two families: *Avsunviroidae* and *Pospiviroidae*. Genus *Pospiviroid* (family *Pospiviroideae*) includes nine phylogenetically related species quite indistinguishable from the biological and symptomatic points of view, and with high genome homology among them. In 2006, the first pospiviroid (*Potato spindle tuber viroid*-PSTVd) was found in ornamental plants (*Solanum jasminoides*) in symptomless infection. Since that time, many others pospiviroid species were found in different ornamentals. Pospiviroids in ornamental plants showed to always be asymptomatic, and easily transmissible to other solanaceous species. This could represent a substantial hazard for the spreading of pospiviroids to susceptible crops (i.e. potato, tomato). The symptomless infection and the high efficiency of transmission make the development of an accurate and reliable molecular diagnostic protocol for *Pospiviroid* detection and species identifica-

tion an important priority. The purpose of this work was the development of a genus-specific diagnostic protocol, by means of RT-PCR amplification using universal pospiviroid primers, which would allow, at the same time, a species-specific characterization, through a restriction fragment length polymorphism analysis (RFLP). The new set of primers is able to amplify the majority of the pospiviroid genomes (about 80%) allowing for the identification of specific species using appropriate restriction endonuclease enzymes (*AluI* and *Sau96I*).

The protocol was tested in a survey where different ornamentals belonging to the *Solanaceous* family were considered, and it was used also in the detection of several samples characterized by mixed pospiviroid infection, giving excellent results.

This study has allowed for the development of an efficient method for general pospiviroid diagnosis and species identification to be used for phytosanitary measures application and has provided an update on pospiviroid diffusion in solanaceous ornamentals.



# Facilitative and antagonistic interactions among pospiviroids infecting solanaceous ornamentals

D. Luison, P. Mangiaracina, E. Costantini, M. Luigi, F. Faggioli, & L. Tomassoli  
Plant Pathology Research Centre, Rome, Italy  
laura.tomassoli@entecra.it

In recent years, viroids have become an increasingly prominent issue for phytosanitary measures in the ornamental industry. In particular, four species belonging to the genus *Pospiviroid* (*Potato spindle tuber viroid* - PSTVd, *Tomato apical stunt viroid* - TASVd, *Chrysanthemum stunt viroid* - CSVd and *Citrus exortis viroid* - CEVd) were found in several European countries to infect the *Solanum jasminoides*, commonly known as potato vine. Even though these viroids cause symptomless infections in potato vine and other ornamentals (e.g., *Lycianthes rantonnei*, *Brugmansia* spp., *Cestrum* spp.), there is a real risk of an uncontrolled spreading of these pospiviroids to vegetable crops such as tomato (*Solanum lycopersicum*). For this reason, studies were carried out to improve our understanding of how these viroids, mainly PSTVd, are transmitted from infected potato vine to healthy ones, and to other ornamental species or to tomatoes.

In a research project (DM 11077/2009) funded by the Italian Ministry of Agriculture, experimental transmission assays were considered crucial to estimate the likelihood of dissemination of PSTVd infection in ornamental nurseries where *S. jasminoides* and other solanaceous are vegetatively propagated and grown. Mechanical inoculation tests by contaminated finger or razor blades, shoots cutting with contaminated tools, and root contact between healthy and infected plants growing in the same pot were performed during two years but no successful transmission of PSTVd occurred to PSTVd-free *S. jasminoides*. On the other hand, PSTVd transmission was achieved in other orna-

mentals (*L. rantonnei*, *Brugmansia* spp., *Datura* spp., *Petunia hybrida*, *Streptosolen jamesonii* and *Cestrum* spp. ) or vegetable species (*Capsicum annum*, *S. lycopersicum*, *S. tuberosum*, *S. melongena*) in the family *Solanaceae*. Following diagnostic investigation ascertained the presence of CEVd in the potato vine lots used for the assay.

To understand the real role of CEVd in the failure to transmit PSTVd on *S. jasminoides*, tomato was chosen as model plant and the viroid-viroid interaction between PSTVd, CEVd and TASVd was studied. Stocks of 10 tomato plants were firstly infected by only one of the three viroids. All isolates were obtained from natural single infected *S. jasminoides* plants identified during domestic phytosanitary monitoring. After viroid presence was confirmed in each plant (21 days post inoculum), cross infection was performed: CEVd or TASVd into PSTVd infected plants, CEVd or PSTVd into TASVd infected plants, and TASVd or PSTVd into CEVd infected plants. First results showed that only TASVd was able to co-infect the already CEVd and PSTVd infected plants. No other co-infections occurred. In all plants, viroid species were identified by RT-PCR/RFLP and Real Time RT-PCR. These preliminary results do not elucidate on the natural mixed infection of PSTVd with CEVd that occurred in *S. jasminoides* plants assayed during our surveys. In contrast, they explain our failure to transmit PSTVd into already CEVd infected plants. Additional experimental tests are now under way and the results will be discussed in the presentation.

# A carlavirus infecting ornamental plants encodes a zinc-finger protein that acts as a plant transcription factor

N.I. Lukhovitskaya<sup>1</sup>, A.D. Solovieva<sup>2</sup>, A.G. Solovyev<sup>3</sup>, & E.I. Savenkov<sup>1</sup>

<sup>1</sup> Department of Plant Biology and Forest Genetics, Uppsala BioCenter SLU, Box 7080, 75007, Uppsala, Sweden, <sup>2</sup> Department of Virology, Biological Faculty, Moscow State University, Moscow, 119992, Russia, <sup>3</sup> A.N.Belozersky Institute of Physico-Chemical Biology, Moscow State University, 119992 Moscow, Russia  
eugene.savenkov@slu.se

Cross-sections and microscopy revealed pronounced cell proliferation in *Chrysanthemum virus B* (CVB, a carlavirus) infected leaves as compared to mock inoculated plants. This aberrant tissue structure is referred as “hyperplasia”, e.g. proliferation of cells within tissue beyond what is ordinarily seen. Hyperplasia is a plant virus disease manifestation, which has not been analyzed so far and very little is known about the mechanism of hyperplasia induction by plant viruses. We demonstrate that a zinc-finger protein p12 encoded by CVB is translocated to the nucleus and acts as a eukaryotic transcription factor (TF) to upregulate

a bHLH-TF-encoding gene (another, a host-encoded transcription factor), which in turn stimulates ectopic cell proliferation and modulates tissue growth in the infected leaves. We will also provide a model for p12-mediated interference with mitotic cell cycle and endocycle. The identification of the first virally encoded effector protein that acts as a eukaryotic transcription factor and modulates growth and development of the host represents an important advance and will lead to characterization of a novel type of virus-host interactions via virus-encoded transcription factors that fine tune host gene expression.

# Investigation on the phytosanitary status of major ornamental hibiscus species in Italy to assess virus infection

A. Manglli<sup>1</sup>, S. Adkins<sup>2</sup>, A. Tiberini<sup>3</sup>, & L. Tomassoli<sup>3</sup>

<sup>1</sup>Dipartimento di Gestione dei Sistemi Agrari e Forestali (GESAF), University of Reggio Calabria, Italy, <sup>2</sup>USDA-ARS-USHRL, Fort Pierce, FL 34945, USA, <sup>3</sup>Plant Pathology Research Centre, Rome, Italy  
laura.tomassoli@entecra.it

The genus *Hibiscus* (family *Malvaceae*) includes about 250 species that vary from annual to perennial herbs, and shrubs to small trees that are native to tropical, sub-tropical and temperate climates. *H. rosa-sinensis* is of major importance in the ornamental industry as potted plants for gardens and terraces due to its attractive flowers. Breeding efforts using *H. rosa-sinensis* and indigenous species during the last century have yielded many hybrids that are in cultivation worldwide. In contrast, *H. syriacus* is mainly planted in urban streets as shrubs and trees flowering in late summer or early fall.

Commercial hibiscus cuttings are often imported into Italy from foreign countries for breeding, plant propagation and container gardening. Frequently, Italian farmers report virus-like symptoms on hibiscus plants that reduce quality and commercial value. Viruses reported to infect *H. rosa-sinensis* include *Hibiscus chlorotic ringspot virus* (HCRSV), *Hibiscus latent ringspot virus* (HLRSV), *Eggplant mottled dwarf virus* (EMDV), and the recently identified tobamoviruses, *Hibiscus latent Singapore virus* (HLSV) and *Hibiscus latent Fort Pierce virus* (HLFPV).

A small study in 2010-2011 examined viruses associated with symptoms observed on hibiscus plants at a major Italian flower company. Symptoms included chlorotic spots or rings, chlorotic mottling, and in some cases, vein necrosis in older leaves and early leaf yellowing and drop. Samples of cultivated *H. rosa-sinensis* hybrids and *H. moscheutos*, and some wild species (*H. arnot*, *H. borianus*, *H. storkly* and *H. densonii*) used for breeding, were collected for diagnostic tests. Additionally, multiple cuttings from 13 imported lots of different cultivars of *H. rosa-sinensis* from Europe, Africa and Asia were sampled before

transplanting. Samples of *H. rosa-sinensis* and *H. syriacus* were also collected from commercial gardens and street trees. After total RNA extraction, samples were assayed by one-step RT-PCR using specific primers in the coat protein gene to detect HCRSV, HLFPV and HLSV. Amplicons of the expected sizes were sequenced and analysed to confirm the virus identification. More than 160 samples were tested by one step RT-PCR. The results revealed an overall high incidence of virus infection. In potted plants, HCRSV was detected in 89 (65.4%), HLFPV in 63 (46.3%) and HLSV in 35 (25.7%) of the tested samples. Mixed infections were also frequent: all three viruses were found in 18% of samples, whereas both HCRSV and HLFPV were present in 25.5%. Moreover, 12 of 13 imported cutting lots were infected: three with HCRSV only and the remainder with mixed infections of two or three viruses. Only HLFPV was found in *H. syriacus* trees (2 of 5 samples). Mixed infections of HCRSV and HLFPV were found in 3 of 4 the wild species (*H. borianus*, *H. storkly* and *H. densonii*). No viruses were detected in *H. moscheutos* and *H. arnot*.

This study showed that viruses are widely distributed in hibiscus in nurseries and commercial gardens surveyed in Italy. Further, it revealed that imported cuttings, for which a phytosanitary certificate is not required, are commonly virus infected. This highlights the importance of phytosanitary measures for vegetative ornamental materials crossing international borders even if not under regulation for quarantine pests or quality requirement marketing.

# A sensitive real-time RT-PCR assay for generic detection of pospiviroids

E.T.M. Meekes<sup>1</sup>, M. Hooftman<sup>1</sup>, R. Dekter<sup>1</sup>, M. Botermans<sup>2</sup>, J.Th.J. Verhoeven<sup>2</sup>, J.W. Roenhorst<sup>2</sup>, & B.T.L.H. van de Vossenber<sup>2</sup>  
<sup>1</sup>Naktuinbouw, Roelofarendsveen, The Netherlands, <sup>2</sup>National Plant Protection Organization, Wageningen, The Netherlands  
e.meekes@naktuinbouw.nl

In many countries phytosanitary regulations apply to *Potato spindle tuber viroid*, because it can evoke serious damage in potato and tomato crops. Because other pospiviroids can cause similar damage and some seem to appear widely spread in ornamental crops, there was a need for a reliable and cost-effective generic testing method. An assay was developed which detects all known species of the genus *Pospiviroid*, using real-time RT-PCR based on TaqMan technology. This GenPospi assay consists of two reactions running in parallel, the first targeting all pospiviroids, except *Columnea latent viroid*, the second specifically targeting the latter viroid (existing assay). The internal control is based on *nad5*, to monitor RNA extrac-

tion and amplification. Method validation on tomato leaves showed that the GenPospi assay detects all pospiviroids up to a relative infection rate of 0.13% (equals 770 times dilution). No cross reactivity was observed, neither with other viroids and viruses nor with leaf material. Repeatability and reproducibility were 100% and the assay appeared robust in an inter-laboratory comparison. The new GenPospi assay has been shown a suitable tool for large-scale screening for all known pospiviroids. It has been validated for tomato leaves but additional data on ornamental crops like *Petunia*, *Calibrachoa*, *Dahlia* and *Vinca* will be presented.

# Emerging and new viruses in ornamentals in Germany

W. Menzel<sup>1</sup>, J. Hamacher<sup>2</sup>, & S. Winter<sup>1</sup>

<sup>1</sup>DSMZ - German Collection of Microorganisms and Cell Cultures, Plant Virus Department, Inhoffenstraße 7 B, 38124 Braunschweig, Germany, <sup>2</sup>Department of Phytomedicine, Institute of Crop Science and Resource Conservation, Nußallee 9, 53115 Bonn, Germany  
wulf.menzel@DSMZ.de

During routine tests (ELISA) of *Impatiens walleriana* and *Impatiens hawkeri* plants with chlorotic symptoms on older leaves and red-violet leaf margins on younger leaves the putative causal agent could not be identified. Following transmission to indicator plants and dsRNA extraction the complete sequence of the RNA 3 of a bromovirus was determined and the virus could be identified as an isolate of *Bacopa chlorosis virus* (BaCV, genus Ilarvirus). BaCV has previously only been reported from bacopa in the USA and no coat protein (CP) sequence has been available prior to our study. Both RNA 3 encoded proteins, CP and movement protein, showed highest sequence identity to parietaria mottle virus, a subgroup 1 ilarvirus. An antiserum was raised against a recombinant CP. The obtained polyclonal antiserum allowed specific detection of BaCV but showed no serological cross reaction with other ilarviruses and was unsuitable for immunoelectron microscopy. The determined host range includes many important flowering plants (impatiens, bacopa, petunia, verbena, calibrachoa, rudbeckia), highlighting the potential threat BaCV might pose for the horticultural industry.

During investigation of *Gaillardia cordata* breeding material several plants reacted strongly (DAS-ELISA) with a commercially available *Chrysanthemum virus B* (CVB) antiserum. In order to confirm the identity of the virus a part of its replicase was sequenced, showing just 26% amino acid sequence identity to CVB. This prompted us to determine the entire genome of this virus isolate. The complete genome was 8659 nt in length (excluding poly-A tail) and contained six open reading frames. The genome organisation resembled that of typical carlaviruses. The replicase (70%) and CP (75%) showed the highest aa sequence identities to *Phlox virus S* (PhVS), which is well below the species demarcation threshold of 80%. The remaining ORFs (TGB1-3, NABP) also showed the highest aa sequence identities to PhVS, ranging from 59% (TGB3) to 77% (NABP).

In addition, the CVB antiserum was tested with other carlavirus isolates available at the DSMZ plant virus collection. Besides CVB and the new carlavirus from *Gaillardia*, it showed a strong cross-reaction (DAS-ELISA) with isolates of *Kalanchoe latent virus*, *Potato virus S*, *Passiflora latent virus* and *Helenium virus S*.

# Preliminary survey and study of the CymMV variability in orchids of Sao Paulo state, Brazil

L.A. Moraes, M.A. Pavan, & R.K. Sakate

Vegetal Production Department - FCA (Faculdade de Ciências Agrônomicas)/UNESP - Botucatu-SP, Brazil  
ladmoraes@fca.unesp.br

Diseases caused by viruses are a major constraint in orchid's production systems health, as they promote considerable damage to its commercial value, since they directly affect its aesthetic appeal as well as productivity. In world terms, the potyvirus *Cymbidium mosaic virus* (CymMV) and the tobamovirus *Odontoglossum ring spot virus* (ORSV) are of greater economic importance, due to their predominant incidence.

A preliminary survey of the viruses present in orchid samples of different generas and from the Sao Paulo cities: Aparecida, Araras, Arthur Nogueira, Botucatu, Bragança Paulista, Campinas, Holambra, Sorocaba, Suzano, Mogi das Cruzes and Ribeirão Preto was performed, totalizing 196 samples analyzed.

The diagnoses were performed through direct ELISA protocol for the detection of *Cymbidium ring spot virus* (CymRSV), as well as indirect ELISA protocol for potyvirus detection, using universal anti serum against this genre. The results were confirmed by RT-PCR with the specific primers, for detection of CymMV and ORSV, respectively: CYMV-F 5'-CTG CCC TGA TCC TGC AGC -3', CYMVK-R 5'- AAA ACC ACA CGC CTT ATT AAG TTT G -3'; ORSV-F 5'- TAT TGT GGT CGG TAA TGG -3'; and ORSV-R 5'- TAT CCG CAG TGA AAA ACC C -3'. In both cases, the primers were drawn based on the total extensions of the cp gene of these viruses.

According to the reading of the plates through 405 nm absorbance, as well as the RT-PCRs electrophoresis, of the 196 samples tested, and 114 were positive for CymMV presence (58.16%), 31 were positive for ORSV (15.8%). In 93.5% of the samples in which ORSV was detected, which means 29 samples, there was mixed infection with CymMV. There were no detection of CymRSV and potyvirus.

CymMV samples sequences showed nucleotide identity of greater than 96% with CymMV Genbank accession numbers AB197937.1, AY571289.1 and AB541560.1, and the phylogenetic tree supposes that, so far, this virus has shown a low variability rate.

These preliminary results denote CymMV major importance in the orchid's production systems in São Paulo state, as shown by its high incidence, and confirm that its genetic variability rate is low, as expected from a mechanical-borne virus.

Financial Support: FAPESP Scientific Program: Detection And Diagnostic Methods

# Detecting pospiviroids in ornamentals: a new, non-degenerate and highly sensitive PCR approach

J. Lamborn & D. Groth-Helms  
Agdia, Inc., USA  
JLamborn@agdia.com

Ornamental plants, along with tomatoes and peppers, represent the majority of all greenhouse grown crops worldwide. Growers are now incorporating both vegetable and decorative plants into their operations, and both are susceptible to infections by a number of shared pathogens including viroids. There are at least six species of viroids that can infect both solanaceous vegetables and ornamentals, including *Chrysanthemum stunt viroid* (CSVd), *Citrus exocortis viroid* (CEVd), *Potato spindle tuber viroid* (PSTVd), *Tomato apical stunt viroid* (TASVd), *Tomato chlorotic dwarf viroid* (TCDVd) and *Tomato planta macho viroid/Mexican papita viroid* (TPMVd/MPVd). With most of these viroids having already been found in North America, including the US, reliable detection

is needed to prevent the spread and aid in effective eradication. Nucleic acid hybridization, thermal cycling amplification and isothermal amplification are a few of the current assays available for viroid detection. Agdia currently provides specific nucleic acid hybridization assays for the detection of *Chrysanthemum stunt viroid* (CSVd) and *Potato spindle tuber viroid* (PSTVd). Agdia Testing Services is now offering a Pospiviroid Group PCR Test using a two-step RT-PCR protocol and Agdia designed primers. This test is proven to detect the six important pospiviroids listed above. This assay is highly sensitive and can be used for both broad screening of pospiviroids, and specific identification of viroids of regulatory interest through sequence analysis.



# Viroid discovery - past achievements, future prospects

R.A. Owens

Plant Pathology Laboratory (USDA/ARS), Beltsville, MD 20705, USA  
robert.a.owens@ars.usda.gov

The 1971 discovery of *Potato spindle tuber viroid* (PSTVd) revealed a previously unsuspected class of subviral pathogens - small, non-coding, covalently closed circular RNA molecules capable of autonomous replication in susceptible plant species. Over the following 10-15 years, a number of other important diseases affecting both herbaceous and woody hosts were shown to be caused by viroids. More than 30 different species of viroids are now known, and not all infections are associated with visible disease. Further complicating control efforts, the original host species for many viroids are unknown. The discovery of

new viroids has been highly dependent on improved technology, and modern diagnostic methodologies like RT-PCR using family- or genus-specific primers have identified a large number of latent viroid infections involving ornamental species. A particularly promising discovery strategy involves large-scale sequencing of small cellular RNA populations and the use of bioinformatic methods to identify the sequences of previously unknown viroids or satellite RNAs. Implications of these findings for the implementation of improved disease control strategies will be discussed.

# Changes in tomato gene expression during *Potato spindle tuber viroid* infection reveal a complex array of changes affecting hormone signaling

R.A. Owens<sup>1</sup>, K.B. Tech<sup>1</sup>, J.Y. Shao<sup>1</sup>, T. Sano<sup>2</sup>, & C. J. Baker<sup>1</sup>

<sup>1</sup>Molecular Plant Pathology Laboratory (USDA/ARS), Beltsville, MD 20705, USA, <sup>2</sup>Plant Pathology Laboratory, Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki 036-8561, JAPAN  
robert.a.owens@ars.usda.gov

Viroids like *Potato spindle tuber viroid* (PSTVd) are the smallest known agents of infectious disease - small, highly structured, circular RNA molecules that lack detectable messenger RNA activity yet are able to replicate autonomously in susceptible plant species. To better understand the possible role of RNA silencing in disease induction, a combination of microarray analysis and large-scale RNA sequence analysis was used to compare changes in tomato gene expression and miRNA levels associated with PSTVd infection in two tomato cultivars plus a third transformed line expressing small PSTVd siRNAs in the absence of viroid replication. Changes in mRNA levels for the sensitive cultivar Rutgers were extensive,

involving more than half of the ca. 10 000 genes present on the array. Chloroplast biogenesis was down-regulated in both sensitive and tolerant cultivars, and effects on mRNAs encoding enzymes involved in the biosynthesis of gibberellin and other hormones were accompanied by numerous changes affecting their respective signaling pathways. In the dwarf cultivar MicroTom, a marked up-regulation of genes involved in response to stress and other stimuli was observed only when exogenous brassinosteroid was applied to infected plants, thereby providing the first evidence for the involvement of brassinosteroid-mediated signaling in viroid disease induction.

# First report of phytoplasmas associated with *Erysimum linifolium* L. stunting

S. Paltrinieri<sup>1</sup>, N. Contaldo<sup>1</sup>, A. Bertaccini<sup>1</sup>, L. Cavicchi<sup>2</sup>, & M.G. Bellardi<sup>1</sup>

<sup>1</sup>DiSTA - Plant Pathology, *Alma Mater Studiorum*, University of Bologna, via G. Fanin, 42, 40127 Bologna, Italy, <sup>2</sup>Plesso Didattico G. Scarabelli (Imola), *Alma Mater Studiorum*, University of Bologna, Via Ascari, 17, 40026 Imola (Bologna), Italy  
assunta.bertaccini@unibo.it

*Erysimum linifolium* L. (syn. *Cheiranthus linifolium* L.) (*Brassicaceae*), or Aegean wallflower, native to the Mediterranean region, is an evergreen perennial compact shrub that offers silvery-green foliage and beautiful spikes of lilac-mauve flowers through long periods, starting in mid-spring. In Liguria region (northern Italy) this species is mostly cultivated to be used in rock gardens or in mixed garden borders. In 2012, a phytoplasma-like disease was observed in a few pot-plants at an ornamental grower of Albenga area (Liguria region). Symptomatic *E. linifolium* showed reduced leaf size, rosetting and stunting; in some cases, shortening of internodes and growing reduction involved only a part of the plant. After first symptoms observation, an increasing percentage of symptomatic plants were found at flowering stage, when affected plants did not bloom. In order to verify phytoplasma presence and to determine their identity, samples from symptomatic and asymptomatic plants were collected and tested, after a chloroform/phenol nucleic acid extraction by direct PCR with primers P1A/P7A followed by nested-PCR with primers F1/B6 and R16F2n/R2. RFLP analyses performed with *Tru1l* and *HhaI* enzymes, allowed the identification, only in the symptomatic plants, of phytoplasmas belonging to subgroup 16SrI-B ('*Candidatus* phytoplasma asteris'). Further confirmation of phytoplasmas identity was obtained after *Tru1l* RFLP analyses on *tuf* gene amplified with cocktail primers.

'*Candidatus* Phytoplasma asteris' (16SrI) is associated with over 100 economically important plant diseases and represent one of the most diverse and widespread phytoplasma groups. Strains that belong to subgroups 16SrI-A, 16SrI-B and 16SrI-C are distributed worldwide and are associated with diseases in more than 80 plant herbaceous species transmitted by more than 30 species of insect vectors. The 16SrI-B represents the largest and most diverse strain cluster in the group in which at least 20 ribosomal subgroups were recognized. Some of the subgroups were only detected in woody hosts such as 16SrI-P identified in poplar in Eastern Europe, 16SrI-S and 16SrI-Q detected in cherry respectively in China and in Lithuania indicating the ability of some of its strains to infect all kind of plant species. The detection and identification of aster yellows phytoplasmas in *E. linifolium* in Italy represents however its first report in this species worldwide. Phytoplasmas belonging to 16SrII group ('*Ca. P. aurantifolia*') were detected in 2010 in *E. cheiri* (syn. *C. cheiri*) a different species cultivated in south-eastern Iran. In this case, infected plants showed witches' broom and phyllody. Considering that *E. linifolium* is propagated by seed, it is very likely that leafhoppers are involved in 16SrI-B phytoplasma spreading in this species. Work on possible insect vector identification is in progress.

# Carlavirus detection 2008 to 2011 in Germany using electron microscopy

K. Richert-Poeggeler<sup>1</sup>, S. Schuhmann<sup>1</sup>, C. Maass<sup>1</sup>, K.C. Eastwell<sup>2</sup>, R.R. Martin<sup>3</sup>, & B. Lockhart<sup>4</sup>

<sup>1</sup>Julius Kühn-Institut, Institute for Epidemiology und Pathogen Diagnostics, Messeweg 11-12, D-38104 Braunschweig, Germany,

<sup>2</sup>Washington State University Irrigated Agriculture Research and Extension Center and Department of Plant Pathology, Prosser 99350, Washington, USA, <sup>3</sup>United States Department of Agriculture, 3420 NW Orchard Avenue, Corvallis, OR 97330, USA,

<sup>4</sup>Department of Plant Pathology, University of Minnesota, St. Paul 55108-6030, Minnesota, USA  
katja.richert-poeggeler@jki.bund.de

Latency of animal and human viruses has been well studied for example in Herpesviruses and Retroviruses. Although latency has been longtime known for plant viruses as the term “latent” in the name of numerous plant viruses indicates, a detailed functional analysis of this phenomenon is missing in plants. Carlaviruses belong to the family *Betaflexiviridae* with the type member *Carnation latent virus*. 43 members are listed by ICTV in 2009. Samples obtained in 2008 to 2011 included the monocot host plants comprising the families *Alliaceae* and *Convallariaceae* as well as dicot host plants out of the families of *Cactaceae*, *Ericaceae*, *Passifloraceae*, *Ranunculaceae*, *Scrophulariaceae* and *Solanaceae*. At least 10 different carlaviruses have been identified using immunoelectron microscopy. In *Alliaceae*, *Cactaceae*, *Ranunculaceae* and *Solanaceae* mixed infections were

frequent. Depending on the host mixed infections consisted of two different carlaviruses or included a carlavirus and a poty- or an allexi-, or a potex-, or a tobamo- or a cucumovirus. Thus symptom expression was variable. A special focus has been paid to carlaviruses in *Helleborus* ssp. Whereas *Carnation latent virus* and *Helleborus net necrosis virus* have been identified in *Helleborus* in Germany in 2008, 2010 and 2011, *Helleborus mosaic virus* has been only found in 2011 in *Aconitum* ssp. Currently ultrathin sections of healthy as well as infected hellebores leaf tissue are analysed for presence of virions and viral induced changes in ultra-cellular structures. In *Solanum jasminoides* and *Vaccinium* ssp. serological identification of carlaviruses was insufficient and therefore additionally RT-PCR followed by sequencing has been employed to verify and/or improve ISEM results.

# Electron microscopic studies of vegetative and generative tissues from *Hosta virus X* infected hosta

K. Richert-Poeggeler<sup>1</sup>, U. Commandeur<sup>2</sup>, K. Kobayashi<sup>3</sup>, C. Maass<sup>1</sup>, & B. Lockhart<sup>4</sup>

<sup>1</sup> Julius Kühn-Institut, Institute for Epidemiology und Pathogen Diagnostics, Messeweg 11-12, D-38104 Braunschweig, Germany, <sup>2</sup> RWTH Aachen University, Institute for Biology VII, Molecular Biotechnology, Worringerweg 1, D-52074 Aachen, Germany, <sup>3</sup> Laboratory of Plant Molecular Biology and Virology, Faculty of Agriculture, National University Corporation Ehime University, 3-5-7 Tarumi, Matsuyama 790-8566, Japan, <sup>4</sup> Department of Plant Pathology, University of Minnesota, St. Paul 55108-6030, MN, USA  
katja.richert-poeggeler@jki.bund.de

*Hosta virus X* (HVX)- hosta patho-system is a promising model system to investigate plant viral latency. HVX belongs to the genus *Potexvirus* in the family *Flexiviridae*. The challenges for HVX detection in asymptomatic hosta led to HVX-infestation of several hosta cultivars worldwide. Successful HVX monitoring in hosta becomes even more complicated due to the observed phenomena of post outbreak latency (recovery). HVX had been detected using immunosorbent electron microscopy both in vegetative and generative organs. Studying virus infection in a perennial plant offers several novel aspects for example: i) virus replication during growing season and winter rest (dormancy) and ii) virus replication in root tissue

in presence and absence of leaves. Using natural and artificial inoculation we could show that both leaf as well as root tissue harbor infectious HVX. With the exception of hosta cultivar “Frances Williams”, HVX detection was more efficient in roots than leaves. Thus the described resistance of H. “Frances Williams” may be due to the incapability of HVX to invade and/or replicate in root tissue. We analyzed different HVX infected hosta using electron microscopy. Particle lengths from 500 to 540 nm were found. Ultrathin sections revealed virions in root as well leaf tissue and alterations of subcellular structures and electron dense bodies in leaves after HVX infection.

# Protective mechanism of gentian plants pre-inoculated with a satellite RNA-containing attenuated *Cucumber mosaic virus* (CMV) strain against virulent CMV

H. Sayama<sup>1,2</sup>, N. Takayanagi<sup>1</sup>, M. Yamada<sup>1,3</sup>, M. Kominato<sup>1</sup>, H. Atarashi<sup>1,2</sup>, & T. Hikage<sup>4</sup>

<sup>1</sup>Nippon Del Monte Co. R&D, 3748 Shimizu-cho, Numata, Gunma 378-0016, Japan, <sup>2</sup>Present address: Kikkoman Co. R & D, 3748 Shimizu-cho, Numata, Gunma 378-0016, Japan, <sup>3</sup>Present address: Sakata Seed Co. R & D, 2-7-1, Nakamachidai, Yokohama, 224-0041 Japan, <sup>4</sup>Hachimantai Floricultural R & D Center, 70 Kamasuda, Hachimantai, Iwate 028-7592, Japan  
hsayama@mail.kikkoman.co.jp

It has been reported that tomato (*Lycopersicon esculentum*) plants pre-inoculated with satellite RNA (SatRNA) containing attenuated CMV strains were protected against virulent CMV and that there exists a direct relationship between attenuation of virus symptoms and accumulation of double stranded satellite RNA (dsSatRNA) in such plants. Large amounts of dsSatRNA were detected in various parts of such pre-inoculated plants including leaves, stems, roots and fruits. On a large scale, in CMV-infested tomato fields, such plants were also protected against virulent CMV invasions. On the other hand in gentian (*Gentiana scabra* var. *buengeri*) plants pre-inoculated with SatRNA-containing attenuated CMV strains, dsSatRNA was detected only in inoculated leaves and no dsSatRNA was observed in the upper leaves. However, upon challenge-inoculation with virulent CMV, dsSatRNA was detected in upper leaves of pre-inoculated gentian plants, and the symptoms observed in the upper leaves were milder than those in non pre-inocula-

ted plants. Those results indicated that accumulation of dsSatRNA in the upper leaves of pre-inoculated gentian plants was triggered by virulent CMV infection and resulted in milder symptoms in the upper leaves. Gentian is a perennial plant. Shoots above the soil disappear in winter because of cold temperatures, but underground roots survive in winter with dormancy. Gentian plants pre-inoculated with SatRNA-containing attenuated CMV strains and then challenge-inoculated with virulent CMV, were stored in 3-5 C for 2 months to create a dormant condition and then kept in 15-20 C to break dormancy. When new shoots sprouted from the roots after dormancy, a large amount of dsSatRNA was detected. These results suggested that dsSatRNA was maintained in dormant roots in winter. In addition it showed that after dormancy SatRNA replication outcompeted viral replication and thereby protected gentian plants from severe symptoms caused by CMV infection.

# A microarray system for multiplex virus detection in ornamentals

C.-F. Tsai, Y.Y. Lin, S.-C. Lu, & C.A. Chang

Institute of biochemical sciences and technology, Chaoyang University of Technology, Taichung, Taiwan, Republic of China  
cachang@cyut.edu.tw

A DNA microarray system that can detect and identify plant viruses of four different economically important virus genera including *Potyvirus*, *Tobamovirus*, *Potexvirus*, and *Carlavirus* within five hours of processing time was developed. Viruses of these four genera all have elongated particles and tend to induce similar systemic mosaic symptoms that frequently hamper correct virus identification. The system contains two major reaction phases. The first phase is a multiplex RT-PCR reaction that combines four respective pairs of genus-specific primers and a primer pair for plant house keeping gene as internal control to amplify the target virus genome. The second phase uses genus-specific oligonucleotides as probes, which are immobilized on biochips to capture the RT-PCR amplicons from the previous reaction. Since the PCR

primers were designed pre-labeled with biotin, the amplified DNA products could therefore be targeted by streptavidin conjugated with alkaline phosphatase (AP). These captured PCR amplicon and its reacted complex would be subsequently visualized on the bluish precipitates of NBT-BCIP substrate digested by AP on the spots where the probes were immobilized. We have tested and shown that this microarray system is feasible for virus detection and quick identification on ornamentals including *Phalaenopsis*, lily, *Hippeastrum*, *Narcissus*, *Lycoris*, *Jasmine*, calla lily and lisianthus. New potex- and carlaviruses on some ornamental crops, which have never been found by the use of conventional methods, were identified using this system.



# A membrane-binding conserved motif in the coat protein of PnMV seems to mediate chloroplast targeting

C. Spetz & D.-R. Blystad

Bioforsk, Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, N-1432 Ås, Norway

carl.spetz@bioforsk.no

Replication of positive-sense single-stranded RNA viruses occurs in specific structures in close association with cellular membranes. Targeting of the viral replication complex (RC) to the site of replication is mediated by the interaction of viral-encoded proteins and host factors. Electron microscope studies have shown that *Poinsettia mosaic virus* (PnMV, family *Tymoviridae*) infection is associated with the presence of vesicular structures in the chloroplasts, which indicates that the replication of PnMV might occur in association with chloroplast-derived membranes.

Using computer assisted homology search, we have identified that the coat protein (CP) of PnMV shows similarity to membrane bound proteins and contains a conserved amino acid sequence motif found in members the Alb3/Oxa1/YidC protein family. Our studies using red fluorescent protein-labelled CP and confocal microscopy show that the viral CP localizes in the chloroplast. In addition, mutational studies on an infections cDNA clone also show that this conserved motif is crucial for viral replication.

# Preliminary description of a new viroid species from dahlia

J.Th.J. Verhoeven<sup>1</sup>, J.W. Roenhorst<sup>1</sup>, E.T.M. Meekes<sup>2</sup>, R. Flores<sup>3</sup>, & P. Serra<sup>3</sup>

<sup>1</sup>National Plant Protection Organization, P.O. Box 9102, 6700 HC Wageningen, The Netherlands, <sup>2</sup>Naktuinbouw, P.O. Box 40, 2370 AA Roelofarendsveen, The Netherlands, <sup>3</sup>Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Universidad Politécnica de Valencia, Valencia 46022, Spain  
j.th.j.verhoeven@minlnv.nl

Recently, we obtained preliminary indications for the presence of a new viroid species in dahlia. More specifically, analysis of dahlia samples by return polyacrylamide gel electrophoresis (PAGE) showed a band corresponding to a nucleic acid in the position expected for a viroid RNA. This result was further confirmed by double PAGE (running the samples first under non-denaturing and then under fully denaturing conditions) using known viroids as size markers.

Subsequent RT-PCR with primers designed to amplify all members of the genus *Pospiviroid* (type species *Potato spindle tuber viroid*, PSTVd) did not yield any

amplicon. However, application of an RT-PCR procedure not demanding any previous information on the sequence because of the use of random primers, resulted in an amplicon of 342 bp. Analysis of the primary and predicted secondary structure revealed a new viroid species sharing properties with pospi-viroids and with *Hop stunt viroid* (HSVd), the type (and single) species of genus *Hostuviroid*. In addition to the molecular characterization of this new viroid from dahlia, we are currently performing inoculation experiments to determine its biological characteristics.

# Occurrence of *Capsicum chlorosis virus* on ornamental crops in Taiwan

C.-C. Chen<sup>1</sup>, C.-A. Chang<sup>2</sup>, S.-D. Yeh<sup>3</sup>, & T.-C. Chen<sup>4</sup>

<sup>1</sup>Division of Plant Pathology, Agricultural Research Institute, Wufeng, Taichung, Taiwan, <sup>2</sup>Graduate Institute of Biochemical Science and Technology, Chaoyang University of Technology, Taichung, Taiwan, <sup>3</sup>Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan, <sup>4</sup>Department of Biotechnology, Asia University, Wufeng, Taichung, Taiwan  
kikichenwolf@hotmail.com

*Capsicum chlorosis virus* (CaCV), a species of the thrips-borne tospovirus, was first identified from the infected *Capsicum* spp. in Queensland, Australia and further recognized widespread in solanaceous crops in Asia. The sequence homology and serological relationships of the S RNA-encoded nucleocapsid protein (NP) are the most important criteria for classification of the genus *Tospovirus*. The NP of CaCV is closely related to that of *Watermelon silver mottle virus* (WSMoV), a prevalent tospovirus species infecting cucurbits in Asia, in both phylogenetic and serological relationships and thus CaCV is classified in the WSMoV serogroup. Reverse transcription-polymerase chain reaction (RT-PCR) using the species-specific primers are recommended to distinguish these two tospoviruses. Serogroup-specific monoclonal antibodies derived from the NSs protein in immunoassays and tospovirus genus-degenerate primers designed from the consensus sequences of L gene for RT-PCR were applied for preliminary diagnosis of tospovirus infections in field. The amplicons were further cloned and

sequenced to verify the viruses. Here, we report that CaCV was identified in several ornamental crops in Taiwan, including orchids (*Phalaenopsis* spp.), gloxinia (*Shinningia hybrida* Hort.), calla lily (*Zantedeschia* spp.), amaryllis (*Hippeastrum hybridum* Hort.) and blood lily (*Haemanthus multiflorus* Martyn.). CaCV causing chlorotic ringspots on *Phalaenopsis* leaves has become an important quarantine virus for the production of orchids in Taiwan. Symptoms of chlorotic ringspots were also found on leaves of the CaCV-infected amaryllis and blood lily. The symptoms on calla lily caused by CaCV were similar to those induced by *Turnip mosaic virus* and *Calla lily chlorotic spot virus*, showing green, yellow or chlorotic spots and strips on the infected leaves. Therefore, diagnosis of CaCV-infected diseases on the basis of symptomatology is difficult. To detect and differentiate CaCV from WSMoV in the field samples by RT-PCR, a CaCV-specific primer pair (CaCV4f/CaCV777c) was designed and successfully used.

# Virus eradication by cryotherapy of *in vitro*-grown shoot tips

R. Wang, W. Bi, B. Li, Z. Zhang, Z. Cui, C. Feng, B. Wang, Z. Yin, & Q. Wang

State Key Laboratory of Crop-Stress Biology for Arid Region, College of Horticulture, Northwest A&F University, Yangling 712100, Shaanxi, P. R. China

qiaochunwang@nwsuaf.edu.cn

Viral diseases cause great economical losses to ornamental crops. Usage of virus-free planting materials is a practical means to efficiently control viral diseases. Virus eradication is a core technique for production of virus-free plants. During the last several decades, various methods such as meristem culture, thermotherapy, thermotherapy followed by meristem culture and micrografting have been successfully developed for virus eradication. Cryotherapy of shoot tips, that is, a brief treatment of shoot tips in liquid nitrogen, which is based on cryopreservation technique, has become a novel biotechnology for

plant virus eradication. Compared with the traditional methods, cryotherapy of shoot tips produces high frequency of virus-free plants and is independent of size of shoot tip size. To date, viruses have been successfully eradicated from staple crops like potato and sweetpotato, and fruit trees like *Prunus*, banana, grapevine and raspberry. The mechanism as to why cryotherapy of shoot tips can efficiently eradicate virus is clear. Cryotherapy of shoot tips is expected to have great potential application to virus eradication of ornamental crops.

# The role of artificial microRNAs in virus resistance

S.M. Wong

Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543  
sciwsm@nus.edu.sg

Traditionally ornamental plants are created through genetic hybridization. However, the variations and disease resistance are generally limited and it is time-consuming to select for desired resistance to combat virus diseases.

Artificial miRNAs (amiRNAs) are of 21 nucleotide in length and they are genetically engineered to silence specific gene(s) of interest. This approach makes use of endogenous miRNA precursor to produce miRNA of sequence unrelated to the miRNAs that are normally produced from the precursors yet equip with gene silencing effect on gene of interest. In 2006, the first amiRNAs have been generated in transgenic *Arabidopsis thaliana* which demonstrated resistance to *Turnip yellow mosaic virus* (targeting P69) and *Turnip mosaic virus* (targeting Hc-Pro). A year later, similar success was shown for *Cucumber mosaic virus* (CMV) (targeting 2b). The virus resistance trait is displayed at the cell level and is heritable. In 2008, a more detailed study showed that the t-RNA-like structure within the 3'UTR of CMV impeded target site access

and restricted amiRNA-RISC-mediated cleavage of the target virus RNA. In 2011, CMV 2a and 2b genes were targeted and resistance was conferred to transgenic tomato plants. Scions originated from the transgenic tomato plant maintain stable resistance to CMV infection after grafted onto a CMV-infected rootstock. However, the grafting assay also suggests that the amiRNA-mediated resistance acts in a cell-autonomous manner and the amiRNA signal cannot be transmitted over long distances through the vascular system.

In this talk, I will cover how to generate amiRNAs using plant miRNAs and show you how the technology works at molecular level. The amiRNAs plays an important role in producing transgenic ornamental plants that are virus resistant. My laboratory in National University of Singapore is currently conducting research in using amiRNAs to confer resistance to *Cymbidium mosaic virus* and *Odontoglossum ringspot virus* in orchids.

## Abstracts of Posters

# The application of ribavirin for elimination of viruses in lily plants growing *in vitro*

D. Sochacki, E. Gabryszewska, & M. Kamińska

Research Institute of Horticulture. Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland  
dariusz.sochacki@inhort.pl

The aim of the research was to obtain virus-free plant material of two cultivars ('Queen of May' and 'Acapulco') and one breeding clone (5/01) of lily (*Lilium* L.) by *in vitro* cultures using chemotherapy with ribavirin (virazole, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide). Two genotypes were dedicated for further research on the induction of polyploids *in vitro*. Four different concentrations of ribavirin added to Murashige-Skoog medium with 0.1 mg NAA l<sup>-1</sup> were used: 5, 10, 20 and 30 mg l<sup>-1</sup>. Medium without ribavirin was used as a control. Bulb scales from *in vitro* virus-positive bulbs were used as initial explants in the experiment. Cultivar 'Acapulco' was infected by *Lily mottle virus* (LMoV), cultivar 'Queen of May' and clone 5/01 were infected by *Lily symptomless virus* (LSV) and *Cucumber mosaic virus* (CMV). Tests were done using DAS-ELISA with specific antibodies (LMoV and LSV from BQ Support, Lisse, The Netherlands and CMV from the Research Institute of Horticulture, Skierniewice, Poland). Regenerated bulbs were tested by DAS-ELISA for LMoV, LSV, CMV and *Lily virus X* (LVX) after 3 months of maintenance on the medium containing ribavirin.

The results of virus eradication have shown that the increase in concentrations of ribavirin have limited the regeneration of bulbs and led to decay of initial scales. The highest survivability of scales and the best regeneration showed clone 5/01, the lowest number of living scales and the lowest regeneration ability showed cultivar 'Acapulco'.

The results of virus testing have shown that virus eradication was successful in 7 among the 69 regenerated bulbs, and in only 4 among 45 regenerated bulbs, for cultivar 'Queen of May' and for 'Acapulco', respectively. Four virus-negative results for 'Queen of May' were obtained on medium with 10 mg l<sup>-1</sup>. The higher number of virus-negative bulbs (9 among 100 regenerated bulbs) have been obtained for genotype 5/01.

All virus-negative bulbs of 'Queen of May' and breeding clone 5/01 were multiplied *in vitro* and used for polyploidization induced by antimitotic agents. Next virus testing by means of DAS-ELISA will be carried out after one and half year after treatment of ribavirin to check the healthy status of the plants. The results leads to conclusion that the effect of ribavirin depends on the concentration of this antiviral agent, kind of virus and their concentration in plant tissue and on the genotype of plant.

## Acknowledgement

This work was supported by The National Centre for Research and Development, Poland, Project No. N R12 0063 06/2009.



# Detection of mixed virus population in freesia plants with necrotic disease

A.M. Vaira<sup>1</sup>, M. Vallino<sup>1</sup>, R. Lenzi<sup>1</sup>, V. Masenga<sup>1</sup>, D. Salvi<sup>2</sup>, & J. Hammond<sup>3</sup>

<sup>1</sup>Istituto di Virologia Vegetale, CNR, Strada delle Cacce 73, 10135 Torino, Italy, <sup>2</sup>Studio Ferrari Salvi, Via Quinto Mansuino 12, 18038 Sanremo (IM), Italy, <sup>3</sup>Floral and Nursery Plants Research Unit, USNA, USDA, 10300 Baltimore Avenue, Beltsville, MD 20705, USA

a.vaira@ivv.cnr.it

Necrotic disorder of freesia (*Freesia refracta* hyb., family *Iridaceae*) was first described in The Netherlands before 1970. In following years, the disorder was widely reported in other European countries including Northern Italy (Vaira & Milne 2008). Very recently, the same necrotic disease was also detected in Virginia, United States (Vaira et al. 2009) and New Zealand (Pearson et al. 2009). Presence of the Ophiovirus *Freesia sneak virus* (FreSV) has been widely associated with the necrotic disease in Europe, the United States and New Zealand but some uncertainty remains (Brandvagt et al. 2008). The freesia leaf necrosis complex has been shown to be soil-borne, transmitted by *Olpidium brassicae*, but other infectious agents (e.g. the Varicosavirus tentatively named *Freesia leaf necrosis virus*) could be naturally transmitted by the same vector, thickening the plot about the disease causal agent. In 2002 Dr. Morikawa was able to differentiate a Mild mottle mosaic- and Streaking disease in tulips: the first was linked to the *Tulip mild mottle mosaic virus* (TMMMMV, genus *Ophiovirus*) infection and the second to an unknown Tulip streaking associated agent, with unstable infectivity (Morikawa 2002). He also showed that development of both diseases was suppressed by fungicidal treatments of the soil, suggesting soil-borne transmission for both agents. In 2005 the same team preliminarily described *Tulip streak virus*, a novel virus strictly associated with tulip streak disease. This new viral agent, morphologically resembling Tenuiviruses or supercoiled Ophioviruses, has a coat protein of c. 30kDa sharing some homology with *Phlebovirus* (*Bunyaviridae*) (Morikawa et al. 2005). Freesia plants showing necrotic disease were collected in Liguria (Northern Italy) during the 2011-2012 growing season. About 40-50% of the plants were affected by typical leaf necrosis and were assayed for FreSV infection by electron microscopy (TEM) and RT-PCR tests. FreSV

was detected using FreSV-specific primers in all six samples obtained by pooling several Freesia plants from different areas of the parcel, and elongated supercoiled virus particles were detected in freesia crude sap by TEM. Freesia tissue was purified using Ophiovirus procedure and the product obtained was used for total RNA extraction and for TEM visualization. Several differently shaped virus particles were visualized in the mixture and total RNA has been used for sequence-independent amplification (SIA) for the identification of RNA virus, following a published procedure (Agindotan 2010). Results of virus detection in infected freesia tissue are reported.

## References

- Agindotan, B.O., et al. 2010. Application of sequence-independent amplification (SIA) for the identification of RNA viruses in bioenergy crops. *J Virol Meth* 169:119-128.
- Brandvagt, B., et al. 2008. Freesia leaf necrosis, the mystery remains. *ISVDOP* 12 p. 45 Haarlem (NL), 20-24 April.
- Morikawa, T. 2002. Soil-borne virus diseases, mild mottle and streaking in tulips. *PSJ Report* 6:21-30.
- Morikawa, T., et al. 2005. Tulip streak virus, a novel virus associated with tulip streak disease. 6th IWGPVFFV Symposium, p. 68 Bologna (IT) 5-7 September.
- Pearson, M.N., et al. 2009. A survey of viruses of flower bulbs in New Zealand. *Australas Plant Pathol* 38:305-309.
- Vaira, A.M., & Milne, R.G. 2008. Ophiovirus. *Encyclopedia of Virology* (Mahy & Van Regenmortel, Editors), pp. 447-454 Oxford: Elsevier.
- Vaira, A.M., et al. 2009. First report of *Freesia sneak virus* in *Freesia* sp. in Virginia, USA. *Plant Dis* 93 (9):965.

# Molecular evaluation of the principal *Ranunculus asiaticus* L. viruses

E. Sacco<sup>1</sup>, C. Borghi<sup>1</sup>, M. Laura<sup>1</sup>, V. Bobbio<sup>1</sup>, M. Rabaglio<sup>2</sup>, M. Kawka<sup>3</sup>, A.M. Vaira<sup>4</sup>, & B. Ruffoni<sup>1</sup>

<sup>1</sup>CRA-FSO, Ornamental Plant Research Unit, Sanremo (IM) Italy, <sup>2</sup>Biancheri Creations® Company, Camporosso (IM), Italy, <sup>3</sup>IUNG, Institute of Soil Science and Plant Cultivation-State Research Institute, Puławy Poland, <sup>4</sup>CNR-IVV, Institute of Plant Virology, Torino Italy  
ermannodomenico.sacco@entecra.it

The buttercup (*Ranunculus asiaticus* L.) belongs to the family *Ranunculaceae* and is a popular ornamental plant cultivated for the sale of cut flowers and for the production of potted or border plants. This species is native to the eastern Mediterranean basin and has become widespread in Western Europe, South Africa, California, Israel and Japan. The hybrids of *Ranunculus asiaticus* are susceptible to infection by several viruses, very often present in mixed infection, and all associated with economically important diseases. The main viral species that infect the buttercup are: *Tomato spotted wilt virus* (TSWV, genus *Tospovirus*), *Impatiens necrotic spot virus* (INSV, *Tospovirus*), *Ranunculus white mottle virus* (RWMV, *Ophiovirus*), *Cucumber mosaic virus* (CMV, *Cucumovirus*), *Tobacco mosaic virus* (TMV, *Tobamovirus*), *Tobacco rattle virus* (TRV, *Tobravirus*), *Tobacco necrosis virus* (TNV, *Tombusvirus*); moreover, several viral species listed in *Potyvirus* genus were detected: *Potato virus Y -Ranunculus strain* (PVY-R), *Turnip mosaic virus* (TuMV), *Ranunculus leaf distortion virus* (RLDV), *Ranunculus mild mosaic virus* (RMMV), *Ranunculus*

*mosaic virus* (RMV) and *Ranunculus latent virus* (RLV, *Potyviridae*, *Macluravirus*). Although the serological diagnosis (ELISA) is in many ways sufficient for the identification of viruses and for the diagnosis of viral diseases, it may find serious limitations in the case of viruses with low titer in the natural host. For this reason molecular analysis has been widely used as a highly sensitive and specific detection method. In the present study, the main viruses infecting *Ranunculus* hybrids, grown in greenhouse in the Liguria region (Imperia province), will be evaluated by double step RT-PCR assays and specific primers, in comparison with ELISA, using leaf tissues as starting material. Preliminary results show the presence of some viral species (TSWV, RLDV, RMV) in the samples analyzed, either in single or in mixed infection, and confirm that the molecular evaluation, due to versatility of use and reliability of results, can be easily used as a tool for viruses detection on large scale propagation materials in order to prevent the spread of the principal viruses and assure the production of virus-free *Ranunculus* plants.