OLV-2 (2.7%), SLRSV (2.3%), OLYaV (1.3%) and ArMV (0.7%) were detected. The most common virus found was CMV which was prevailed with a high incidence of 24.7% in olive orchards. The use of one step RT-PCR has revealed efficient and reliable to detect most of the eight olive viruses found in Egypt. Surprisingly, the infection rate found is lower than the expected, if we take into consideratin previous surveys conducted in the Mediterranean area. Last, this technqiue could be useful to be applied in the decetion of olive viruses for production of certified plant along with the certification programs.

Oral Session III

The microarray detecting six fruit-tree viruses

Lenz, Ondrej, Petrzik, Karel, Spak, Josef
Biology Centre ASCR, v.v.i., Institute of Plant Molecular Biology
Contact: lenz@umbr.cas.cz

An oligonucleotide microarray for the detection of *Apple mosaic virus* (ApMV), *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV), *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV) and *Plum pox virus* (PPV) was developed. Specific fragment of viruses were PCR amplified by using Cy3-labelled primers and hybridized onto microarray and detected simultaneously. Advantages and drawbacks of the method will be discussed.

This project was supported by a grant from the Ministry of Education, Youth and Sport of the Czech Republic no.: OC 853.001, and by a grant from the Academy of Science of the Czech Republic no.: AV0Z50510513.

Validation of a microarrays protocol for detection and genotyping of PPV reference samples

Pasquini, Graziella
CRA-PAV Centro di Ricerca per la Patologia Vegetale via C.G. Bertero, 22 00156 Rome, Italy
Contact: graziella.pasquini@entecra.it

A genomic strategy for PPV identification has been recently developed (Pasquini et al., 2008). The method is based on using a 70-mer oligonucleotide DNA microarray chip capable of simultaneously detecting and genotyping PPV strains. Universal and specific probes have been identified and used with a sensitive protocol of hybridization using an indirect fluorescent labelling of cDNA product with cyanine that enhanced the sensitivity of the virus detection without the use of the PCR amplification step About 30 samples belonging to a PPV isolates collection, including M, D, EA, and C strains, have been used for the validation and standardization of the protocol. The sensitivity, specificity, repeatability and reproducibility of the protocol have been tested.

Real time PCR quantitative analysis of plant viruses in stone fruit trees tissues

Jarosova, Jana, Kundu, Jiban Kumar
VURV, Drnovska 507, 16106 Prague
Contact: j.jarosova@vurv.cz

Real Time PCR assays aiming at quantifying the level of plant infection by pathogens have been increasing for the last few years. Within microbiology, the application of Real Time PCR has had the biggest impact upon the field of virology. However, Real-time PCR application in fundamental plant virology studies is still lagging behind. The use of relative and absolute quantification is discussed in this study. Also, case studies including Plum pox virus, Prune dwarf virus and Apple chlorotic leaf spot virus are presented.

Simultaneous detection of the main stone fruit viruses and viroids by non-isotopic molecular hybridization polyprobe

Sánchez-Navarro, Jesús[1], De la Torre, Rodolfo[2], Pallás, Vicente[1]
[1]Instituto de Biología Molecular y Celular de plantas, Universidad Politécnica de Valencia-CSIC, Avenida de los naranjos s/n, 46022 Valencia, Spain
[2]FES-IZTACALA-UNAM. Unidad de Biotecnología y prototipos (UBIPRO). Avenida De los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Mexico. 54090
Contact: jesanche@ibmcp.upv.es

Stone fruit are affected by a large number of economically important and common viruses and viroids. Relevant pathogens include the Ilarvirus *Apple mosaic virus* (ApMV), *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV) and *American plum line pattern virus* (APLPV); the potyvirus responsible of the 'Sharka' disease, *Plum pox virus* (PPV), the Trichovirus *Apple chlorotic leaf spot virus* (ACLSV), the Foveavirus *Apricot latent virus* (ApLV), the ampelovirus *Plum bark necrosis stem pitting associated virus* (PBNSPaV) and the viroids *Peach latent mosic viroid* (PLMVd) and the *Hop stunt viroid* (HSVd). A critical step in the sanitary status of the crop is the detection of the virus/viroid in the early stage of the infection. In this sense, the efforts in the diagnostic methods have been addressed to improve the sensitivity but also to reduce the processing time/cost of the analysis by performing the simultaneous detection of several pathogens in a single assay. The multiple detection of several pathogens by using non-radioactive molecular hybridization technique and a unique riboprobe 0 'Polyprobe' carrying partial sequences of different viruses fused in tandem has probed to be very attractive (Herranz et al., 2005. J. Virol. Methods 124, 49-55). In the present study we have used the polyprobe technology to detect eight viruses and two viroids affecting the stone fruit crop.

Detection of cherry leafroll virus, prune dwarf virus and prunus necrotic leafroll virus in prunus pollen

Horner, Mary[1], Van den Brink, Roy[2], Hough, Ellena[1], Eastwell, Kenneth[3], Howell, William[3]
[1]Plant and Food Research, Hawke's Bay, Private Bag 1401 Havelock North, New Zealand
[2]Plant and Food Research, Palmerston North, Private Bag 11600 Palmerston, North New Zealand
[3]Washington State University- IAREC, Prosser 99350
Contact: mhorner@hortresearch.co.nz

Current protocols requires that pollen imported into New Zealand and used in breeding programmes needs to undergo lengthy testing of progeny in a Level 3 Quarantine Facility for potential transmission of viruses. A reliable method was developed to directly test the pollen material by Reverse transcription polymerase chain reaction (RT-PCR). Experiments testing various maceration methods on pollen material and the effect of various associated antioxidant agents upon Ribonucleic acid (RNA) extraction and of polyphenolic inhibition of RT-PCR were investigated. An optimum protocol for RNA extraction from pollen was developed using a ball bearing silica extraction method in association with a modified extraction buffer. This protocol reliably detected the presence of *Prunus necrotic ringspot virus, Prune dwarf virus* and *Cherry leafroll virus* in pollen or anther material. *Cherry leafroll virus* was reliably detected in pollen samples containing 0.01 % infected pollen. If adopted by Biosecurity New Zealand, this method, which could be used to directly analyze the pollen as it enters New Zealand, should simplify testing protocols and reduce compliance costs and time associated with the importation of pollen material.

Reverse transcription loop-mediated isothermal amplification (RTLAMP): A novel method for the detection of *Peach latent mosaic viroid* (PLMVd)

Boubourakas, I.N.[1], Fukuta, S.[2], Fagioli, F.[3], Luigi, M.[3], Barba, Â.M.[3], Kyriakopoulou, P.E.[1]
[1]Agricultural University of Athens, Department of Plant Production Science, Plant Pathology Laboratory, 11855 Athens, Greece
[2]Biotechnology Group, Aichi Agricultural Research Center, 1-1 Sagamine, Yazako, Nagakute, 480-1193 Aichi, Japan
[3]CRA - Centro di Ricerca per la Pathologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy

A reverse transcription loop-mediated isothermal amplification method (RTLAMP) for the detection of Peach latent mosaic viroid (PLMVd) was developed. LAMP is a novel nucleic acid amplification method, quite simple, preformed under isothermal conditions (60-65ºC), with high specificity, efficiency and rapidity. It is characterized by the use of four different primers: F3 (forward outer primer), B3 (backward outer primer), FIP (forward inner primer), BIP (backward inner primer), specifically designed to recognize 6 distinct regions on the target sequence. Four primer sets (OLD, OLD1, NEW and FUKUTA'S) were designed. Based on preliminary experiments, the set OLD1 was selected for further evaluation and proved to be highly specific. Simple and accelerated RT-LAMP was preformed using non-degenerate and degenerate F-Loop and B-Loop primers respectively. In the case of the accelerated protocol, the viroid could be detected within only 31 min using as template T-RNA or trace of plant tissue, taken by sterilized toothpick, compared to 180 min with a RT-PCR assy. In addition, the RT-LAMP method was found to be 100 times more sensitive than the RT-PCR. Using RT-LAMP

PLMVd was detected in naturally infected peach, plum apricot, pear, wild pear and quince plants as well as in an Italian peach calico infected plant.

Sensitive detection and strain discrimination of plum pox virus using Rt - Real Time Pcr - Fret Method

Malinowski, Tadeusz
Research Institute of Pomology and Floriculture, Pomologiczna 18, 96-100 Skierniewice, Poland
Contact: tmalin@insad.pl

A new method of Plum pox virus detection and strain identification / discrimination, based on real time PCR with fluorescence resonance energy transfer (FRET) probes, has been developed. One 'universal' donor probe as well as D and M (Rec) specific acceptor probes labelled with different fluorophores are utilised for one-tube detection and typing of amplicons during the reaction. Two different channels are used for simultaneous detection of D and M (Rec) type isolates. Post-reaction melting curve analysis provides further data and allows also positive identification of EA type isolates, based on different melting curve profiles. The method is fast, sensitive and provides a way of quantification of PPV types in mixed infections.

Application of scanning electron microscopy for diagnosis of phytoplasmas in single and mixed infections in papaya

Lebsky, Vladimir[1], Poghosyan, Arevik[1], Silva-Rosales, Laura[2]
[1]Centro de Investigaciones Biologicas del Noroeste (CIBNOR), Mar Bermejo 195, Col. Playa Palo de Sante Rita, A.P.128, La Paz, BCS, 23090, Mexico
[2]Centro de Investigacion y de Estudios Avanzados del IPN, Campus Guanajuato, Km. 9.6 Lib. Nte. Carr. Irapuato-León, Irapuato, Gto. 36500, Mexico
Contact: arevik04@cibnor.mx

Virus and virus-like diseases are a major threat to papaya production in Mexico, where it is a leading commercial crop in the States of Michoacan, Oaxaca, Jalisco, Nayarit, Yucatán, Veracruz and Chiapas. Additionally, the State of Baja California Sur is the main producer of organically grown papaya destined for USA market. Infection with papaya ringspot virus (PRSV), papaya mosaic virus (PMV) and phytoplasma has been reported from different Mexican states. Some symptoms of yellow type diseases such as mosaics, stunting, bunchy top and leaf chlorosis, necrosis and malformations are somewhat similar in appearance, but provoked by distinct pathogens. A set of complex diagnostic procedures are needed for correct and precise diagnosis of pathogens so that timely and correct control measures can be taken.

Using scanning electron microscopy (SEM) technique phytoplasmas were detected in the phloem tissues of field and greenhouse-indexed papaya plants from Baja California Sur. The 32 regional varieties as well as cv. Maradol with numerous symptoms of dieback, mosaics, bunchy top and yellow crinkle were analyzed. The pathogen was detected in stems, leafstalks, roots, axillary leaflets, leaf veins and in germinated seeds within the fruit. No viral infection was revealed by test-plants and molecular techniques. Analysis by a SEM technique of papaya samples from Veracruz and Irapuato, both field-grown and mechanically infected with PRSV and PMV in various combinations, revealed phytoplasmas in phloem of most of the tested samples. In some cases, along with phytoplasma, rod-shaped bacteria were also distinguished. Further investigations would determine phylogenetic relations between phytoplasmas from samples collected from different states and provide more information about mixed infections and disease epidemiology.

Oral Session IV

New viruses identified in fig trees exhibiting fig mosaic disease

Tzanetakis, Ioannis[1], Martin, Robert[2]
[1]University of Arkansas, Dept. of Plant Pathology
[2]USDA-ARS, Corvallis, OR
Contact: itzaneta@uark.edu

Though fig mosaic disease has been known for decades but the causal agent has been elusive. Here we present data on the incidence of at least four new viruses isolated from fig trees exhibiting mosaic