International Workshop on Viroids and Satellite RNAs (IWVdS)

Beijing · China,

August 23rd-25th, 2013

Sponsored by

Institute of Plant Protection, Chinese Academy of Agricultural Sciences (IPP-CAAS)

Istituto di Virologia Vegetale del CNR (Italy)

Beijing Society for Plant Pathology (BSPP)

Inner Mongolia Potato Engineering and Technology Research Center, Inner Mongolia University

Supported by

State Key Laboratory of Biology of Plant Diseases and Insect Pests (SKLBPI)

Chinese Society for Plant Pathology (CSPP)

The Natural Science Foundation of China (NSFC)

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General Information

Letter of Invitation

Dear Friends & Colleagues,

It is our pleasure to invite you and your collaborators to attend the International Workshop on Viroids and Satellite RNAs (IWVdS) in Beijing, China. This event, which will take place on August 23rd～25th 2013, would provide a stimulating platform for exchanging research developments and tracking technical progress on viroids and satellite RNAs research.

The IWVdS is a Pre-Conference included in the program of the 10th International Congress of Plant Pathology (ICPP), to be held in Beijing from 25th to 31st August 2013, thus allowing participants to attend both IWVdS and ICPP minimizing time and costs.

IWVdS has been jointly sponsored by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences (IPP-CAAS), the Institute of Plant Virology, Italian National Research Council (IVV-CNR), the Beijing Society for Plant Pathology (BSPP), the Inner Mongolia Potato Engineering and Technology Research Center, Inner Mongolia University, and is supported by the State Key Laboratory of Biology of Plant Diseases and Insect Pests (SKLBPI), the Chinese Society for Plant Pathology (CSPP) and the Natural Science Foundation of China (NSFC).

The financial supports of these institutions allowed us to lower the registration fees as much as possible. The registration fees include two-day accommodation, access to the oral presentation session, lunch, dinner, coffee breaks and conference abstract book.

We sincerely invite you to come together with us, and make this workshop enjoyable and profitable!

Welcome to Beijing in August 2013!

On behalf of the IWVdS organizing committee
Dr. Shifang Li and Dr. Francesco Di Serio

Conveners

Dr. Shifang Li, Institute of Plant Protection, Chinese Academy of Agricultural Sciences (China)

Dr. Francesco Di Serio, Istituto di Virologia Vegetale del CNR (Italy)

Scientific Committee

Prof. Francesco Di Serio (Italy)
Prof. Lianhui Xie (China)
Prof. Ricardo Flores (Spain)
Prof. Robert Owens (USA)
Prof. Shifang Li (China)
Prof. Shouwei Ding (USA)
Prof. Teruo Sano (Japan)
Dr. Yiming Bao (USA)

Local Organizing Committee

Prof. Changyong Zhou (China)
Prof. Chenggui Han (China)
Prof. Dewen Qiu (China)
Prof. Julian Chen (China)
Dr. Lixia Wang (China)
Mrs. Lixin Chen (China)
Dr. Meiguang Lu (China)
Prof. Ruofang Zhang (China)
Prof. Shifang Li (China)
Dr. Tao Zhou (China)
Prof. Xueping Zhou (China)
Prof. Zujian Wu (China)

Venue

The International Workshop on Viroids and Satellite RNAs (IWVdS) will take place at the Beijing International Convention Center (BICC), China, from 23rd to 25th August, 2013.

Detailed Venue information: http://www.icppbj2013.org/file/invitation.asp

The BICC website: http://www.bicc.com.cn

Address: No.8, Beichen East Road, Chaoyang District, Beijing, 100101, P. R. China.

Beijing International Convention Center (BICC) is the second largest convention facility in Beijing, which specializes in staging national and international conferences, exhibitions and other large events. The convention center has served almost 1,000 different international and domestic conventions, exhibitions and meetings each year since its opening in 1990.

BICC is located in the leisure and meeting center of the Asian Games Village, which is within walking distance of the Olympic Village. It is nine kilometers away from the famous Tiananmen Square, the center of the city, and twenty-five kilometers away from the Beijing Capital International Airport. BICC is close to popular shopping centers, entertainment hotspots, restaurants, and sporting facilities. It is convenient to go to some of the Beijing's world famous attractions from BICC: the Summer Palace, the Great Wall and the Ming Tombs. BICC has been awarded the title of Best Venue for hosting international conferences.

Accommodation

The Organizing Committee has secured accommodation for participants at Beijing Continental Grand Hotel (rated 4*). The room rate includes breakfast.

Beijing Continental Grand Hotel is designed as a special four-star hotel connecting with the BICC (conference venue) by a walkway. It has 500 standard rooms and various suites as well as quite a few restaurants. It also offers services including foreign currency exchange, shopping arcade, airline ticket sales, beauty salon, massage, sauna, fax, typing, photocopying, laundry. Internet access is also provided at a cost.

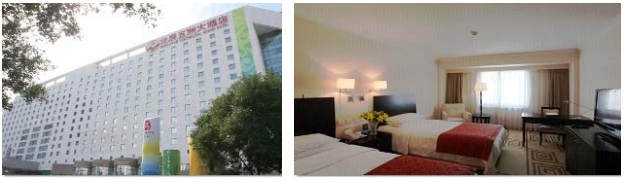
Rates (Service charge included):

Deluxe: RMB780/Single (one breakfast included)

RMB860/Twin (two breakfasts included)

In the flourishing Asian Olympic Village area, on the North Fourth Ring road, along the Capital Axis line, next to the Bird's Nest (Beijing National Stadium), the Water Cube (Aquatic Center). Address: No.8 Beichen East Road, Chaoyang District, Beijing, 100101, P. R. China.

- To ICPP venue (BICC): 200 m, 2 minutes by walk.
- To Beijing Capital International Airport: 25.0 km, 35 minutes by taxi (taxi charge: around RMB 100/one way).



Weather

August in Beijing is sunny, warm and humid. The average daily temperature is 25～35℃ (77～95F).

Insurance

The registration fees do not include insurance for the participants regarding accidents, sickness or loss of personal property. You are advised to make necessary arrangements for a short-term health and accident insurance before leaving your home country.

Currency Exchange

In China, only RMB is used. Exchange centers can be found at airports, banks and hotels. The exchange rate is set by the Bank of China, which is now about US$ 1.00=RMB 6.15. When exchanging money, you should keep your receipt in case you want to change any RMB back to foreign currency when leaving China.

Credit cards (Visa, Master, American Express, Diners Club and JCB) are accepted in many department stores and hotels. It is possible to draw cash from ATM machine by above credit cards.

The Bank of China and most of the hotels can cash traveler cheques issued by most foreign banks or financial institutions.

Arrival in Beijing

From the Beijing Capital International Airport to the venue is 25.0 km and it takes about 35 minutes by taxi. The charge is around RMB100 for one way.

Most important: Please print the following picture and show it to the TAXI driver, who may take you to the venue.

请送我到五洲大酒店，谢谢！

地址：北京市朝阳区北辰东路8号

电话： +86-10-84985588

Check-in

Reception staffs will do their best to help you check-in at 8:00 am～20:00 pm on August 23rd, at the Beijing Continental Grand Hotel.

Those who have not paid registration fee online will need to pay it on-site.

After check-in, you will get an information pack, meal vouchers, and a room card.

On-site registration fee can only be paid by cash (RMB): 3,000 RMB for every delegate and 1,900 RMB for each family member.

Please exchange RMB in advance at airport or bank.

Matters Need Attention for Lodging

IWVdS will provide free lodging for registered delegates at Beijing Continental Grand Hotel (rated 4*) for two days from the noon of August 23rd to the noon of August 25th, 2013. Any time before or after that, you will need to pay by yourself. If you wish to reserve a room (at this hotel) for extra nights, please contact us in advance. If you wish to try economical hotels at any time before or after the workshop, please reserve rooms in advance by yourself.

Preliminary Schedule

Time	Venue	
Aug 23rd, 2013 8:00 ～ 20:00	Beijing Continental Grand Hotel	Registration
Aug 24th, 2013 8:30 ～ 19:30	Beijing International Convention Center	Scientific program
Aug 24th, 2013 20:00 ～ 22:00	Beijing Continental Grand Hotel	Farewell banquet
Aug 25th, 2013 12:00	Beijing Continental Grand Hotel	Workshop end check out

Scientific Program

The conference will be held on August 23rd～25th, 2013, in Beijing. The conference covers a wide range of topics on viroids and satellite RNAs.

All meetings will take place in room 305 at Beijing International Convention Center on August 24th, 2013.

For the lecturers and speakers, please copy your PPT files to the computer between 14:00 pm and 20:00 pm on August 23rd at the registration site in the Beijing Continental Grand Hotel, or 7:30 am～8:20 am and 13:00～13:50 pm on August 24th at the Venue.

Conveners: Dr. Shifang Li, Dr. Francesco Di Serio **Venue: Meeting Room 305, 3rd Floor, BICC.** **Date: August 24th, 2013**	
8:30～ 9:00	**Opening ceremony** Dr. Shifang Li, Dr. Francesco Di Serio Dr. Dewen Qiu, Dr. Xueping Zhou
KEYNOTE LECTURES I 9:00～10:30	**Chairman: Dr. Shifang Li**
9:00～9:30	**1.** **Robert A. Owens** (**Keynote speaker**): Why are viroids restricted to plant hosts: Current status of a long～standing conundrum
9:30～10:00	**2.** **Xueping Zhou** (**Keynote speaker**): Advances in understanding *Begomovirus* Satellites
10:00～10:30	**3.** **Shouwei Ding** (**Keynote speaker**): Development of next-generation technologies for the identification and discovery of viruses and viroids

10:30 ～ 11:00	**Coffee break and taking photos**
SESSION I 11:00～12:45	**Identification and characterization of new/emerging viroids and viroid-like RNAs and risk assessment** **Chairmen: Dr. John W. Randles and Dr. Qingfa Wu**
11:00～11:15	**4.** **Kaishu Ling**: Characterization and detection of emerging viroids in North American greenhouse tomatoes
11:15～11:30	**5.** **John W. Randles**: First report of the detection of *Grapevine yellow speckle viroid* 1 RNA in bottled wine
11:30～11:45	**6.** **John W. Randles**: Constructing elements of the disease cycle of *Coconut cadang-cadang viroid* (CCCVd) from its epidemiology
11:45～12:00	**7.** **Tao Zhou**: Identification and molecular characterization of *Apple dimple fruit viroid* in China
12:00～12:15	**8.** **Shifang Li**: Identification of new viroid-like circular RNAs from grapevine and apple
12:15～12:30	**9.** **Mohammadreza Mohammadi**: Characterization of variants of *Coconut cadang-cadang viroid* from coconut palm in Malaysia
12:30～12:45	**10.** **Stancanelli Giuseppe**: Pest risk assessment of *Pospiviroids*: How biological and epidemiological features influence plant quarantine risk
12:45 ～14:00	**Lunch**
KEYNOTE LECTURES II 14:00～15:00	**Chairman: Dr. Francesco Di Serio**
14:00～14:30	**11.** **Ricardo Flores** (**Keynote speaker**): Recent insights into viroids and viroid-host interactions

14:30～15:00	**12.** **Detlev Riesner** (**Keynote speaker**): Potential mRNA targets of viroid-specific small RNA
SESSION II **15:00～16:15**	**Plant responses to viroids and satellite RNA's infections** **Chairmen: Dr. Gustavo Gomez and Dr. Teruo Sano**
15:00～15:15	**13.** **Francesco Di Serio**: Deep sequencing and degradome analyses: tools for further dissecting viroid-host molecular interplay
15:15～15:30	**14.** **Gustavo Gomez**: Transcriptional alterations in cucumber plants infected by *Hop stunt viroid*
15:30～15:45	**15.** **Charith Raj Adkar-Purushothama**: Comparison of small RNAs derived from *Potato spindle tuber viroid* strains of different pathogenicity
15:45～16:00	**16.** **Teruo Sano**: Characterization of *Hop stunt viroid* adaptation mutations emerged during persistent infection in hops
16:00～16:15	**17.** **Wanxia Shen**: Mechanisms of satellite RNA-mediated attenuation of viral disease symptoms in plants
16:15～16:30	**Coffee break**
SESSION III **16:30～17:45**	**Genetic diversity and replication** **Chairmen: Dr. José-Antonio Daros and Dr. Matilde Tessitori**
16:30～16:45	**18.** **Peter Palukaitis**: Sequence diversity of *Chrysanthemum stunt viroid*: multiple polymorphic positions throughout the genome
16:45～17:00	**19.** **Matilde Tessitori**: Host effects on the progeny of *Citrus dwarfing viroid*
17:00～17:15	**20.** **Wenxing Xu**: Sequence analysis of *Apple scar skin viroid* Chinese isolates revealed nine nucleotides closely related to the dapple symptom

17:15～17:30	**21.** **Aneta Wiesyk**: Stability of variable domain of *Potato spindle tuber viroid*
17:30～17:45	**22.** **Jos é -Antonio Daròs**: RNA circularization during viroid replication
17:45～18:00	**Coffee break**
SESSION IV **18:00～19:30**	**Detection methods and control of viroids** **Chairmen: Dr. Peter Palukaitis and Dr. Dongmei Jiang**
18:00～18:15	**23.** **Sathis Sri Thanarajoo**: Detection of *Coconut cadang-cadang viroid* (CCCVd) variants in oil palm using real-time PCR
18:15～18:30	**24.** **Dongmei Jiang**: Rapid detection methods for viroids in the genus *Coleviroid*
18:30～18:45	**25.** **Dianqiu Lv**: Situation, detection and control of *Potato spindle tuber viroid* in China
18:45～19:00	**26.** **Maja Ravnikar:** Touch screen-operated test to detect *Potato spindle tuber viroid* that is quicker than a coffee break
19:00～19:15	**27.** **Congliang Deng**: Nucleic acid extraction method based on silica-coated magnetic particles for RT-qPCR detection of plant RNA virus/viroid
19:15～19:30	**28.** **Zhibo Zhang**: Elimination of *Chrysanthemum stunt viroid* (CSVd) from infected Argyranthemum maderense 'Yellow Empire'
20:00	**Welcome reception and Farewell banquet** **(2nd Floor of the Beijing Continental Grand Hotel)**

Abstracts of Scientific Program

KEYNOTE LECTURES I

9:00～10:30, August 24th, 2013

Chairman: Shifang Li

1. Why are viroids restricted to plant hosts: Current status of a long-standing conundrum

R. -A. Owens
Molecular Plant Pathology Laboratory, USDA/ARS, Beltsville, MD 20705 USA [retired]
Email: owensj301@hotmail.com

More than a quarter century ago, T.O. Diener (discoverer of the viroid) summarized their significance for molecular biology in a series of five questions. As described by other keynote speakers, the molecular mechanisms of viroid replication are now understood in some detail, and the nature of molecular signals mediating the interaction of viroids with various host proteins (including those involved in RNA transcription and RNA-mediated gene silencing) are currently under active investigation. Because viroids are non-coding RNAs, the mechanism(s) by which they induce disease in infected plants are also of great interest – with potentially important implications for gene regulation in all eukaryotic cells. Two final questions are less amenable to experimentation but of considerable theoretical interest: How did viroids originate? And why are they restricted to higher plants? Several years later, when Diener proposed that viroids and certain circular satellite RNAs are "relics of pre-cellular evolution" formed in the RNA world, virus genomics and taxonomy were still in their infancy. Recent advances in virus genomics have greatly stimulated interest in the origins and evolution of viruses, and at least two groups have emphasized the linkage between the evolution of viruses and that of their hosts. Furthermore, evidence has been presented indicating that major transitions during the formation of eukaryotic cells created new opportunities for virus evolution. One such transition distinguishing plant from animal cells was the formation of the chloroplast via fusion of a free-living cyanobacterium with a non-photosynthetic eukaryotic cell. Implications of this newer information will be discussed.

2. Advances in Understanding Begomovirus Satellites

X.-P. Zhou
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Biotechnology, Zhejiang University, Hangzhou 310058, China
Email: zzhou@zju.edu.cn

Begomoviruses are numerous and geographically widespread viruses that cause devastating diseases in many crops. Monopartite begomoviruses are frequently associated with betasatellites or alphasatellites. Both betasatellite and alphasatellite DNA genomes are approximately half the size of begomovirus DNA genomes. Betasatellites are essential for induction of typical disease symptoms. The *βC1* genes encoded by the betasatellites have important roles in symptom induction, in suppression of transcriptional, and in posttranscriptional gene silencing, and they can affect jasmonic acid responsive genes. Host plants of begomoviruses have evolved diverse innate defense mechanisms against the βC1 protein to counter these challenges. Alphasatellites have been identified mainly in monopartite begomoviruses that associate with betasatellites and have no known contributions to pathogenesis of begomovirus-betasatellite disease complexes. Applications of current molecular tools are facilitating viral diagnosis and the discovery of novel species of geminiviruses and satellite DNAs and are also advancing our understanding of the global diversity and evolution of satellite DNAs.

3. Development of next-generation technologies for the identification and discovery of viruses and viroids

S.-W. Ding
Department of Plant Pathology and Microbiology, University of California-Riverside
Email: dingsw@ucr.edu

SESSION I 11:00～12:45

Identification and characterization of new/emerging viroids and viroid-like RNAs and risk assessment

Chairmen: John W. Randles and Qingfa Wu

4. Characterization and detection of emerging viroids in North American greenhouse tomatoes

K.-S. Ling[1], R. Li[1] and S. Sombat[1,2]
[1]*U.S. Department of Agriculture-Agricultural Research Service, U.S. Vegetable Laboratory, 2700 Savannah Highway, Charleston, SC 29414, USA.* [2]*Plant Protection Research and Development Office, Department of Agriculture, Phaholyothin Rd., Chatuchak, Bangkok 10900, Thailand.*
Email: kai.ling@ars.usda.gov

Tomato is an economically important vegetable in many countries around the world, with major production centers in China, the U.S., Spain, Italy, India, Turkey, and Egypt. Although most tomato production is field grown, there is a growing trend in protective production (greenhouse). Nearly 40% of fresh tomatoes in the U.S. supermarkets are produced in greenhouses. In recent years, at least three distinct viroids, including *Tomato chlorotic dwarf viroid* (TCDVd), *Mexican papita viroid* (MPVd) and *Potato spindle tuber viroid* (PSTVd), have been identified on greenhouse tomatoes in Canada, Mexico and the United States. The intensive production practices and the protective growing environment appeared to facilitate the outbreaks of viroid diseases. Mixed infection of viroids with other common greenhouse tomato viruses (i.e., *Pepino mosaic virus*) posed additional challenges to achieve an accurate identification for the actual causal agent, thus complicated the situation in disease management. To achieve a better understanding of these emerging viroid diseases, it was necessary to characterize the molecular and biological properties of the viroids and to develop sensitive detection methods. Here, we present analysis of natural population genetics of tomato viroids in North America, generation of infectious cDNA clones for MPVd, and development of genus and species-specific detection methods, including real-time RT-PCR and loop-mediated isothermal amplification. With an increasing concern in a possible seed-transmission, we characterized the seed-transmissibility of PSTVd in tomato and developed a standard seed health test. Finally, we will discuss the possibility of using disinfectants to manage the spread of viroid diseases in tomato.

5. First report of the detection of *Grapevine yellow speckle viroid* 1 RNA in bottled wine

N. Habili and J.-W. Randles
Waite Diagnostics, School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, SA 5005, Australia
Email: john.randles@adelaide.edu.au

Of the five viroids known to infect grapevine, *Grapevine yellow speckle viroid* 1 (GYSVd-1) occurs in all Australian commercial wine grape varieties (Chardonnay, Shiraz, Cabernet and Merlot). While methods for the extraction of DNA from wine are known, none has been developed for RNA. To test whether viroid RNA can pass through the fermentation process we developed a rapid protocol for the extraction of total nucleic acids from wines using a silica-based matrix. Both DNA (23 s rRNA gene) and viroid RNA were detected in extracts from wine samples. RT PCR amplicons of the expected size (220 bp), using primer pairs PBCVd100C 5'-AGACCCTTCGTCGACGAC and PBCVd94H 5-TGTCCCGCTAGTCGAGCGG specific for the central conserved region of the genus *Apscaviroid*, were isolated from red and white wines. Sequencing confirmed that viroid amplicons from vines and wines were similar. BLAST analysis showed that sequences representing both GYSVd-1 and GYSVd-2 were present in wine. The GYSVd-1 sequence had highest identity with an isolate from vineyards in Washington State, USA. The *Apscaviroid* generic primers amplified viroid in a 2004 Cabernet Sauvignon wine from the Yarra Valley (Victoria, Australia), the oldest wine tested. RAPD analysis detected DNAs up to 5kbp in wine. However, no significant matching was observed between the RAPD profiles of cv. Cabernet Sauvignon DNA and the DNA found in its wine. This suggests that various DNA molecules occur in wine. Deep sequencing of wine cDNA is in progress to seek sequences from other viroids or viruses.

6. Constructing elements of the disease cycle of *Coconut cadang-cadang viroid* (CCCVd) from its epidemiology

J.-W. Randles, A. Alfiler, C.-M. Carpio, D. Hanold, E. Pacumbaba, L. Perera, M.-J.-B Rodriguez, G. Vadamalai and B. Zelazny.
School of Agriculture Food and Wine, University of Adelaide, Waite Campus, Urrbrae, South Australia 5064, Australia.
Email: john.randles@adelaide.edu.au

CCCVd causes cadang-cadang disease of coconut palms in the Philippines, but its epidemiology is poorly understood and management is based on viroid diagnosis and exclusion. Sporadic epidemics occur, yet principal reservoirs of viroid and the mode of natural transmission required for constructing the disease cycle remain unknown. Epidemics develop from interactions between pathogen, plant, vector, and environment. Their patterns of development provide clues to modes, timing and rates of spread of pathogens. Several patterns of spread with natural and artificial sources will be described, and trials to test the role of insect, seed or pollen transmission outlined. CCCVd influences disease expression, with molecular remodelling and mutation associated with different stages of the disease and severity of symptoms. While northern blotting and ribonuclease protection assays have shown that viroid variants occur naturally, the lack of a good assay host has precluded experimentation on the infection, replication and pathogenicity processes. Next generation sequencing approaches have yet to be developed for molecular profiling of viroid populations in infected palms. Recent confirmation that CCCVd is associated with an orange leaf-spotting syndrome in oil palm raises the possibility of using symptoms to map the natural spread of CCCVd in an oil palm model. As oil palm can be routinely cloned and cultured axenically, viroid-host interactions could be more conveniently studied than in coconut. The oil palm system could then provide a basis for studying the disease cycle of cadang-cadang in coconut.

7. Identification and molecular characterization of *Apple dimple fruit viroid* in China

T. Ye, S.-Y. Chen, R. Wang, L. Hao, H. Chen, N. Wang, L.-Y. Guo, Z.-F. Fan and T. Zhou
Department of Plant Pathology, China Agricultural University, Beijing 100193, China
Email: taozhoucau@cau.edu.cn

In a survey of apple virus diseases in China, typical symptoms of *Apple dimple fruit viroid* (ADFVd)-infections were observed. The presence of ADFVd in the symptomatic fruits and several leaf samples collected from young trees was confirmed by Reverse-transcript PCR and Dot-blot hybridization. Full sequences of four ADFVd variants were obtained. Sequence alignment and RNA structure analysis showed two nucleotides variations and loops alterations in the genome of Chinese ADFVd variants compared with isolates and variants previously reported in Italy. Phylogenetic analysis revealed ADFVd isolates and variants reported worldwide were clustered in relation to geographical locations, and all the Chinese variants belong to the same clade. This is the first report of identification and molecular characterization of ADFVd in China.

8. Identification of new viroid-like circular RNAs from grapevine and apple plants

Z.-X. Zhang[1], S.-S. Chen[1,2], S.-S. Qi[3], S.-W. Ding[4], Q.-F. Wu[3] and S.-F. Li[1]
[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China;[2]Department of Fruit Science, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, china. [3]School of Life Sciences, University of Science and Technology of China, Hefei, 230027, China; [4]Department of Plant Pathology and Microbiology, Institute for Integrative Genome Biology, University of California, Riverside, CA 92521
Email: sfli@ippcaas.cn

Small interfering RNAs (siRNA) are produced abundantly in plants and animals infected by pathogens due to host defense mediated by RNA silencing. In principle, pathogens including virus and viroid could be identified though assembly of siRNA obtained by deep sequencing. Deep sequencing analysis becomes a routine diagnostic tool in plant pathology. Viroid-like RNAs have been identified from grapevine and apple trees using a program of progressive filtering of overlapping small RNAs (PFOR), which is a useful tool in discovery of new circular RNAs independent of sequence. The circular RNA from grapevine may be a new species of the genus *Apscaviroid* because of the following characteristics: i) it forms a predicted rod-like secondary structure (the metastable structures hairpin I and II); ii) it has the central conserved and the terminal conserved regions, the characteristic of members of the genus *Apscaviroid*; iii) it shows a maximum of 79% nucleotide sequence identity with other viroids, which is far below the main species demarcation limit of 90%. Furthermore, sequence alignment of the RNA with *Citrus viroid* VI (CVd VI) and Persimmon viroid 2 (PVd 2) showed association of sequence duplication with enlargement of viroid genome. The new circular RNA detected in apple that consists of 434 nucleotides and has ribozyme activity. Identification of this RNA confirmed the presence of ASSD-RNA-2 purified from apple with apple scar skin disease thirty years ago. However, a survey of the RNA revealed that the presence of the ASSD-RNA-2 not always completely accompany with *Apple scar skin viroid* (ASSVd) in apple plants.

9. Characterization of variants of *Coconut cadang-cadang viroid* from coconut palm in Malaysia

M.-R.Mohammadi[1], S. Meon[1], M.-Y. Wong[1], J.-A. Daros[2] and G. Vadamalai[1]
[1]Institute of Tropical Agriculture (ITA), Universiti Putra Malaysia (UPM), 43400, Serdang, Selangor, Malaysia; [2]Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV), Avenida de los Naranjos s/n, 46022 Valencia, Spain;
Email: mr.mohammadi@hotmail.com

Coconut cadang-cadang viroid (CCCVd), the causal agent of the lethal cadang-cadang disease of coconut in the Philippines, has been associated with orange spotting disorder in oil palm in Oceania and South East Asia and variants of CCCVd have been detected and characterized from oil palm in Malaysia. In order to investigate the occurrence of CCCVd on coconut palm, leaf samples from two coconut palms showing severe yellow spotting symptom were collected and tested through reverse transcription-polymerase chain reaction (RT-PCR) using CCCVd specific primers. CCCVd was detected in both palms based on amplification of DNA product of expected CCCVd246 molecule size. Sequence analysis of the amplicons confirmed the molecules as CCCVd and showed that they carry 100 percent homology with 246nt oil palm variant of CCCVd and show substitutions of C31→U and G70→C in pathogenicity and central conserved region respectively, compared to coconut variant from the Philippines. This is first report of characterization of CCCVd from coconut palm in a region out of the Philippines and also from a host other than oil palm in Malaysia.

10. Pest risk assessment of pospiviroids: how biological and epidemiological features influence plant quarantine risk

G. Stancanelli, T. Candresse, F. Di Serio, R. Flores, A. Fox, J.-T.-J. Verhoeven and S. Winter
European Food Safety Authority, Plant Health Unit, Via Carlo Magno 1A, 43126 Parma, Italy.
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The Scientific Panel on Plant Health (PLH) of the European Food Safety Authority conducts risk assessment of plant pests and pathogens, providing scientific advice to the Commission, Parliament and Member States of the European Union (EU) and supporting science-based decisions on plant health and quarantine issues. In 2011 and 2012 the PLH panel conducted pest risk assessments for the EU of pospiviroids: *Potato spindle tuber viroid*, *Citrus exocortis viroid*, *Columnea latent viroid*, *Mexican papita viroid*, *Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid*, *Tomato planta macho viroid*, *Chrysanthemum stunt viroid* and *Pepper chat fruit viroid.* The PLH Panel also identified and evaluated risk reduction options. The key biological and epidemiological features of pospiviroids, including a relatively broad host range, the long distance spread via vegetatively propagated crops and, at least in some instances, seed- and/or insect-mediated transmission, have been analysed to assess the likelihood of introduction and spread as well as the potential impact of pospiviroids on solanaceous crops and on other ornamental species in the EU. The results of the assessments are presented and discussed in terms of how these features of pospiviroids determine their quarantine risk.

KEYNOTE LECTURES II

14:00～15:00

Chairman: Francesco Di Serio

11. Recent insights into viroids and viroid-host interactions

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Viroids, since their discovery some 50 years ago, have been a source of surprising findings that include, apart from their discovery itself (which opened a first window into a novel subviral world), the hammerhead structures (the smallest and best studied ribozymes), and the RNA-directed *de novo* methylation of genomic sequences in plants (now viewed as a manifestation of RNA silencing). Most viroids inciting diseases in economically relevant plants have been probably discovered because of the availability of techniques for the specific detection (double or sequential PAGE) and characterization (RT-PCR and deep sequencing) of these small circular RNAs; however, a new viroid affecting pepper was found not so long ago (in 2009). The situation is most likely different in non-symptomatic cultivated and wild plants, as illustrated by the identification of *Dahlia latent viroid* this same year. In the replication context, progress has been made regarding the third step of the cycle, the circularization of RNA strands; this step is catalyzed in members of the family *Avsunviroidae* by an isoform of the tRNA ligase with a predicted chloroplastic transit peptide, and in members of the family *Pospiviroidae* by the nuclear DNA ligase 1 redirected to act on RNA (emphasizing once again the extreme resilience of viroids, able to change the template and substrate specificity of some of their host enzymes). How viroids move has been also addressed, with data showing that the apparently unstructured loops in the rod-like secondary structure of *Potato spindle tuber viroid* and closely-related members of the family *Pospiviroidae,* are instead stabilized by alternative non-canonical pairs and that specific loops are critical for systemic trafficking; other data indicate that intracellular trafficking is mediated by certain viroid domains. Advances have been also achieved on the mechanism of pathogenesis: two small RNAs containing the pathogenic determinant of a chloroplast-replicating viroid guide cleavage of a host mRNA (encoding a chloroplastic heat-shock protein) as predicted by RNA silencing. Pathogenesis, however, may additionally operate through other biochemical routes. Finally, the extremely high mutation rate of a member of the family *Avsunviroidae* (the highest reported for any replicating entity), adds further credence to the notion that considers viroids as molecular fossils of the RNA world presumed to have preceded the advent of cellular organisms based on DNA and proteins. This summary of recent discoveries highlights that, far from having revealed all their secrets, viroids can still provide novel and exciting surprises in quite distinct areas of Biology.

12. Potential mRNA targets of viroid-specific small RNA

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The systemic infection of host plants by *Potato spindle tuber viroid* (PSTVd) is accompanied by the accumulation of viroid-specific small RNAs (vsRNA) that might (mis) regulate the host's gene expression via transcriptional (TGS) or post-transcriptional gene silencing (PTGS) mechanisms and lead to the viroid/host specific symptoms. Several tomato cultivars (f.e., *Solanum lycopersicum* L. cv. Heinz and Rutgers) show severe symptoms up to necrosis due to PSTVd infection, whereas other cultivars (like cv. Moneymaker and UC82B) show no or only very mild symptoms. Furthermore, the severity of induced symptoms depends on the variant of PSTVd, which differ by only a few mutations; these mutations appear frequently by evolution and adaptation to high temperatures and/or different hosts.
We performed deep-sequencing of small RNAs from tomato cultivars (Heinz, Rutgers, Moneymaker, UC82B) that were either mock-inoculated or infected with PSTVd variants QFA (mild), RG1 (intermediate), C3, or AS1 (lethal). In the deep-sequencing datasets we identified frequent vsRNAs, and selected from the tomato whole-genome data possible mRNA targets of vsRNA using stringent criteria. We selected a few of the predicted targets for qRT-PCR quantification in symptomatic AS1-, C3- and QFA-infected tomato in comparison to healthy plants. The analyses revealed disbalancing of the genes, some were down-regulated including strongly depressed auxin and ethylene response regulators (ERF4, NPH3), kinases involved in plant defense (PP2A, SERK1), leaf development transcription factor (TCP3) and R2R3Myb. Strong up-regulation was observed for other analyzed genes, particularly in H/ACA complex subunit 1 responsible for ribosome biogenesis.
Our theoretical and experimental results on PSTVd are consistent with the hypothesis on Pospiviroid-mediated plant pathogenesis via involvement of PTGS or TGS mechanisms depressing genes regulating plant development. Furthermore, vsRNAs derived from PSTVd variants inducing symptoms of different severity might aim at multiple and different targets.
Acknowledgments: The work was supported by co-operative projects FP7-REGPOT-2012-2013-1 MODBIOLIN No. 316304 and by GACR P501/10/J018.

SESSION II 15:00～16:45

Plant responses to viroid and satellite infections

Chairmen: Gustavo Gomez and Teruo Sano

13. Deep sequencing and degradome analyses: tools for further dissecting viroid-host molecular interplay

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RNA silencing is an RNA-based network regulating gene expression and defense against invasive nucleic acids in most eukaryotes, including plants. Involvement of RNA silencing in viroid-host interaction became evident when viroid derived small RNAs (vd-sRNAs) of 21-24 nt, structurally similar to host microRNAs (miRNAs) and small-interfering RNAs (siRNAs), were detected in tissues infected by nuclear and chloroplast-replicating viroids. Based on these findings, it was proposed that vd-sRNAs, similarly to miRNAs, might target host mRNAs for degradation (or translation inhibition), thus leading to symptom expression in the infected plants. In the last few years, the availability of high-throughput sequencing technologies is allowing in-depth characterization of vd-sRNAs accumulating in host tissues during infection, hence contributing significantly to further dissect possible functional roles of RNA silencing in the plant-viroid interplay. Based on these technologies, we recently characterized vd-sRNAs derived from a chloroplast replicating viroid (*Peach latent mosaic viroid,* PLMVd). Moreover, by semi-quantitative RT-PCR and RNA ligase-mediated rapid amplification of cDNA ends, we have shown that two vd-sRNAs (containing the pathogenicity determinant strictly associated with an albinism) target for degradation a host mRNA, thus providing the first experimental evidence that vd-sRNAs indeed function like miRNAs. Interestingly, the targeted mRNA codes for a protein (cHSP90) involved in chloroplast biogenesis, which is the developmental pathway specifically compromised in the albino tissues infected by PLMVd variants generating the two vd-sRNAs (Navarro et al., Plant J. 2012). Altogether these data support involvement of RNA silencing in PLMVd pathogenesis and a possible more general role of vd-sRNAs in modulating host gene expression during viroid infection. To get a deeper insight into this question, we have integrated data from high-throughput sequencing of vd-sRNAs accumulating in tissues infected by chloroplast- and nuclear-replicating viroids with the respective degradome analyses. We will present data and discuss implications of the RNA degradation patterns potentially elicited by vd-sRNAs during viroid infection.

14. Transcriptional alterations in cucumber plants infected by *Hop stunt viroid*

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Viroids are plant-pathogenic non-coding RNAs able to interfere with yet poorly known host-regulatory pathways and cause phenotypic alterations recognized as diseases. The way by which these tiny RNAs coerce the host to express symptoms remains to be totally deciphered. In recent years, diverse studies have proposed close interplay between viroid-induced pathogenesis and RNA silencing, supporting the notion that viroid-derived small RNAs (vd-sRNAs) mediate the post-transcriptional cleavage of endogenous mRNAs by acting as elicitors of symptoms expression. Although the evidence supporting the role of vd-sRNAs in pathogenesis is robust, the possibility that this phenomenon can be a more complex process, also involving viroid-induced alterations in plant gene-expression at transcriptional levels, has been considered. Here we show that in cucumber plants infected with the *Hop stunt viroid* the viroid-induced pathogenesis is also associated with transcriptional host alterations. This study reports a previously unknown mechanism associated with viroid infection in plants and provides new insights into aspects of host alterations induced by viroid infectious cycle.

15. Comparison of small RNAs derived from *Potato spindle tuber viroid* strains of different pathogenicity

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Viroids are the smallest known plant pathogens. They are single-stranded, covalent closed circular RNAs with the size range of 246-400 nucleotides. Viroids do not encode any pathogen-specific proteins and therefore viroids propagation is fully dependent on the host biochemical machinery. They exclusively infect plants and autonomously replicates via RNA/RNA pathway using host machinery. As might be expected from their highly base-paired structure and RNA–RNA mode of replication, viroids have been shown to induce RNA silencing. Accumulation of viroid-specific small RNA (vd-sRNA) has been reported upon infection in host plant. Further, involvement of such vd-sRNA has been correlated with symptom production in host. *Potato spindle tuber viroid* (PSTVd) is a type member of the viroid family *Pospiviroidae*. Only a few nucleotide changes between PSTVd strains are sufficient to induce remarkably different symptoms in infected tomato plant, i.e. *Solanum lycopersicum* cv. Rutgers. In this present study, tomato plants were infected with PSTVd mild and PSTVd severe strain, independently. After 35 days of post inoculation, small RNA was extracted from the leaves and subjected to high-throughput sequence. By bioinformatics, vd-sRNA and plant miRNA were analyzed from PSTVd mild and PSTVd severe infected plants. The vd-sRNA was mapped against respective viroid genome. A remarkably difference in the distribution of vd-sRNA hot spots has been noted. Furthermore, comparison of specific miRNAs from these infected plants with the controls revealed un-even distribution ratios suggesting their involvement in symptom expression in host plant.

16. Characterization of *Hop stunt viroid* adaptation mutations emerged during persistent infection in hops

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Hop stunt epidemic was incited by HpSVd originated from cultivated grapevines. During the epidemics, HpSVd-grape variant underwent convergent evolution in hops and resulted in a new adaptation mutant, HpSVd-hop, with five nucleotide changes at 25, 26, 54, 193 and 281. In view of "survival of the fittest", it is anticipated that the mutations could have conferred higher replication/accumulation competence in hop. To verify this, we first compared infectivity of HpSVd-hop and -grape in hop, grapevine and cucumber. The result was partially matched to the prediction; HpSVd-hop abolished the ability to replicate stably in grapevine and reduced the ability to replicate/accumulate in cucumber. Namely, the five mutations emerged in hop gave an adverse effect upon HpSVd replication/accumulation in the original host, which can be explained by "trade-off" effect. Meanwhile, HpSVd-hop didn't show advantage to HpSVd-grape in replicate/accumulate even in hop; they were co-existed evenly in hops without out-competing the other. We finally analyzed changes of HpSVd small RNA populations accumulated in HpSVd-hop and –grape infected plants. They were well consistent ether in hop and in cucumber, however, the number of HpSVd small RNAs originated from anti-genomic strand containing the nucleotide 54 or 281 were exclusively downed in plants infected with HpSVd-hop. The specific reduction of these small RNAs gave us a speculation that two mutations in 54 and 281 at least were incited by structural restraint to escape from RNA silencing. This was supported by the data that the hop adaptation mutations actually initiated from the nucleotide 54 and 281.

17. Mechanisms of satellite RNA-mediated attenuation of viral disease symptoms in plants

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Viral satellite RNAs (satRNAs) are small non-coding RNAs that depend on their associated virus (helper virus) for replication and spread in plants. Some satRNAs exacerbate disease phenotypes by inducing their own symptoms in infected host plants, but the majority attenuates the symptoms caused by their helper viruses. How satRNAs modulate viral disease symptoms has been a longstanding question. In the current study we investigated how satRNAs attenuate helper virus-caused disease symptoms and provided a plausible model that involves satRNA-derived short interfering RNAs (siRNAs). Using Nicotiana plants and *Cucumber mosaic virus* (CMV) Y-satellite RNA (Y-Sat) as an experimental model, we demonstrate that satRNA infection releases the suppression of hpRNA-induced transgene silencing by the CMV-encoded RNA silencing suppressor 2b or tombusvirus-encoded RNA silencing suppressor P19. This is achieved by saturating P19 with satRNA-derived small interfering RNAs (siRNAs). We also demonstrate that satRNA infection minimizes the induction of miR168 by RNA silencing suppressors expressed from infecting viruses (CMV 2b) or from a transgene (HcPro of potyvirus), and this diminished miR168 induction is correlated with reduced disease phenotypes. Taken together, our results suggest that satRNAs attenuate viral symptoms by sequestering helper virus-encoded RNA silencing suppressors with satRNA-derived siRNAs, thereby preventing the suppressors from interfering with host microRNA function that is essential for plant development. Our findings have implications for symptom moderation by other subviral agents such as defective interfering viral RNAs that are associated with both plant and animal viruses.

SESSION III 16:30～17:45

Genetic diversity, Replication and Detection methods

Chairmen: Dr. José-Antonio Daros and Dr. Matilde Tessitori

18. Sequence diversity of *Chrysanthemum stunt viroid*: multiple polymorphic positions throughout the genome

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The extent of natural sequence variation among isolates of *Chrysanthemum stunt viroid* (CSVd) was examined by sequencing cDNA clones, by "deep sequencing," and by comparison with the global sequences obtained from the GenBank. The nucleotide sequences from cDNA clones of three CSVd isolates (one each from USA, China and Australia) were compared. The US and Australian isolates were found to be quasi-species, while the Chinese isolate contained only a single variant. Sequence variation also was examined within two Korean isolates by comparing the sequences obtained either from ~100 cDNA clones of each vs. by "deep sequencing" using 454 pyrosequencing technology. Both approaches showed that there was little variation within the populations of molecules examined, with the major variations detected at largely the same positions in both Korean isolates. This lesser variation may be a reflection of a recent introduction of these isolates to Korea. Finally, a comparison of the nucleotide sequences of 117 isolates and cDNA clones obtained from 16 countries showed that in some cases identical CSVd isolates were found in several countries and from multiple locations within the same country. CSVd isolates differed as much in sequence between countries as within countries. Sequence variation was observed at 103 nucleotide positions scattered through the CSVd genome. However, seven sites (positions 47, 49, 50, 64, 65, 254 and 298) showed variation in a large proportion of various CSVd isolates, with the minority nucleotide alternatives occurring in ~14 % to 32 % of the populations.

19. Host effects on the progeny of *Citrus dwarfing viroid*

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Identifying possible sources of genetic variability is significant in the case of *Citrus dwarfing viroid* (CDVd) which has been proposed as a dwarfing agent for high-density citrus plantings. In order to mimic the natural conditions of the field, a natural CDVd isolate (CMC) was used as an inoculum source for long-term and short-term bioassays in different citrus host species including both rootstock and grafted citrus species. Characterization of progenies (derived populations) indicates that the genetic stability of CDVd was high in certain hosts (Trifoliate orange, Troyer citrange, Etrog citron, Navelina sweet orange), which preserve viroid populations similar to the original CMC isolate even after 25 years. By contrast, CDVd variant populations in Interdonato lemon and Volkamer lemon were completely different to those in the inoculated sources, highlighting how influential the host is on the genetic variability of CDVd populations. The results allow to preliminarily discuss the implications for risk assessment of the use of CDVd as a dwarfing agent even if specific experiments for the genetic and pathogenetic evolution of single variant are in progress.

20. Sequence analysis of *Apple scar skin viroid* Chinese isolates revealed nine nucleotides closely related to the dapple symptom

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In recent years, apple fruits of *Malus pumila* Mill cv. 'Fushi' have been damaged by a dapple disease showing yellow spots on their surface induced by *Apple scar skin viroid* (ASSVd), and decreased their market value significantly by cutting down 2 to 3 folds than the normal fruits in price in China. Many ASSVd variant sequences have been reported, while whether there are some genomic motifs within the viroid sequence modulating their pathogenicity keeps unknown. Thirteen 'Fushi' apple fruit samples were collected from three orchards in Yantai, Shandong province, and twelve 'Fushi' apple fruit samples were purchased in a vegetable market in Shanxi province, China. RT-PCR identification of ASSVd within these fruit samples demonstrated that ten samples were positive and demonstrated a high incidence of ASSVd infection in these regions, even without consideration of five samples preferentially collected due to their dapple phenotypes. Five to ten clones for each isolate derived from the positive samples were sequenced, aligned and analyzed phylogeneticly, four isolates derived from the samples exhibiting the symptom of yellow spots were clearly separated from the others without showing the symptom in phylogenetic tree, in which the four isolates were clustered together, and in sequence alignment by showing unique nucleotide variation including of A_{38}, A_{43}, A_{44}, T_{47}, A_{247}, T_{256}, T_{256}, T_{285}, and G_{286}. Although strict experiments are required to confirm our conclusion, the results provide basic information for further identification of the pathogenecity determiner for ASSVd.

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21. Stability of variable domain of *Potato spindle tuber viroid*

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Viroids present simple models to study the role of RNA structures during replication and systemic trafficking. These small RNAs do not encode proteins and are not encapsidated and therefore express all biological function directly from RNA. *Potato spindle tuber viroid* (PSTVd), as the type member of the family Pospiviroidae, adopts a rod-like secondary structure with five domains. This elongated conformation, comprising loops and bulges, is biologically significant. To examine the function and the degree of tolerated changes within the lower strand of variable domain we have constructed two different mutants using available restriction site of Ava II (220-224 nt. position). Mutants that contain insertion (PSTVd-AvaK) or deletion (PSTVd-AvaMB) of three nucleotides were infectious and causing severe/intermediate or mild/intermediate disease symptoms, respectively. Analysis of sequences obtained from PSTVd-AvaK infected tomato plants revealed existence of PSTVd-AvaK variants at an early stage of infection (4-5 weeks post inoculation). However, at 7-8 wpi reversion to parental sequence were observed. In the case of PSTVd-AvaMB, besides reversion to parental sequence and PSTVd-AvaMB sequence, new mutants were observed. All of them have contained additional mutations outside the AvaII mutated site or extra 3 nt. at Ava II site (different than parental). Obtained mutants were further analysed. Most of them were infectious but not genetically stable, moreover this stability were stronger in tomato roots than in leaves.

22. RNA circularization during viroid replication

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Viroids are small circular RNAs (246-401 nt) that infect plants. Despite their small size and lack of protein-coding any ability, they are able to complete a complex infectious cycle in the host plant. The more than 30 viroid species currently known are classified in two families: *Pospiviroidae*, including viroids that replicate in the nucleus through an asymmetric rolling-circle mechanism, and *Avsunviroidae*, including viroids that replicate in the chloroplast through a symmetric rolling-circle mechanism. During replication, the infecting viroid circular genomic RNA (attributed + polarity) is copied by a host RNA polymerase to produce oligomeric RNAs of complementary polarity (−). These oligomeric (−) RNAs, either directly (*Pospiviroidae*) or after cleavage and circularization (*Avsunviroidae*), serve as templates to produce new oligomeric RNAs of (+) polarity, which are processed to monomers and finally circularized. In the family *Pospiviroidae*, viroid RNA synthesis is carried out by the host RNA polymerase II, whereas oligomeric RNA cleavage is mediated by a type III RNase. In the *Avsunviroidae*, viroid RNA synthesis is carried out by a nuclear-encoded chloroplastic RNA polymerase and RNA cleavage by the viroid hammerhead ribozymes. Despite circularity being one of the viroid's distinctive property, the factors mediating RNA circularization during viroid replication have remained unknown.

In our work, we obtained a chromatographic fraction of tomato leaf proteins able to efficiently circularize the monomeric linear replication intermediate of *Potato spindle tuber viroid* (PSTVd, family *Pospiviroidae*) opened at position G95-G96, with 5'-phosphomonoester and 3'-hydroxyl termini. This activity showed specificity with respect to the terminal groups and the position at which the RNA substrate was opened, and required Mg^{2+} and ATP. Mass spectrometry analysis of this protein fraction allowed identifying the host DNA ligase 1 as the candidate enzyme to mediate the circularization reaction. Using a recombinant version of tomato DNA ligase 1 expressed in *Escherichia coli*, we confirmed the capacity of this enzyme to circularize the PSTVd monomeric linear replication intermediate. By means of RNA silencing assays, we demonstrated its participation in PSTVd circularization *in vivo*.

On the other hand, we cloned and expressed in *E. coli* the chloroplastic isoform of eggplant tRNA ligase. This recombinant protein was able to efficiently circularize the monomeric linear (+) and (−) RNAs of *Eggplant latent viroid* (ELVd) and of the other three members of the family *Avsunviroidae*. The reaction was also highly

specific of the hammerhead ribozymes cleavage sites. Virus-induced gene silencing of tRNA ligase in *Nicotiana benthamiana* plants expressing transitorily ELVd RNAs supported the participation of this enzyme in the circularization of the viroids within the family *Avsunviroidae* during replication.

In summary, our work indicates that viroids recruit host enzymes to mediate RNA circularization during replication. Whereas members of the family *Pospiviroidae* subvert the activity of DNA ligase 1 forcing it to ligate RNA, members of the family *Avsunviroidae* take advantage of the activity of the chloroplastic isoform of tRNA ligase.

SESSION IV 18:00～19:30

Detection methods and control of viroids

Chairmen: Dr. Peter Palukaitis and Dr. Dongmei Jiang

23. Detection of CCCVd variants in oil palm using real-time PCR.

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Coconut cadang-cadang viroid (CCCVd) is the causal agent of the lethal cadang-cadang disease in the Philippines. In Malaysia, several sequence variants of CCCVd were found in oil palms but in low concentration. Existing molecular methods used for detection of this viroid is time-consuming and insensitive. Therefore, SYBR-green based real-time PCR assay has been developed to rapidly detect CCCVd variants in oil palm which is of low concentration. The optimal annealing temperature for this assay was at 60ºC. A standard curve was generated using a positive control (plasmid with Oil Palm CCCVd 246nt insert) with 10-fold serial dilution series ranging from 10^{-1} to 10^{-5} copies/µl. Oil palm CCCVd variants were successfully detected from infected field samples within 1 hour, whereas healthy palm showed no amplification. The detected samples were validated through sequencing and confirmed to be a variant of CCCVd with 246nt.

24. A rapid detection method for viroids in the genus *Coleviroid*

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Viroids are the smallest autonomous infectious nucleic acids known today. *Coleus blumei* (coleus) is an ornamental plant grown worldwide and it can be infected by several viroid species in the genus *Coleviroid*, family *Pospiviroidae*. Up to now, six main viroids infecting coleus have been reported: *Coleus blumei viroid*-1～*Coleus blumei viroid*-6 (CbVd-1～CbVd-6). As members of the family *Pospiviroidae*, all of the six known coleus viroids share a common central conserved region (CCR) sequence. Here, a simple and low-cost hybridization method was developed for coleus viroids. A universal cRNA probe (8CCR-probe), which was performed using an octamer of 32-nucleotide sequence derived from the CCR region of coleus viroids, can be used to detect at least four coleus viroids by hybridization simultaneously. Dot-blot hybridization result showed that the sensitivity of 8CCR-probe was similar to the specific probes for each CbVds. Northern hybridization results revealed that all the four coleus viroids in the coleus samples, which were displayed in the Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) result, could be distinguished readily and simultaneously using the 8CCR-probe. Batch detection assay showed that results of the hybridization analysis using the 8CCR-probe were agreed with the results obtained using specific probes. This detection method for coleus viroids is reliable, rapid and low-cost, and it is an effective way to survey the occurrence of coleus viroids or select viroid-free coleus seedlings. In addition, this method also provides reference for the development of rapid detection method for other viroids, and even viruses.

25. Situation, detection and control of *Potato spindle tuber viroid* in China

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26. Touch screen-operated test to detect PSTVd that is quicker than a coffee break

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Increased global trade with fresh plant material offers additional possibilities for plant pathogens to spread around the world efficiently. One such pest is *Potato spindle tuber viroid* (PSTVd), affecting mainly potato and tomato production. Early detection of PSTVd infected material at the entry points (such as ports, airports, border crossings) can prevent its spread and possible consequent economic loss. Early detection of PSTVd is particularly important because of its easy mechanical transmission and long survival out of plant host e.g. we have shown that it can survive for more than 1 month in water. The desired method should be fast and simple and should perform well in and outside diagnostic laboratories. Here we describe accurate detection of PSTVd using single tube RT-LAMP isothermal amplification that gives results in 30 minutes including all hands-on work and sample preparation. The test can be performed in a touch screen operated portable device that detects amplification in real-time based on fluorescence. The use of intercalating dye enables an additional confirmation: melting curve analysis of the amplicon. It can be performed in one tube together with DNA target detection e.g. *Ralstonia solanacearum*, using melting curve analysis for discrimination between both pathogens.
LAMP PSTVd test was tested with over a broad range of PSTVd isolates successfully detecting all of them. Due to high sequence similarities it also gives signal with some *Tomato chlorotic dwarf viroid* isolates and *Tomato planta macho viroid* isolates, of which both can be distinguished from the PSTVd amplicon based on melting curve analysis. While the test is approximately ten times less sensitive than real-time PCR it is ten times more sensitive than conventional RT-PCR. Recently, the performance of the test in combination with an improved RNA extraction protocol has been evaluated on a more complex matrix of tomato seeds. The potentials and risks of different methods used will be compared and discussed.

27. Nucleic acid extraction method based on silica-coated magnetic particles for RT-qPCR detection of plant RNA virus/viroid

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To develop a reliable and rapid nucleic acid extraction method and detecting technique for certification and quarantine programs on plant materials, the extraction method based on silica-coated magnetic particles (SMPs method) and RT-qPCR assay were described. *Arabis mosaic virus* (ArMV), *Lily symptomless virus* (LSV), *Hop stunt viroid* (HSVd) and *grape yellow speckle viroid 1* (GYSVd-1)was selected as research objects and the sequences including amplification regions of RT-qPCR were transcribed in vitro as standard RNA templates. The standard curves covered six or seven orders of magnitude with detection limits of 100 copies per each assay. The extraction efficiency of SMPs method was evaluated by recovering spiked ssRNAs from plant samples and compared to the commercial kits, i.e., TRIzol and RNeasy Plant mini kit. The results showed that the recovery rate of SMPs method was roughly equal to the commercial kits (TRIzol and RNeasy Plant mini kit) when spiked ssRNAs were extracted from lily leaves, whereas it was higher than commercial kits when spiked ssRNAs were extracted from grapevine leaves. SMPs method was used to extract viral nucleic acid from 30 lily-leaf samples and compared to other two methods, of which each 15 had been respectively ArMV-positive and LSV-positive. The positive results with these three methods were in accordance and the differences on detecting ArMV was not statistically significant, but on LSV detection, the mean viral load of SMPs method was just $0.5\log_{10}$ lower than RNeasy Plant mini kit and had no significant with TRIzol. Nucleic acid was extracted with SMPs method from 19 grapevine-leaf samples in contrast with commercial kits and subsequently HSVd and GYSVd-1 were screened by RT-qPCR. The results suggested that SMPs method was superior to other methods on both positive rate and the load of viroids regardless of HSVd or GYSVd-1. In conclusion, SMPs method was able to efficiently extract the nucleic acid of RNA viruses or viroids for RT-qPCR detection, with particular emphasis on viroids.

28. Elimination of *Chrysanthemum stunt viroid* (CSVd) from infected Argyranthemum maderense 'Yellow Empire'

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Chrysanthemum stunt viroid (CSVd) was first reported in the US in 1940s and has to date been found to be widespread in the world wherever chrysanthemum is grown. CSVd can cause considerable economic losses in the chrysanthemum industry. In Norway, CSVd causes serious problems in the production of *Argyranthemum,* a genus closely related to *Chrysanthemum*. In our project, different methods have been used to eliminate CSVd, including meristem culture, cryotherapy and chemotherapy combined with meristem culture. In our study, none of these methods have worked to eradicate CSVd from infected *Argyranthemum maderense* 'Yellow Empire' (YE). To understand why none of these methods worked, we decided to determine the distribution of CSVd by in situ hybridization in YE, and also in *Argyranthemum frutescens* 'Border Dark Red' (BDR): an alternative cultivar used for comparison. Our localization studies showed that in YE, CSVd is distributed all over the phloem, and also invades the leaf primordia 1 and 2 and even the first cellular layer of the apical meristem. On the other hand, in BDR, CSVd was found to invade the phloem and up to leaf primordium 3, but not the leaf primordia 1 or 2 nor the apical meristem. The methods we have employed rely on viroid-free meristematic tissue for regeneration of healthy material. Thus, our results might explain why none of the methods we used was able to eliminate CSVd from YE. In addition, our in situ hybridization studies showed that the cellular distribution of CSVd can be different even in two closely related plant species.
We are currently exploring a different approach. Our preliminary results show that by maintaining CSVd infected material at low temperature (5 ^{o}C) for a prolonged time can result in a lower amount of CSVd infected cells. Consequently, we are investigating if low temperature treatment combined with meristem culture and/or cryotherapy can result in CSVd eradication.

General Abstracts

1. Oligonucleotide microarray detection of viroids at genus level

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A major challenge in agricultural industry is the development of techniques that can screen plant materials for viroid infection. The microarray technique shows promise in this regard, as their high throughput nature can potentially detect a range of viroids using a single test. In this paper, we present a microarray that can detect a wide spectrum of all the 8 reported viroid genera including 37 known plant viroid species. The array was constructed using an automated probe design protocol which generated a minimal number of probes to detect viroids at the genus level. The designed arrays showed a high specificity and sensitivity when tested with a set of standard virus samples. Microarray screening was applied on an infected citrus sample. *Hop stunt viroid* infection was identified as the major disease causing pathogen for the infected citrus sample.

**These authors contributed equally to the paper.*

2. Molecular characterization of *Apple scar skin viroid* from pear grown in China

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Apple scar skin viroid (ASSVd) is the type species of genus *Apsacaviroid* in family *Pospiviroidae* The ASSVd infections can induce severe apple fruit cracking, scarring and distortion or color dappling, depending on the cultivar. In pear, its infections are usually symptomless except for rusty skin and Japanese pear fruit dimple. Previously, pear plants carrying ASSVd was suggested as a pathogen source for apple scar skin or dappling occurred widely in China. In this study, we surveyed four different pear-growing regions in China. Totally, 239 symptomless pear plants were analyzed for the presence of ASSVd by RT-PCR with a set of ASSVd-specific primer, which amplified the full cRNA of ASSVd. Forty one plants were ASSVd positive. Two or three clones of each RT-PCR product from 14 plants were sequenced. The obtained sequences had 90.7–100% similarity with each other, and 87-99% similarity with the viroid sequences available in Genbank. The sizes of full cRNA varied from 330 nt to 335 nt long. The sequence variants of 330 nt (21 out of 41 sequences) were predominant, and the second predominant variants were 334 nt (13 clones). The most of plants tested contained ASSVd variants with two different sizes. Phylogenetic analysis revealed that the obtained sequences grouped into two clusters, of which one consisted of sequences from pear ASSVd isolates and the other one also contained different apple isolates from China, Korea and Japan. Those results indicated that the sequence divergence of ASSVd might have a host relationship.

3. Detection of four grapevine viroids and Sequence Analysis

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Viroids detection was preformed by RT-PCR using the universal primer designed from the conserved region of the *Apscaviroid* group and four viroids (GYSVd1, GYSVd2, AGVd, HSVd) were acquired from the grapevine samples of Liaoning, Shandong, Gansu and Ningxia province. The viroids were successfully detected in majority samples and infection rates of GYSVd1, GYSVd2, AGVd and HSVd were 41.7% (20/48), 22.9% (11/48), 75% (36/48), and 83.3% (40/48), respectively. Sequence analysis showed that GYSVd1 isolate from Liaoning province (GU170805) and Australian Type I was clustered into the same group, sharing 99% sequence identity. This Liaoning GYSVd1 isolate showed sequence identities with Australia and Japan Type II, China GYSVd3, and America and Japan Type III were 97%, 89%, and 89%, respectively, and had far relationship with all the GYSVd2 isolates. The Liaoning AGVd isolate (HM211854) shared 97% sequence identity with other AGVd isolates. The sequence identity of HSVd isolate from Shandong province (HM357802) was higher than 98% comparing with Beijing and Japan grapevine isolates, and lower than 97% with isolates from potato, hop and citrus.
Key words: grapevine viroids; RT-PCR detection; sequence analysis

4. NCBI resources for viroid genomes and classifications

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As part of the Viral Genome Project at National Center for Biotechnology Information (NCBI), complete viroid genomes are collected from all viroid sequences in GenBank. A reference sequence record is created for each species, and other complete genomes within the same species are tagged as neighbors. The reference sequence records are curated by NCBI staffs, who work closely with the ICTV Study Group for viroids, to maintain high standards of annotation and taxonomic classification. The viroid reference sequences are available at http://www.ncbi.nlm.nih.gov/genomes/GenomesGroup.cgi?taxid=12884&opt=Viroid. One of the tools to help the taxonomic classification of viroids (and some virus groups) is PASC (for Pairwise Sequence Comparisons), which was developed in NCBI. PASC calculates the pairwise identities of viroid sequences within a family and displays their distributions, and can help determine demarcations at different taxonomic levels such as variant, species, genus and subfamily levels. PASC can be used to classify new viroid genomes to the proper taxa. PASC can also identify viroid records that were mis-assigned in the NCBI taxonomy database. PASC for the *Avsunviroidae* family and the *Pospiviroidae* family are available at http://www.ncbi.nlm.nih.gov/sutils/pasc/viridty.cgi?cmdresult=main&id=395 and http://www.ncbi.nlm.nih.gov/sutils/pasc/viridty.cgi?cmdresult=main&id=396 respectively.

5. Analysis of sequence variation of *Potato spindle tuber viroid* in viroid-infected potato and tobacco plants

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The genetic stability of *Potato spindle tuber viroid* (PSTVd) from three potato areas of Heilongjiang in China was analyzed. PSTVd samples of PKS 1-6 from Keshan was cloned and sequenced. Compared with severe strains(KF 440-2 and RG1) and intermediate strain (PSTVd int) reported in Genebank, PKS 1-6 had more differences in sequence(identity were only 97.21%, 97.77% and 98.61%, respectively), whereas PKS 1-6 was more constant in sequence with mild strains(KF-5, GI 333357 and GB X76844) (identity were 99.16%, 97.72% and 99.44%, respectively). To inoculate PKS 1-6 isolate to *N. Benthamiana* plants and analysis the sequence of tobacco samples infected PSTVd, three of four PKS 1-6 progenies in *N. Benthamiana* had mutations, and one maintain parental sequence. The mutant region located in the CCR (at position 81, 82 and 267), Var (at position 127 and 136), and Path(at position 56). For sequence analyses of PSTVd samples collected in Fujin potato field, five in six clones had the identical sequence with PKS 1-6, and only PFJ-14 variant had 2 mutants at position 4(T_L) and 296(Path). But for sequence analyses of PSTVd from Taikang, four in eleven clones were identical compared with PKS 1-6. A total of 22 clones were sequenced. Among them, 12 mutations had been observed. In all clones, the number of mutant nucleotides was between 0～3, and the homology was between 99.16%～100%, and the lowest free energy difference was between 159.10%～173.6% kcal/mol. The mutation mainly located in CCR and Var(variant frequent were 38.1% and 28.9%, respectively), followed by Path region(variant frequent was 14.3%) and T_L/T_R region(variant frequent was 9.5%).

6. Improved nucleic acid spot hybridization detection technique for *Potato spindle tuber viroid*（PSTVd）

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Potato spindle tuber viroid (PSTVd) is one of the major diseases that threaten the potato production in China. Rapid and sensitive detection technique is the key mean of controlling this disease. Nucleic acid spot hybridization (NASH) is one of the most important detection techniques for PSTVd. But, most small laboratories or companies in China lack the equipments for the detection. In order to extend this technique widely in China, we established an efficient hybridization system, in which a highly sensitive and specific dimeric probe labeled with digoxin was applied and CDP star was used as the substrate of reaction. The steps of sample denaturation and pre-hybridization were simplified, and a simple RNA extraction method was utilized. Hybridization oven were substituted for UV lamp in a clean bench to fix RNA and shaking incubator to hybridize the sample. The samples of leaves, tubers and sprouts could be detected successfully. The minimum concentration of PSTVd detected by this system was 0.05 pg. A kind of simple, rapid and sensitive NASH kit for PSTVd detection was also developed successfully and used in routine detection without any expensive equipments.

7. CSVd and CChMVd quasispecies populations assessed via 454 pyrosequencing

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Chrysanthemum stunt viroid (CSVd) and *Chrysanthemum chlorotic mottle viroid* (CChMVd) were identified from chrysanthemum plants showing stunting, distortion, and chlorotic leaf symptoms in Korea. Pyrosequencing technology is a powerful tool to determine rapidly sequences of viroid quasi-species without requiring the traditional approach of cloning procedures. In this study, we investigated population of both CSVd and CChMVd using pyrosequencing technology and we assessed the effectiveness of pyrosequencing technology with conventional Sanger-sequencing method for analyzing quasi-species population of the two viroids. CSVd-specific RT-PCR products from a single CSVd-infected chrysanthemum and CChMVd-specific RT-PCR products from a double- infected (CSVd and CChMVd) chrysanthemum plant were analyzed and directly sequenced by the high-throughput platform 454-pyrosequencing. A total of 114,673 nucleotide (nt) variations from CSVd and 93,268 from CChMVd were identified by 454-pyrosequencing. High variation of nucleotide sequences was observed on 47, 49, 50 and 298 nt in the left half of the secondary structure proposed for the Korean isolates of CSVd. No mutations were found in the central part of the upper conserved region. On the other hand, nt sequences of CChMVd showed highly variable sites; at nt positions 38, 93, 136, 138, 154, 156, 200, 231, 292, 335, 346 and 373, all around the secondary structure of CChMVd. These results were verified by sequence analyses of the same RT-PCR products using conventional Sanger-sequencing method. These data suggested that 454-pyrosequencing is a useful technology to access rapidly the quasi-species population of CSVd and CChMVd variants being generated from infected chrysanthemum species. The detail relationship between population structure of the two chrysanthemum viroids and sequence variations of their genomes is discussed.

8. Development of a polyriboprobe for detecting pospiviroids

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Spread of viroids belonging to the genus *Pospiviroid* (family *Pospiviroidae*) has been recently recorded in ornamentals and vegetables in several countries of the European Union (EU). Major concerns derive from the identification of *Potato spindle tuber viroid* (PSTVd) and *Chrysanthemum stunt viroid* (CSVd), which are quarantine pests in EU. Effectiveness of control measures against viroid spread, which are based mainly on the use of viroid-free propagation material and on the interception of infected plants, requires fast, reliable, sensitive and economic detection methods. Methods allowing simultaneous detection of several viroid species largely contribute to contain costs. In this context, a single molecular probe (polyprobe), composed of assembled sequence fragments from most viroid species belonging to *Pospiviroids* genus (family *Pospiviroidae*) appears very appropriate for detecting them by molecular hybridization because only one single transcription reaction is needed. Based on bioinformatics analyses of pospiviroid genomic RNAs, we have developed a polyprobe (POSPIprobe) that detects at least eight different pospiviroid species, including PSTVd and CSVd. Partial sequences from four different pospiviroids were selected and cloned directionally to generate a single vector that was used for synthesizing the POSPIprobe by *in vitro*-transcription. POSPIprobe specificity for eight pospiviroids was confirmed by Northern-blot, while its sensitivity was tested by dot-blot assays, providing similar results to that obtained using single probes separately. Dot-blot analyses with the POSPIprobe was validated by simultaneously testing 68 samples from tomato, chrysanthemum, *Argyranthemum frutescens* and pepper infected by different pospiviroids, confirming the high potential of this probe in quarantine, certification and survey programs. These data have been recently published in *Journal of Virological Methods* 2012.

9. Viroid-like RNAs from cherry: the first satellite RNAs of micoviruses?

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Several years ago, two cherry small circular RNAs (cscRNA1 and cscRNA2) and 10–12 double stranded RNAs (dsRNAs) of presumable viral origin were identified in sweet and sour cherry plants grown in Italy. These RNAs were found to be closely associated to a disease termed cherry chlorotic rusty spot (CCRS). The Italian CCRS disease is symptomatologically very similar to the Amasya cherry disease (ACD) described previously in Turkey, and both diseases have been closely associated with similar mycelium-like structures and with double-stranded (ds) RNAs of mycoviruses from the genera *Chrysovirus*, *Partitivirus* and *Totivirus.* More recently it has been realized that cherry trees from Spain affected by cherry leaf scorch (CLS), a fungal disease reported to be caused by *Apiognomonia erythrostoma,* family *Gnomoniaceae* (order *Diaporthales*), show a very similar symptomatology (translucent-chlorotic leaf spots evolving into rusty areas). However, although CLS- and CCRS-affected trees have been associated with similar mycelia, fungal fructifications, fungal genes and mycoviral dsRNAs sharing high sequence similarity — further supporting a close relationship between these two disorders— the involvement of *A. erythrostoma* in CCRS has not been conclusively shown (Carrieri et al., J. Plant Pathol. 2012). Here we report that a small viroid-like RNA similar to cscRNA1 associated with CCRS is also present in CLS-affected trees, thus providing an additional link between them. Comparisons between cscRNAs from CLS and CCRS isolates have shown several common features, including sequence identity (83%), a quasi rod-like conformation with short bifurcations at both termini, and the presence of hammerhead ribozymes in both polarity strands. However, the CLS isolate lacks the recombinant molecules of smaller size (cscRNA2). Although the biological nature of cscRNAs remain to be conclusively shown, the identification of at least cscRNA1 in different cherry cultivars and geographic areas (Spain and Italy) in close association with the same mycoviral dsRNAs, strongly support the satellite nature of the cscRNAs, which could thus be the first mycovirus satellite RNAs.

10. Bioinformatics tools for detecting viroids in deep sequenced small RNA libraries

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Viroid RNAs are targeted by Dicer-like enzymes and, as a consequence, viroid-derived small RNAs (vd-sRNAs) with structural features similar to the small interfering RNAs (siRNAs) and microRNAs (miRNAs) accumulate in the infected tissues. Several studies have demonstrated that deep sequencing of small RNAs (sRNAs) from viroid-infected plants provides vd-sRNAs covering the entire genome, thus indicating that the source viroid can be in principle reconstructed. Based on this premise, we have developed an approach for identifying known and unknown viroids and viroid-like (circular) RNAs in sRNA libraries. The workflow is based on stable open source tools and can be used for different levels of viroids and viroid-like (circular) RNA identification. Since vd-sRNAs are mainly of 21 and 22 nucleotides, we retrieved the sRNAs with these sizes and run an assembly with the program Velvet. The resulting contigs are then further processed to search for overlaps using the CAP3 program. At this stage we checked whether the 'supercontigs' can be circularized. In a second step, we examined circularized and non-circularized sequences with the PatSearch program for the presence of conserved sequence/structural motifs in viroids, like hairpin I (family *Pospiviroidae)* and hammerhead ribozymes (family *Avsunviroidae)*. The output of the workflow is a set of circular sequences with or without viroid-related structural description, and a set of linear sequences with structural description and, therefore, a catalog of potential viroids and viroid-like RNAs. With respect to a previous method designed to identify circular RNAs in sRNA libraries, our approach has the advantage of linking together several bioinformatics tools widely used in other contexts, with parameters that can be easily modified and adapted to a specific search for circular RNA species or secondary structures such as those of viroids.

11. Molecular characterization of *Coleus blumei viroid-5* isolated from China and Indonesia

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Viroids are the smallest pathogens known today. *Coleus blumei* (coleus) was found originally in Indonesia, and it is an ornamental plant grown worldwide. Coleus is susceptible to infection by several viroid species in the genus *Coleviroid*, family *Pospiviroidae*. Up to now, six main viroids infecting coleus have been reported: *Coleus blumei viroid*-1 through 6 (CbVd-1～CbVd-6). Although CbVd-1 has been reported in many countries in the world after it was first reported in Brazil in 1989, the information of CbVd-5 is limited after its first report in 2009. In this study, 92 coleus samples were collected from Beijing, China and three samples were collected from Indonesia. Detection results of hybridization using CbVd-5 cRNA probes and reverse transcription-polymerase chain reaction (RT-PCR) using CbVd-5 specific primers (CbVd-5-P_F: 5'-TGACTAGAACAGTAGTAAAG-3'; CbVd-5-P_R: 5'-AATTGAGGTCAAACCTCTTT-3') demonstrated that 18 out of 92 samples from China and all the three samples from Indonesia were positive to CbVd-5. Although the infection rates of CbVd-5 were very high in the previous reports, the detection rate of CbVd-5 from Beijing samples was only 19.6%. After RT-PCR, sequence analysis for one sample selected randomly from China and Indonesia respectively revealed that the similarities of CbVd-5 between the sequences we obtained and the reference sequence (Accession NO. NC003683) were 98.54 ～ 100%, which demonstrated that CbVd-5 sequences from China and Indonesia were very conserved. To our knowledge, this is the first report of CbVd-5 from Indonesia. This study extends the regional information of CbVd-5.

12. Molecular and biological characterization of *Potato spindle tuber viroid* and *Dahlia latent viroid* in dahlia cultivated in Japan

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Dahlia (*Dahlia* x *hybrida*), a perennial, ornamental flowering plant, harbors three viroids; *Chrysanthemum stunt viroid* (CSVd; Nakashima *et al.,* 2007), *Potato spindle tuber viroid* (PSTVd; Tsushima *et al.,* 2011) and *Dahlia latent viroid* (DLVd; Verhoeven *et al.,* 2012). All of these viroids were symptomless in dahlia plant, but has a potential risk for the other sensitive host plants, such as chrysanthemum (CSVd), potato (PSTVd), and tomato (PSTVd). Dahlia isolate of PSTVd had a nine nucleotide mutations comparing to PSTVd-intermediate isolate and showed very mild stunting and leaf curling on infected tomato plants; i.e, mild strain to tomato. Since two of the viroid (i.e, CSVd and PSTVd) had already been reported in Japan, here we further investigated the infection of DLVd in dahlia cultivated in Japan. By using two-dimensional (2D)-PAGE analysis, we have detected a circular viroid-like RNA band from some of dahlia samples examined, which was different in migration rate in the gel from CSVd and PSTVd. Reverse transcription – polymerase chain reaction (RT-PCR) using DLVd-specific primer sets successfully amplified DNA fragment of the expected size. Nucleotide sequencing revealed that it is a DLVd and had a unique U insertion in U-rich region at the nucleotide position 277 ~ 282. DLVd was reported to have a narrow host range (Verhoeven *et al.,* 2012). We have also inoculated the isolate to several herbaceous host plants including hop (*Humulus lupulus*), but have not yet obtained any positives.

13. Polymorphic positions of *Grapevine yellow speckle viroid-2* (GYSVd-2) in Iranian sequence variants

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To date, GYSVd-2 has been found only in few countries and grapevine is the only known natural host of GYSVd-2, which has restricted distribution in world viticulture. In this study, three GYSVd-2 isolates from three distinct regions of northwest Iran were molecularly characterized. The Iranian GYSVd-2 variants were composed of 360-363 nucleotides sharing a high sequence identity of 96-100% with each other, 97-99% with the reference sequence (NC-003612) and 99-100% with other GYSVd-2 sequences. In total, six new variants were identified with the most nucleotide changes with respect to the reference variant located in the terminal left (TL) domain, the proposed rod-like secondary structure. Three and five variants showed the deletion of A356 and U363 in the TL domain, respectively. Also, substitutions of G359 by U, U360 by C and C361 by U in five variants were found. In contrast, limited nucleotide changes were observed in other domains. In silico analysis showed that some of the nucleotide substitutions would affect the predicted secondary structures but possible biological roles on viroid replication and pathogenesis are unknown and would require further investigations.

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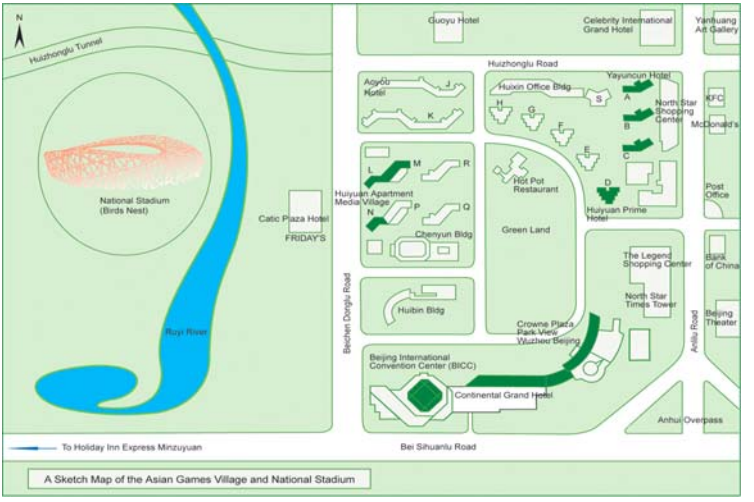
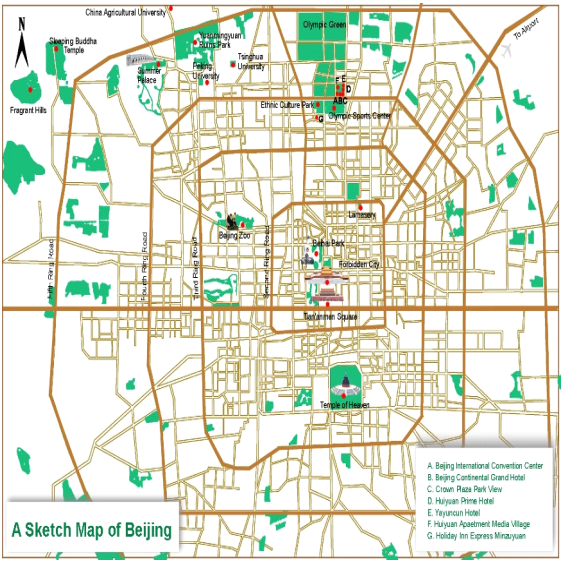
Map of Venue and Beijing





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