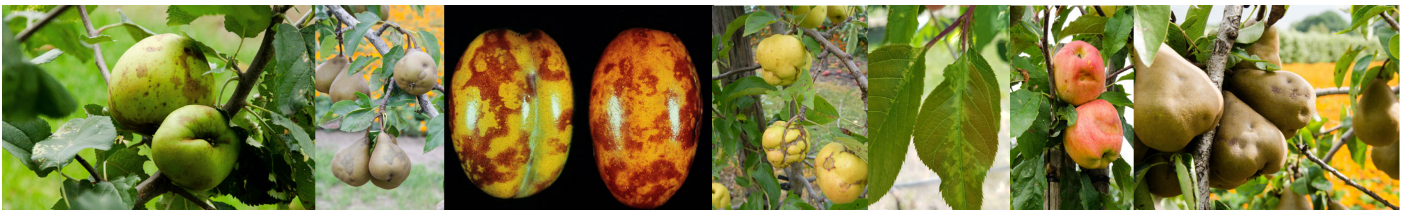
25th

ICVF

2023 The Netherlands

"Let's keep our fruits healthy"



Book of Abstracts

25th International Conference on Virus and other graft transmissible diseases of Fruit crops
Wageningen, 9-13 July 2023

The 25th International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops

9-13 July 2023 Wageningen, the Netherlands

Scientific committee:

Wilhelm Jelkmann	**Josef Spak**	**Nicola Fiore**
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Francesco di Serio	**Maher al Rwahnih**	**Vicente Pallas**
Hano Maree	**Miroslav Glasa**	
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Organizing committee:

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Gabriëlla Harris	Coordinator logistics Netherlands Food and Consumer Product Safety Authority, Wageningen

Welcome to the 25th International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops (ICVF)

Dear colleagues,

The organizing committee has the honor to welcome you to attend the 25th International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops held in Wageningen, The Netherlands.

The topic of viruses and other graft transmissible diseases dates back to a meeting in 1954 in Wädenswil, Switzerland. This meeting was organized by a small group of international plant pathologists interested in a few virus diseases of deciduous fruit trees then known to occur in Europe and North America. The subsequent meetings became wider in scope and increasingly concerned with viruses and virus diseases of fruit trees and small fruit crops worldwide. Furthermore, the scope included diseases caused by phytoplasmas and viroids.

The last time this meeting was held in the Netherlands was in 1955, also in Wageningen. We of the organizing committee are very grateful that 68 years later this scientific community can meet again in Wageningen.
Furthermore we are relieved that after postponing the meeting due to COVID, we can finally meet again in a face-tot face meeting.

We hope you will enjoy,

Best wishes,
The local organizing committee

25th

2023 The Netherlands

25th International Conference on Virus and other graft transmissible diseases of Fruit crops
Wageningen, 9-13 July 2023

"Let's keep our fruits healthy"

We sincerely thank the sponsers of the 25th International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops:

Netherlands Food and Consumer Product Safety Authority
Ministry of Agriculture, Nature and Food Quality

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BUREAU BEX

25th International Conference on Virus and other graft transmissible diseases of Fruit crops

Wageningen, The Netherlands, 9 to 13 July 2023

Sunday	**9 July WICC**
19:30-20:00	Registration
20:00-21:30	Welcome reception
Monday	**10 July WICC**
8.30 – 9.30	Registration & Poster set-up
9.30 – 11.00	**OPENING SESSION**
09.30-09.45	*Gerard Jongedijk* WELCOME ADDRESS
09.45-10.00	*Richard Harrison* WORD OF WELCOME TO THE NETHERLANDS, WAGENINGEN (WUR)
10.00-11.00	*Wilhelm Jelkmann* 40 YEARS RESEARCH VIRUSES AND VIRUS-LIKE ORGANISMS IN FRUIT CROPS
11.00 – 11.30	*Coffee/tea break*
11.30 – 12.30	**HTS FROM RESEARCH TO DIAGNOSTICS** Chair: *Sébastien Massart & Armelle Marais-Colombel*
11:30-12:15	***Sébastien Massart*** **1ST KEYNOTE: HTS FROM RESEARCH TO DIAGNOSTICS**
12:15-12:30	*Nuria Fontdevila* MANAGING THE DELUGE OF NEWLY DISCOVERED PLANT VIRUSES AND VIROIDS
12.30 – 13.30	*Lunch*
13.30 – 14.45	**HTS FROM RESEARCH TO DIAGNOSTICS (CONTINUED)**
13:30-13:45	*Rachelle Bester* PEAR STONY PIT DISEASE: THE SOUTH AFRICAN EXPERIENCE.
13:45-14:00	*Armelle Marais* PRUNUS-INFECTING LUTEOVIRUSES: MORE VIRUSES AND STILL MANY QUESTIONS.
14:00-14:15	*Marcel Westenberg* HOW HIGH THROUG-PUT SEQUENCING CAN BE USED AS A GENERIC TOOL TO IDENTIFY VIRUSES IN A REGULATORY FRAMEWORK.
14:15-14:30	*Francesco Di Serio* SELF-CLEAVING VIROID-LIKE RNAS IDENTIFIED BY PLANT METATRANSCRIPTOMICS: THE QUESTION OF THE ACTUAL HOST.
14:30-14:45	*ShiFang Li* DISCOVERY OF A NOVEL ROSE-ASSOCIATED HAMMERHEAD VIROID IN CHINA ROSE (*ROSA CHINENSIS* JACQ.) BY HIGH-THROUGHPUT SEQUENCING.
14.45 – 15.15	***Group Picture*** *and Coffee/tea break*
15.15 – 17.00	**POSTERS**
17.00 – 19.00	**Wageningen City Tour (walking)**

Tuesday 8.00 – 9.00	**11 July** **Omnia WUR Campus** Travelling to WUR Campus, Omnia Building
9.00 – 10.30	**NEXT GENERATION CERTIFICATION** Chair: *Thierry Candresse & Varvara Maliogka*
09:00-09:45	***Thierry Candresse*** **2ND KEYNOTE: NEXT GENERATION CERTIFICATION**
09:45-10:00	*Josef Špak* EXPERIENCE WITH HTS TESTING OF CERTIFIED APPLE ROOTSTOCKS AND GRAFTS FOR THE PRESENCE OF VIRUSES AND VIROIDS.
10:00-10:15	*Almash Jahan* UNVEILING VIRUSES AND VIROID IN APPLE GERMPLASM AND ORCHARDS IN HUNGARY USING HIGH THROUGHPUT SEQUENCING.
10:15-10:30	*Dimitre Mollov* OPTIMIZING CERTIFICATION AND QUARANTINE PROTOCOLS FOR *FRAGARIA* AND *RUBUS* GERMPLASM INTRODUCTION AND MOVEMENT.
10.30 – 11.00	*Coffee/tea break*
11.00 – 12.15	**NEXT GENERATION CERTIFICATION (CONTINUED)**
11:00-11:15	*Maher Al Rwahnih* DEVELOPMENT OF REVISED DIAGNOSTIC PROTOCOL FOR QUARANTINE AND CERTIFICATION OF PRUNUS GERMPLASM AT FOUNDATION PLANT SERVICES IN DAVIS, CALIFORNIA, USA.
11:15-11:30	*Annelien Roenhorst* IDENTIFICATION OF EUROPEAN UNION QUARANTINE PHYTOPLASMAS: A CHALLENGE FOR DIAGNOSTIC LABORATORIES IN PLANT HEALTH.
11:30-11:45	*Oscar Hurtado-Gonzales* NEXT GENERATION QUARANTINE OF IMPORTED FRUIT TREES IN USDA APHIS.
11:45-12:00	*Jean-Philippe Renvoisé* HIGH THROUGHPUT SEQUENCING (HTS): AN AID AND IMPROVEMENT TO CONVENTIONAL FRUIT TREE PROPAGATION AND QUARANTINE SCHEMES.
12:00-12:15	*Kerstin Zikeli* GERMANY-WIDE APPLE HAMMERHEAD VIROID SCREENING IN APPLE REVEALS HIGH AHVD GENOME SEQUENCE VARIABILITY AND LEADS TO AN OPTIMIZED AHVD SPECIFIC REVERSE TRANSCRIPTION REAL-TIME PCR.
12.15 – 13.15	*Lunch*
13.15 – 14.45	**EMERGING DISEASES** Chair: *Ioannis Tzanetakis & Ni Hong*
13:15-14:00	***Ioannis Tzanetakis*** **3RD KEYNOTE: EMERGING DISEASES**
14:00-14:15	*Dimitre Mollov* BLUEBERRY DECLINE ASSOCIATED WITH NEW AND EMERGING VIRUSES.
14:15-14:30	*Hano Maree* CHARACTERIZATION OF A NEW VIROID ASSOCIATED WITH MARBLING AND CORKY FLESH IN JAPANESE PLUMS.
14:30-14:45	*Jana Fránová* CHARACTERIZATION OF STRAWBERRY VIRUS 1, A NEW STRAWBERRY CYTORHABDOVIRUS, ITS DISTRIBUTION, ANTIBODY PREPARATION AND EFFECT ON FRUIT YIELD IN THE CZECH REPUBLIC.
14.45 – 15.15	*Coffee/tea break*
15.15 – 17.00	**Campus Tour**
17.00 – 18.00	Travelling back to WICC

Wednesday 12 July TECHNICAL EXCURSION and CONFERENCE DINNER **Departure from Wageningen 07.15 h !** **Field excursion by bus to the province of Limburg (fruit tree nursery, test centre Naktuinbouw, Clean Fruit Plants, Vermeerderingstuinen Nederland, ISFC Delphy)** **The technical excursion will continue with the CONFERENCE DINNER.** **Please note: we will not return to Wageningen before the conference dinner.** **Please wear comfortable clothes and shoes for the whole day.**	
Thursday 13 July WICC	
08.30 – 09.45	**VECTOR TRANSMISSION AND VIRUS-VECTOR-INTERACTION** Chair: *Piotr Trębicki & Francesco Di Serio*
08:30-09:15	***Piotr Trębicki*** **4TH KEYNOTE: VECTOR TRANSMISSION AND VIRUS-VECTOR-INTERACTION**
09:15-09:30	*Weier Cui* PLANTHOPPER *CIXIOSOMA* SP. (*HEMIPTERA: CIXIIDAE*) TRANSMITS '*FRAGARIA × ANANASSA*' PHYLLODY PHYTOPLASMA (16SRXIII) TO PERIWINKLE (*CATHARANTHUS ROSEUS*).
09:30-09:45	*Christine Seinsche* VECTOR MONITORING OF QUARANTINE PESTS AND REGULATED NON-QUARANTINE PESTS IN FRUIT CROPS AND VITICULTURE.
09:45-10:00	*Kadriye Cağlayan* SPREAD OF BLUEBERRY MOSAIC-ASSOCIATED VIRUS (BLMAV) FROM CULTIVATED BLUEBERRIES TO WILD PLANTATIONS OR VICE-VERSA IN NATURAL ECOSYSTEMS.
10.00 – 10.30	*Coffee/tea break*
10.30 – 11.00	Business meeting
11.00 – 12.15	**POSTERS**
12.15 – 13.15	*Lunch*
13.15 – 14.30	**VIRUS (PATHOGEN) HOST INTERACTION** Chair: *Carolyn Malstrom & Hano Maree*
13:15-14:00	***Carolyn Malstrom*** **5TH KEYNOTE: VIRUS (PATHOGEN) HOST INTERACTION**
14:00-14.15	*V.Pallas/J. Sanchez* GENERATION OF INFECTIOUS CLONES OF PRUNUS NECROTIC RINGSPOT VIRUS (PNRSV) AND PRUNE DWARF VIRUS (PDV) TO ANALYZE POTENTIAL SYNERGISTIC EFFECTS OF THEIR MIXED INFECTIONS.
14:15-14:30	*Jan Böhm* GENOME ANALYSIS OF '*CANDIDATUS* PHYTOPLASMA RUBI' HIGHLIGHTS SEPARATED POSITION OF 16SRV PHYTOPLASMAS.
14.30 – 15.00	*Coffee/tea break*
15.00 – 16.15	**VIRUS (PATHOGEN) HOST INTERACTION (CONTINUED)**
15:00-15:15	*Kahraman Gürcan* INVESTIGATION OF MORE LOCI IN LINKAGE GROUP 1 IN APRICOT (*PRUNUS ARMENIACA*) AS CANDIDATE RESISTANCE MARKERS AGAINST TO PLUM POX VIRUS.
15:15-15:30	Aisha Taskuzhina VIRAL INFECTIONS ENDANGER THE SURVIVAL OF THE WILD APPLE POPULATION.
15:30-15:45	*Arnaud Blouin* LITTLE CHERRY VIRUS 2 AND “NOT SO LITTLE CHERRY VIRUS 1”: RESULT OF A 8 YEARS FIELD TRIAL.
15:45-16:00	*Ioannis Tzanetakis* DEVELOPMENT OF AN INFECTIOUS CLONE OF BLACKBERRY CHLOROTIC RINGSPOT VIRUS AND ITS DELIVERY TO BLACKBERRY.
16:00-16.15	*Beatriz Navarro* CITRUS CONCAVE GUM-ASSOCIATED VIRUS AND CITRUS VIRUS A: COGUVIRUSES WITH A WIDE HOST RANGE CODING FOR A WEAK RNA SILENCING SUPPRESSOR.
16.15 – 16.30	**CLOSING**

LIST OF POSTERS

POSTERS	
MONDAY 15.15 – 17.00	
THURSDAY 11:00 – 12:15	
P 1	*Fu, L.N., Xu, W.X.* IDENTIFICATION AND CHARACTERIZATION OF NOVEL VIROIDS IN PEAR
P 2	*Fontdevila, N., Gailly, C., Lateur, M., Steyer, S., Dubuis, N., Blouin, A.G., Massart, S.* CHARACTERIZATION OF PYRUS VIRUS A (PyVA), A NOVEL VELARIVIRUS IDENTIFIED IN PEAR TREES
P 3	Quito-Avila, D.F. , Cornejo-Franc,o J.F., Villamore, D.E., Tzanetakis, I., Martin, R.R., Alvarez, R.A., Mollov, D. OCCURRENCE OF VIRUSES INFECTING FRAGARIA AND RUBUS SPP. IN ECUADOR
P 4	Brans, Y. , Audergon, J-M., Delmas, M., Feugey, L., Leonetti, J., Pailly, O., Unbekandt, C., Latour, F., Chabanon-Dutemps, S., Crouzet, M.-P. HOT WATER TREATMENT FOR THE ELIMINATION OF VIRUSES, PHYTOPLAMAS AND BACTERIA FROM FRUIT TREE BUDWOOD (Results of the THERMOFRUIT project)
P 5	Brans, Y., Latour, F. Chabanon-Dutemps, S. ASSESSING A GREENHOUSE PROTOCOL TO IDENTIFY SUSCEPTIBILITY OF APRICOT AND PLUM TO EUROPEAN STONE FRUIT YELLOWS PHYTOPLASMA
P 6	Brans, Y., Latour, F., Castaing, J. TOWARDS THE CHARACTERIZATION OF BIOLOGICAL PROPERTIES AND SYMPTOMATOLOGY OF HTS-IDENTIFIED VIRUSES
P 7	Voruna, M., Mihaljica, M., Vakić M., Elbeaino T., Delić, D. MOLECULAR ANALYSIS OF RUBUS YELLOW NET VIRUS ON RASPBERRY FROM BOSNIA AND HERZEGOVINA
P 8	Delić, D., Stainton D. , Radulović, M., Vakić, M., Tzanetakis I.E. DETECTION OF BLACKCURRANT LEAFROLL ASSOCIATED VIRUS 1 (BCLRAV-1) IN BLACK AND RED CURRANTS IN BOSNIA AND HERZEGOVINA
P 9	Bennypaul, H., Sanderson, D., Donaghy, P., Abdullahi, I. DEVELOPMENT OF A REAL-TIME PCR ASSAY FOR THE DETECTION AND IDENTIFICATION OF RUBUS STUNT PHYTOPLASMA IN *RUBUS* SPP.
P 10	He, C.Y., Xing, F., Zhang, Z.X., Zhan, B.H., Habili, N., Li, S.F., Wang, H.Q. THE DOWN-REGULATION OF MITOCHONDRIAL ATP SYNTHASE SUBUNIT O FACILITATES APPLE NECROTIC MOSAIC VIRUS INFECTION
P 11	Desiderio, F., Galbács, N., Zsuzsanna, Várallyay, É. INVESTIGATING THE PRESENCE AND DISTRIBUTION OF PRVF IN HUNGARIAN SOUR AND SWEET CHERRY
P 12	Ruiz-García, A.B., Morán, F., Malagón, J., Paz, S., Ruiz-Torres, M., Herrera-Mármol, M., Olmos, A. CHARACTERIZATION OF SPANISH OLIVE VIROME BY HIGH THROUGHPUT SEQUENCING
P 13	Olmos, A., Morán, F., Canales, C., Ruiz-García, A.B. APPLE STEM PITTING VIRUS, APPLE STEM GROOVING VIRUS, APPLE CHLOROTIC LEAF SPOT VIRUS, AND APPLE HAMMERHEAD VIROID IN LOQUAT IN SPAIN

P 14	Ruiz-García, A.B., Olmos, A., Marais, A., Faure, C., Candresse, T. APPLE DIMPLE FRUIT VIROID IS A NATURAL HOST IN POMEGRANATE
P 15	L. Corachán-Valencia, M. Rubio, P. Martínez-Gómez, I. Batlle, J. A. Sánchez-Navarro and V. Pallas. INTRA- AND INTER-VARIETAL GENETIC DIVERSITY OF ILARVIRUSES PRESENT IN AUTOCHTHONOUS ALMOND VARIETIES [PRUNUS DULCIS (MILLER) WEBB] IN THE SPANISH MEDITERRANEAN REGION
P 16	Huamán-Pilco, A., Cui, W., Fiore, N. and Zamorano, A. NEW EMARAVIRUS FOUND IN THEOBROMA CACAO L. IN AMAZONAS, PERÚ
P 17	Zhang, Z., Wang, Q., Zhang, Y.L., Ren, Q.T., Gao, Y.J., Wang, G.P. and Hong, N. THE NATURAL TRANSMISSION AND GENE FUNCTION OF PEAR CHLOROTIC LEAF SPOT-ASSOCIATED VIRUS
P 18	Pál Salamon, Evans Duah Agyemang, Francesco Desiderio, Emese Demian, Zsuszsanna Nagyné-Galbács, Andras Takacs, Eva Varallyay CLEMATIS VITALBA IS AN ALTERNATIVE HOST PLANT OF PRUNUS VIRUS I.
P 19	Fránová, J., Koloniuk, I., Lenz, O., Přibylová, J., Sarkisova, T, Špak, J., Tan, L.J., Vinokurov, K., Zemek, R., Blystad, D.-R., Hamborg, Z., Sapkota, B., Spetz, K., Trandem, N., Čmejla, R., Rejlová, M., Sedlák, J., Valentová, L., Bilavčík, A., Bobrova, O., Faltus, M., Hammond, D.S.H., Zámečník, J., Holub, J., and Skalík, J. SURVEY OF RASPBERRY VIRUSES, ASSOCIATED ARTHROPODS AND RECOVERY OF IN VITRO RASPBERRY CULTURES IN THE CZECH REPUBLIC AND NORWAY
P 20	Achs, A. and Šubr, Z. TARGETING TO APOPLAST IMPROVES THE ACCUMULATION AND STABILITY OF C-TERMINAL HALF OF SARS-CoV-2 NUCLEOPROTEIN EXPRESSED IN NICOTIANA BENTHAMIANA BY PLUM POX VIRUS-BASED VECTOR
P 21	Alaxin, P., Predajňa, L., Achs, A., Šubr, Z., Mrkvová, M. and Glasa, M. ARGENTINE TANGO OF TWO HARMONIZED HOP STUNT VIROID GENETIC GROUPS IN GRAPEVINE (VITIS VINIFERA L.) – FIRST REPORT FROM SLOVAKIA.
P 22	Luigi M., Donati L., Sciarroni R., Gentili A., Taglienti A., Tiberini A., Faggioli F., Ferretti L. MOLECULAR CARACHTERIZATION OF CARLAVIRUS ISOLATES INFECTING HOP IN ITALY
P 23	Achs, A., Alaxin, P., Predajňa, L., Mrkvová, M., Šubr, Z. and Glasa, M. BOTH PRUNUS AND NON-PRUNUS PLANTS CAN HOST A NOVEL ILARVIRUS – PRUNUS VIRUS I.
P 24	Malandraki, I., Beris, D., Rampou, A., Vassilakos N., Varveri C. INSIGHTS INTO THE VIROME OF GREEK POME FRUIT ORCHARDS AS REVEALED BY RNA-SEQ
P 25	Ordek, Kıvılcım, Gazel, Mona, Roumi, Vahid, Caglayan, Kadriye COMPLETE GENOME SEQUENCES OF DIVERGENT RASPBERRY BUSHY DWARF VIRUS ISOLATES FROM RUBUS SPP. IN TURKEY
P 26	Luigi M., Cesari E., Brunetti A., Pilotti M., Taglienti A., Gentili A., Ferretti L., Botti S., Rossato M., Lopatriello G., Carlomagno M., Faggioli F. THE APPLICATION OF HTS TECHNOLOGY TO THE CASE STUDY OF SLRSV-INFECTED RAGGIOLA OLIVE CULTIVAR
P 27	Luigi M., Taglienti A., Corrado C.L., Cardoni M., Botti S., Bissani R., Casati P., Passera A., Miotti N., De Jonghe K., Everaert E., Olmos A., Ruiz-García A.B., Faggioli F. VALIDATION OF A DUPLEX REAL TIME RT-PCR FOR THE DETECTION OF PLMVD AND COMPARISON OF DIFFERENT NUCLEIC ACID EXTRACTION PROTOCOLS.

P 28	Orfanidou, C.G., Katsiani, A., Sassalou, C.L., Beta, C., Panailidou, P., Drogoudi, P., Katis, N.I., Maliogka, V.I. ELUCIDATING THE VIROME OF THE MAIN PRUNUS SPECIES GENOTYPES CULTIVATED IN GREECE
P 29	Elien Guldentops, Maaike Heyneman, Stefaan P.O., Werbrouck, Kris De Jonghe ASSESSMENT OF ONT SEQUENCING (MINION) FOR PLANT VIRUS DETECTION AND COMPARISON WITH ILLUMINA-BASED SEQUENCING
P 30	Tzanetakis, I.E., Singh, S., Stainton, D.RAPID DEVELOPMENT OF AFFORDABLE, VIRUS-MIMICKING ARTIFICIAL POSITIVE CONTROLS (VIMAPC) THAT MIMIC TITER AND TROPISM
P 31	Stainton, D., Villamor, E.V, D., Sierra Mejia, A., Srivastava, A., Mollov, D., Martin, R., R., Tzanetakis, I., E. BLUEBERRY VIRUS L – A NEW LUTEOVIRUS WHICH IS HIGHLY PREVALENT IN THE UNITED STATES
P 32	Zuļģe, N., Stalažs, A., Drevinska, K. and Moročko - Bičevska, I.SURVEY FOR OTHER BLACKCURRANT REVERSION VIRUS VECTORS
P 33	Menzel, W., Knierim, D., Margaria, P. and Winter, S. INVESTIGATIONS INTO THE VIRUS STATUS OF BLUEBERRIES IN GERMANY WITH A SPECIAL FOCUS ON THE SO CALLED "OFF TYPE"
P 34	Stöhr P, Berwarth C, Zikeli K, Jelkmann W DEVELOPMENT OF AN IMPROVED LITTLE CHERRY VIRUS 2 (LChV-2) REVERSE TRANSCRIPTION-PCR DETECTION METHOD
P 35	Zikeli, K., Berwarth, C., Faus, S., Leible, T., Jelkmann, W. DETECTION AND CHARACTERIZATION OF THE FIRST STRAWBERRY POLEROVIRUS-1 ISOLATE IN STRAWBERRY IN GERMANY WITH HTS AND VECTOR TRANSMISSION BY STRAWBERRY APHID
P 36	Fontdevila, Núria, Tahzima, Rachid, Rong, Wei, Blouin, Arnaud G., Reynard Jean-Sébastien, Bobev, Svetoslav G., Caglayan, Kadriye, Candresse, Thierry, Castaing, Julie, Foucart, Yoika, Jelkmann, Wilhelm, Zikeli Kerstin , Mavrič Pleško, Irena, Meekes, Ellis, Minafra, Angelantonio, Rott, Mike, Shifang, Li, Leichtfried, Thomas, Vončina, Darko, Okić, Arnela, Botermans Marleen, de Koning Pier , De Jonghe, Kris, Massart, Sébastien UNCOVERING THE WORLDWIDE DIVERSITY OF LITTLE CHERRY VIRUS 1 AND 2 AND THE ASSOCIATED VIRUSES IN PRUNUS SPP.
P 37	Jevremović, D., Vasilijević, B., Katanić, V., Paunović, S.OCCURRENCE OF TWO LITTLE CHERRY VIRUSES IN STONE FRUITS IN SERBIA
P 38	Jevremović, D., Vasilijević, B., Katanić, V., Paunović, S. MOLECULAR DETECTION AND CHARACTERIZATION OF CHERRY VIRUS A IN PRUNUS SPECIES IN SERBIA
P 39	Yuekun Yang, Shamei Lv, Ying He, Tingting Wang, Guoping Wang, Ni Hong and Liping Wang ROLE OF LONG NONCODING RNAS INVOLVED IN RESISTANCE TO BOTRYOSPHAERIA DOTHIDEA INFECTION IN PEAR
P 40	Renvoisé, J.-P., Gonzalez, T., Cammas, M., Bachès, M., Bachès, B., Oihabi, A., de Saint Roman M., Bazin, S., Calado, G., Carlut, E., Chambon, F., Fournier, A., Garnier, S., Luguin, A., Pion, A., Strbik, F., Gerbault, J.-E., Al Hameid, A., Beheiry, H., Bloquel, E., Julhia, L., Froelicher, Y & Belval, L SANITARY DIAGNOSIS FOR THE QUALITATIVE DEVELOPMENT OF THE CITRUS SECTOR OF ALULA
P 41	Zhou J.L., Lin S.J., Liu G.Y. , Yu J.L., Liu H., Ding J.H. , Wang G.P., Hong N., Hartung J.S., Ding F. GENETIC POLYMORPHISM OF CANDIDATUS LIBERIBACTER ASIATICUS BASED ON A SEC-DEPENDENT SECRETED EFFECTOR PROTEIN

P 42	Fowkes, R. A., Skelton, A., McGreig, S., Adams, P. I. and Fox, A. BASELINE SURVEILLANCE OF RUBUS VIRUSES IN THE UK
P 43	Zhou J.L. 1, Lin S.J., Liu G.Y., Yu J.L., Liu H., Ding J.H., Wang G.P., Hong N., Hartung J.S., Ding F. GENETIC POLYMORPHISM OF CANDIDATUS LIBERIBACTER ASIATICUS BASED ON A SEC-DEPENDENT SECRETED EFFECTOR PROTEIN

ORALS

SESSION 1

HTS FROM RESEARCH TO DIAGNOSTICS

Keynote speaker: Sébastien Massart

Chair: *Sébastien Massart & Armelle Marais-Colombel*

MANAGING THE DELUGE OF NEWLY DISCOVERED PLANT VIRUSES AND VIROIDS: AN OPTIMIZED SCIENTIFIC AND REGULATORY FRAMEWORK FOR THEIR CHARACTERIZATION AND RISK ANALYSIS

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The advances in high-throughput sequencing (HTS) technologies and bioinformatic tools have resulted in new opportunities for the discovery and unbiased diagnosis of plant viruses and viroids, as well as an increase in the discovery and publication of new sequences of viral origin (1). However, the biological characterization following the discovery of a new virus is a complex and long process. Hou et al. (2020) analyzed 78 publications mentioning the discovery of new viruses and viroids from 32 fruit tree species since 2011 (2). In this study, we present how this review used the available information to assess the implementation of the proposed framework for the evaluation of biosecurity, commercial, regulatory, and scientific impacts of plant viruses and viroids identified by HTS technologies (3). Even though the previous framework was widely used to guide the characterization of newly identified plant viruses, this review shows that continuation of the biological characterization of a new virus is rarely presented in later citing publications.

We now propose an improved and adapted framework that takes into account trends in virus discovery and characterization, and that integrates novel approaches and bioinformatic tools recently published, or that are currently under development.

This revised framework is better adapted to the current rate of virus discovery and provides an improved prioritization for filling knowledge and data gaps. It consists of four distinct steps adapted to include a multi-stakeholder feedback loop. The key improvements include better prioritization and organization of the various steps, earlier data sharing among researchers and involved stakeholders for providing context to new virus findings (i.e., host range, diversity, and distribution), public database screening, and exploitation of genomic information to predict biological properties.

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PEAR STONY PIT DISEASE: THE SOUTH AFRICAN EXPERIENCE

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Introduction

The overall health of any fruit industry is reliant on high quality planting material free of pests and pathogens. To avert the proliferation of virus-infected material, detection assays must exhibit robustness, sensitivity, and specificity. Recently, numerous instances of pear stony pit disease (PSPD) like symptoms have been detected in various pear cultivars in South Africa. This has underscored the necessity to scrutinize the prevalence of the disease and the efficacy of current detection assays in identifying apple stem pitting virus (ASPV). While there is a strong suspicion that ASPV may be closely associated with PSPD, conclusive evidence is still lacking. It is worth noting that specific species of stink bugs can also cause similar damage to fruit (2).

Materials and Methods

Pear stony pit disease-like trees were visually identified in several pear orchards distributed over two different geographical regions in the Westen Cape of South Africa. Samples were collected from symptomatic trees and tested for ASPV using an RT-PCR assay. A subsample of these samples were sent for high-throughput sequencing (HTS) to construct virome profiles. Based on the HTS data, a new ASPV detection assay was developed and validated using the samples of the survey. Orchards were also surveyed for the presence of stink bugs.

Results and Discussion

Pear stony pit disease-like trees were visually identified in orchards representing six pear cultivars. Using the initial RT-PCR assay, ASPV was identified in 71% of symptomatic trees, however in the HTS data divergent ASPV variants were identified and resulted in the development of a new RT-PCR assay that could detect more ASPV variants. The HTS data analyses also lead to the first detection of citrus virus A (CiVA) and apple rubbery wood virus 2 (ARWV-2) in South African pear trees. The presence of stink bugs, specifically from the genus *Antestiopsis*, was only identified in one of the geographical regions surveyed. After fruit symptom evaluations, two different symptom categories were identified. Either large, light brown internal markings that were not present deep within the fruit's flesh and which had a <60% association with ASPV and mostly identified in the region where stinkbugs were present or the very defined pits on the pear surface with small internal dark shapes that penetrated deep into the fruit's flesh which had a >90% association with ASPV. Therefore, currently, the best practice recommendation for symptom management includes planting ASPV free plant material and eliminating stink bugs from orchards.

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PRUNUS-INFECTING LUTEOVIRUSES: MORE VIRUSES AND STILL MANY QUESTIONS

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Introduction

Luteoviruses (family *Tombusviridae,* genus *Luteovirus*) have been shown to infect many crops, in which they often cause economically destructive diseases. Before the present work, six *Luteovirus* species have been identified in *Prunus* species, rose and apple, without any clear association with symptoms or disorders in the infected trees.

Materials and Methods

In the frame of a virome characterization study in *Prunus*, more than 500 trees have been analysed by high-throughput sequencing (HTS) of purified double-stranded RNAs. In order to maximize the host genetic diversity, *Prunus* accessions from five cultivated species (*Prunus armeniaca, P. persica, P. domestica, P. avium* and *P. cerasus)* and related species (as *P. serulata*, *P. cerasifera*), gathered in the INRAE Prunus Biological Resource Center have been submitted to HTS indexing. Wild or ornamental *Prunus* otherwise collected were also analyzed. For this purpose, double-stranded RNAs, proxy of viral infection by RNA viruses, were purified and sequenced by Illumina in a multiplexing format. In parallel, datamining of *Prunus* public transcriptomic RNASeq Sequence Read Archives was also performed.

Results and Discussion

Bioinformatic analyses as well as further molecular characterization efforts allowed to identify six novel luteoviruses infecting cultivated (peach) and ornamental/wild (almond and wild cherries) *Prunus* species[1,2] (Khalili et al., 2020; 2023). A survey conducted at the European level on peach-infecting luteoviruses [i.e. the two already known luteoviruses: nectarine stem pitting-associated virus (NSPaV) and peach-associated luteovirus (PaLV), and the novel peach-associated luteovirus 2 (PaLV2)] provided data on their wide geographical distribution in Europe, their significant prevalence and their genetic diversity. Moreover, biological indexing on GF305 peach indicator seedlings showed that NSPaV, PaLV, and PaLV2 are graft-transmissible. No clear association of any of these three viruses with symptoms could be observed, either in graft-inoculated GF305 plants, or in the broad range of field-grown peach varieties studied. These results thus suggest that these three viruses are likely asymptomatic in peach. These indexing experiments have allowed to obtain GF305 indicators infected by various combinations of peach-infecting luteoviruses, which constitute an excellent material for future studies of the aphid transmission and potential pathogenicity of these three viruses.

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HOW HIGH THROUGPUT SEQUENCING CAN BE USED AS A GENERIC TOOL TO IDENTIFY VIRUSES IN A REGULATORY FRAMEWORK
-
HTS TEST VALIDATION WITHIN AN ISO17025 ACCREDITED PLANT HEALTH LABORATORY

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Introduction

The use of High Throughput Sequencing (HTS) for the detection and identification of plant viruses and viroids is widely used. To use HTS in a regulatory framework, a laboratory needs to have a validated HTS test to be able to become ISO 17025 accredited for this test. So far, validation of HTS mainly focused on the bioinformatics pipeline, however these pipelines are only a part of HTS tests. We have described and validated an HTS test from biological material till the reporting of the identified viruses and were, so far we know, the first plant health laboratory in Europe with an ISO 17025 accreditation for HTS.

Materials and Methods

Tomato brown rugose fruit virus in infected tomato leaves was used as a model system for the validation of the HTS test. Performance criteria analytical sensitivity, repeatability and reproducibility were determined similarly as for traditional molecular methods as described in the EPPO standard PM7/98 specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity (EPPO, 2021). Analytical specificity, selectivity and robustness were assessed in a more generic way. This approach allowed the addition of detection and identification of other viruses and viroids by HTS to the scope of accreditation.

Results and Discussion

The HTS test was demonstrated to be fit for purpose for at least 180 viruses and viroids, including viruses with both DNA and RNA genomes, in a variety of hosts, including fruit crops. The ISO 17025 accredited HTS test allows us to detect and identify potentially all EU-regulated viruses and viroids, instead of implementing and validating specific tests (ELISAs, PCRs) for each regulated pest. Therefore, a single HTS test is providing an efficient and appropriate tool to screen for multiple viruses and viroids in survey and certification programs. We will present challenges and practical solutions in implementing and validating an HTS test within an ISO 17025 accredited plant health laboratory.

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SELF-CLEAVING VIROID-LIKE RNAS IDENTIFIED BY PLANT METATRANSCRIPTOMICS: THE QUESTION OF THE ACTUAL HOST

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Viroids and viroid-like satellite RNAs are infectious, circular, non-protein coding RNAs reported in plants. Some viroids (family *Avsunviroidae*) and some viroid-like satellite RNAs share self-cleaving activity mediated by hammerhead ribozymes (HHRzs) endowed in both RNA polarity strands. More than twenty five years ago, two self-cleaving viroid-like RNAs containing HHRzs in each polarity strands were identified in RNA preparations from cherry trees and subsequently reported to be very likely associated with a fungus infecting cherry leaves (Minoia et al., 2014). Later on, based on high-throughput sequencing technologies, new viroid-like RNAs from fig and citrus trees were reported Olmeva-Velarde et al., 2020; Navarro et al.,2021). It was proposed that they were very likely not infecting the plant, but another organism, maybe a fungus only transiently associated with the plants from which the sequenced RNA libraries were generated, thus suggesting a wider host range than plants for viroids and viroid-like RNAs.
Here, we report that more than 20.000 potential new viroid-like RNAs containing self-cleaving ribozymes have been identified in a study in which about 200,000 publicly available metagenome/metatranscriptomic sequencing libraries in the Sequence Read Archive were screened for circular and ribozyme-containing RNAs. Some of these viroid-like RNAs were non-protein coding RNAs containing HHRzs in both polarity strands, thus structurally resembling those previously reported in cherry, fig and citrus. Other viroid-like RNAs contained different self-cleaving ribozymes and/or were able to code for protein(s), such as viral RNA-dependent RNA polymerases involved in their own replication. Interestingly, representative members of both protein and non-protein coding viroid-like RNAs were shown replicate in fungi, thus extending the host range of viroid-like RNAs to this life kingdom. Altogether these data show the existence of a previously unknown layer of microbial biodiversity based on circular self-cleaving RNA genomes (ribozycirculome), likely associated with fungi or with other organisms, that must be taken into consideration when metatranscriptomics data from plants are analyzed.

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DISCOVERY OF A NOVEL ROSE-ASSOCIATED HAMMERHEAD VIROID IN CHINA ROSE (*ROSA CHINENSIS* JACQ.) BY HIGH-THROUGHPUT SEQUENCING

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Introduction

Viroids are small, single-stranded, non-protein coding and circular RNA molecules enabling to infect many dicot and monocot plant species. Based on the site of replication, accumulation of their genomes, structural features and replication mode, viroids are classified into two families *Pospiviroidae* and *Avsunviroidae*, which include members that are located in nucleus and chloroplast, respectively (1). China rose (*Rosa chinensis* Jacq., family *Rosaceae*) is an important ornamental plant, and as previously shown it is affected by some viruses, including apple mosaic virus (ApMV), arabis mosaic virus (ArMV), prunus necrotic ringspot virus (PNRSV), strawberry latent ringspot virus (SLRSV) and tobacco streak virus (TSV) (2). However, there has not been any report of viroid infection in China rose. High-throughput sequencing (HTS) technology has proven an efficient approach for the identification of both known and novel viral and subviral pathogens.

Materials and Methods

Leaves from China rose were collected from Beijing, China. The cDNAs of small-RNA libraries were prepared and sequenced using an Illumina TrueSeq Small RNA Sample Preparation Kit (Illumina, San Diego, CA, USA) at LC-Bio Science (Hangzhou, China). Complete nucleotide sequences were cloned and sequenced.

Results and Discussion

In the present study, a novel rose-associated hammerhead viroid in China rose (*Rosa chinensis* Jacq.) was discovered by HTS, and was tentatively named China rose hammerhead viroid (CRHVd). This viroid consists of 485 nucleotides which is significantly larger than the currently known viroids. CRHVd is predicted to have a branched conformation. Despite the low nucleotide sequence identity with known viroids, CRHVd contains a hammerhead ribozyme in each polarity strand, typical characteristic of members of the family *Avsunviroidae*. The infectivity of dimeric RNA transcripts generated by in vitro transcription of a synthetic CRHVd cDNA, was demonstrated by directly injecting into the stems of young China roses. A field survey revealed a low CRHVd incidence (4.8%, 12/252) in rose plants in China.
To the best of our knowledge, CRHVd is the only viroid known to infect rose plants.

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SESSION 2

NEXT GENERATION CERTIFICATION

Keynote speaker: Thierry Candresse

Chair: *Thierry Candresse & Varvara Maliogka*

EXPERIENCE WITH HTS TESTING OF CERTIFIED APPLE ROOTSTOCKS AND GRAFTS FOR THE PRESENCE OF VIRUSES AND VIROIDS

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Introduction
High-throughput sequencing (HTS) of plant RNA has been proposed as a plausible alternative to biological indexing of virus-like pathogens, which is generally recognized for the certification of propagative apple material and it is mandatory in the European Union.

Materials and Methods
Our experiments included HTS testing of the certified M9 apple rootstocks (Standard CZ, Elite, Basis 3 and Basis 3 T337, purchased from 3 providers) and budwood (grafts) from mother plants of cultivars Golden Delicious, Boskoop Red, Šampion, Jonagored Supra, Selena, and Idared. The mother apple plants were kept in technical isolation in the insect-proof greenhouse at the Research Institute of Pomology Ltd., Holovousy. HTS libraries were prepared from enriched double-stranded RNA and processed with NovaSeq 6000. The results were verified by RT-(q)PCR and Sanger sequencing.

Results and Discussion
Apple hammerhead viroid-like RNA (AHVd) was detected in Šampion, Jonagored Supra, while in Idared it was present in a mixed infection together with Citrus concave gum-associated virus (CCGaV). Boskoop Red and Golden Delicious were free of any virus-like sequence, similarly as M9 Elite rootstocks. In some Basis 3 rootstocks we revealed AHVd, CCGaV and Solanum nigrum ilarvirus 1 (SnIV-1), in some Basis 3 T337 rootstocks we identified CCGaV, SnIV-1, apple luteovirus 1 (ALV-1) that are relatively novel viruses in apple (Várallyay et al., 2022). Furthermore, we revealed sequences of Apple rubbery wood virus 1 as well as sequences of three tentative novel viruses, which are subjects of further studies. Results on the identification of virus-like pathogens and repeated testing in a field nursery will be discussed. Our results suggest application of more rigorous HTS testing for the certification of rootstock and budwood source plants as a powerful tool to determine their health status and improve sanitary measures for higher quality of apple propagative materials. Granted by the project QK1910065 of the Czech Ministry of Agriculture.

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UNVEILING VIRUSES AND VIROID IN APPLE GERMPLASM AND ORCHARDS IN HUNGARY USING HIGH THROUGHPUT SEQUENCING

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Introduction

High-throughput sequencing (HTS) has accelerated both the pace of discovery and the identification of new viral species. This technique provides insight into viruses and viroids in any fruit and that's why results of surveys using this method can give valuable information to a broad community of breeders, nurseries, apple growers and phytosanitary administrations.

Materials and Methods

To investigate the virus status of apple orchards and germplasm collections, HTS was applied as an unbiased diagnostic method. Samples used in this research were collected from five orchards and three germplasm collections at distinct geographical locations in Hungary. Six separate locations were used to create a total of 16 sRNA sequencing libraries, which represented 18 different cultivars. These libraries were sequenced and the reads were analyzed using CLC Genomic workbench. The RT-PCR or Northern blot investigation supported the HTS results.

Results and Discussion

During our virological survey we discovered a widespread distribution of Apple hammerhead viroid (AHVd), Apple luteovirus 1 (ALV-1), Citrus concave gum-associated virus (CCGaV)[1] (Várallyay *et al*., 2022) and Apple rubbery wood virus 2 (AWRV2) in apple trees. In 9 out of the 16 libraries, AHVd was found and we validated its presence either by Northern blot or RT-PCR. A range of single nucleotide polymorphisms (SNPs) were identified in AHVd sequences in the loop part of the structure, where base pairing is not essential for the proper viroid function. Sequences of variants of AHVd from the same cultivar differed slightly and clustered together, inferring a common origin and evolution in infected trees. ALV1 was detected both in the production orchards and in the germplasm collection suggesting its widespread distribution. Alignment of six ALV-1 sequences revealed a genomic region (~4500–~4700 nt) with several indels of variable length. CCGaV RNA1 and RNA2 were detected in five libraries by sRNA HTS in five different species. CCGaV isolates showed high conservation within their sequenced region. We also found the presence of Apple rubbery wood virus 2 (AWRV2), that has not been described in Hungary before and validated its presence in several samples by RT-PCR. Thus, we conclude that HTS has not only proven very successful for virus discovery to resolve disease etiology in many apple trees but also uncovered the complexity of metaviromes in field-collected samples. This will utterly help in the propagation of disease-free germplasm for virus-free apple trees across Hungary.

This work was supported by the NKFIH, grantK127951, AJ is a PhD student at MATE Doctoral School of Plant Science.

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Várallyay *et al*., Viruses 2022, 14, 2347.

OPTIMIZING CERTIFICATION AND QUARANTINE PROTOCOLS FOR FRAGARIA AND RUBUS GERMPLASM INTRODUCTION AND MOVEMENT

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With globalization and the trade of plant germplasm, the potential for major plant disease outbreaks is constantly increasing. To slow the spread, plants with potential diseases are quarantined until determined to be free of economically important pathogens[3] (Martin et al. 2016). Plant viruses can be difficult to detect through visual inspection, which requires tests to increase reliability, reproducibility, sensitivity, and specificity. Systemic diseases and mainly viral pathogens have been tested by grafting to bioindicator plants (biological indexing) for decades[5] (Villamor et al. 2019). Reading indicator symptoms can be a challenge and requires experienced evaluator. Some plants do not display symptoms unless infected with multiple viruses in a complex such as blackberry yellow vein disease complex (Martin et al. 2013) or strawberry necrotic shock virus[4] (Martin and Tzanetakis 2006). Bioassays have high associated costs, as they require facilities to grow and maintain indicators and personnel to run them[2] (Huttinga 1996). High Throughput Sequencing (HTS) and bioassays can detect the same viruses, but HTS can also detect viruses that would go undetected in a bioassay and can detect previously unknown viruses[1] (Al Rwahnih et al. 2015). Additionally bioassays can take several years compared to a couple of weeks for HTS. In this research we used 75 Fragaria and 75 Rubus plants that are known to be infected with different viruses as part of the USDA ARS National Clonal Germplasm Repository curated collection and the USDA Virology Lab, Corvallis OR positive control collection. Symptomatic virus infected plants with single infection or mixed virus infections were grafted onto each strawberry bioindicator lines UC 4, 5, 10 and 11 developed by the University of California breeding program. After grafting, symptoms were observed and recorded each week until dormancy. In addition, the 75 virus-infected plants are being tested by PCR and subjected to HTS. Similarly, 75 known virus-infected Rubus plants (single and mixed infections) were grafted onto *Rubus occidentalis* 'Munger' as the widely used biological indicator for virus indexing of Rubus viruses. RNA extraction from all plants will be used for RT-PCR and HTS tests. Preliminary results suggest that HTS can detect viruses that are not showing symptoms on grafted plants. This research will result in updated and optimized protocols for nursery certification testing, as well as aid regulatory agencies in optimizing regulations related to quarantine protocols and the importation and release of germplasm to stakeholders.

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DEVELOPMENT OF REVISED DIAGNOSTIC PROTOCOL FOR QUARANTINE AND CERTIFICATION OF *PRUNUS* GERMPLASM AT FOUNDATION PLANT SERVICES IN DAVIS, CALIFORNIA, USA

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Foundation Plant Services (FPS), located in Davis, California, USA, is a source of elite propagation materials of commercially important *Prunus* scion and rootstock cultivars as part of the California Department of Food and Agriculture's (CDFA) Deciduous Fruit and Nut Tree Registration and Certification (R&C) Program. The R&C Program targets the elimination of specific viruses that are spread through grafting or propagation. FPS also facilitates the introduction, quarantine, and release of imported *Prunus* for the US fruit tree industry under a Controlled Import Permit (P588) from the United States Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine (USDA APHIS PPQ). Both CDFA and USDA APHIS PPQ regulations have required the grafting of candidate selections to specific *Prunus* biological indicators that were observed for symptom development to assess the disease status of plant material.

We conducted side-by-side studies comparing the efficacy of woody and herbaceous indexing to polymerase chain reaction (PCR) and high throughput sequencing (HTS) in *Prunus*, rose, and grape diagnostics. FPS integrated this research knowledge into an improved protocol that adopts the most reliable and efficient techniques using HTS and real time quantitative RT-qPCR/qPCR, which are known to be highly sensitive tests. The revised protocol uses informative HTS testing of source material to provide customers with feedback on the phytosanitary status of the plant material and determines if virus elimination therapy is necessary. Once plants are propagated and have developed appropriate tissue to test, initial HTS/PCR testing provides information on viruses that may be present in the plant material in the summer to fall. Final HTS/PCR testing after a six-month minimum period of dormancy provides a third and final test, testing for the presence of viruses and verifying earlier test results or detecting pathogens previously undetected due to low titer. The USDA-APHIS-PPQ and CDFA have approved the use of this revised diagnostic testing protocol to replace biological indexing with a combination of HTS and PCR testing for release of plant material.

The entire process from introduction to release of material using biological indicators ranges from two to six years. The revised protocol can reduce the regulatory testing process to one-year, excluding the time required to establish plants. This expedited timeline with quick access to clean plant material is critical for certification programs and enables the US industry to remain competitive and responsive to consumer needs.

IDENTIFICATION OF EUROPEAN UNION QUARANTINE PHYTOPLASMAS: A CHALLENGE FOR DIAGNOSTIC LABORATORIES IN PLANT HEALTH

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The Plant Health Regulation (EU) 2016/2031 of the European Union (EU) on protective measures against pests of plants aims to prevent the introduction and spread of harmful organisms in the EU territory. Annex II of this regulation lists the Union quarantine pests ((EU) 2021/2285), i.e. those pests not known to occur (part A) and those known to occur (part B) in the EU territory. The implementation of this regulation implies that National Reference Laboratories should be able to detect and identify the listed pests. For phytoplasmas this appeared not to be an easy task, based on the results of an interlaboratory comparison. In particular the identification of phytoplasmas at strain level was problematic and differed between laboratories. The different outcomes, however, could not be ascribed to a single cause, but resulted from a combination of issues concerning 1) the interpretation of the regulation, 2) taxonomic issues, and 3) technical restrictions. A few examples will be further detailed and explained. Furthermore, potential solutions will be presented that aim to achieve a more harmonized approach between laboratories. These include both pragmatic and more fundamental approaches, for which the merits and weaknesses will be discussed. The European Union Reference Laboratory for Pests of Plants on Viruses, Viroids and Phytoplasmas has informed the European Commission on the diagnostic issues and provided potential solutions to improve the harmonization of identification of phytoplasmas within the European Union.

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NEXT GENERATION QUARANTINE OF IMPORTED FRUIT TREES IN USDA APHIS

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Introduction

Fruit tree varietal improvement relies on access to valuable imported germplasm. The Plant Germplasm Quarantine Program (PGQP) of the USDA APHIS, located in Beltsville, Maryland, is the largest federal quarantine program in the United States, and the main gateway to importing fruit trees safely. PGQP now screens all implorted fruit tree germplasm for viruses, viroids, and phytoplasmas, including uncharacterized agents, using PCR and HTS-based methods. If pathogen(s) are detected, germplasm is treated to generate pathogen-free trees before their released for planting in the U.S. PGQP has sequenced and analyzed over 500 fruit tree importations using in-house sequencing and bioinformatic capabilities since 2019. Lessons learnt, new viruses identified, and relevant observations and consideration will be discussed.

Materials and Methods

All imported fruit trees were received as dormant budwood and grafted on virus-free rootstocks. Total RNA was extracted using the RNeasy Plant Mini kit (Qiagen) with an amended extraction buffer, quantified by Qubit (ThermoFisher), and inspected on TapeStation 4200 (Agilent). RNA-seq libraries were prepared using the TruSeq Stranded Total RNA Library Plant Kit (Illumina) with Ribo-Zero™ for ribodepletion. Libraries were sequenced using various sequencing kits on the NextSeq500 or NextSeq2000 (Illumina) at the USDA-APHIS PGQP facilities. Bioinformatic analysis was conducted with the internally developed pipeline (Phytopipe). The same total RNA extract is subject to RT-PCR for the detection of a wide range of regulated viruses and viroids. PCR confirmation of HTS-results was done using custom-designed or publicly available primers when needed.

Results and Discussion

The fruit tree quarantine program of PGQP has fully incorporated HTS-based diagnostics as another tool for virus indexing while reducing or eliminating the use of some woody or herbaceous indicators. This, together with newly updated policy regulations, have resulted in a shorter quarantine time and a more thorough diagnostics of imported fruit trees. The wide use of HTS metagenomics has also allowed the discovery and later characterization of new viruses as well as the report of new fruit tree hosts for previously discovered viruses.

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HIGH THROUGHPUT SEQUENCING (HTS): AN AID AND IMPROVEMENT TO CONVENTIONAL FRUIT TREE PROPAGATION AND QUARANTINE SCHEMES

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Introduction

One of the major achievements of fruit tree certification or quarantine programs is to evaluate the sanitary status of plant material and ensuring the propagation of high value material. The emergence of high throughput sequencing (HTS) offers new opportunities for diagnosis of plant viruses and viroids and in particular the characterisation of their virome. HTS use is a major challenge in certification and quarantine contexts with a need to demonstrate accurate and reliable results prior to routine implementation for the sanitary control and selection of fruit trees cultivars.

Materials and Methods

For this, the CASDAR Virvalid[1] project had two main objectives. The first was to compare an HTS method based on dsRNA extraction with conventional detection methods (biological indexing, ELISA, molecular hybridization (MH), PCR), using samples of known virological status, chosen among apricot, cherry, peach, pear, plum and apple trees with single or mixed infections by viruses and/or viroids. A second objective was to compare analytical sensitivities of these various methods using the same range of samples with a known viral status.

Results and Discussion

Best inclusivity was obtained with HTS (90%), compared to 85% for PCR/MH and 71% for ELISA whereas diagnostic specificity was the same for all methods (100%). Inclusivity, calculated with viruses only, reached 100% for HTS. In addition, unexpected infections were detected by HTS demonstrating that this method was clearly challenging the conventional methods for its versatility and ability to detect latent infections. Depending on the virus, HTS analytical sensitivity ranged from 1 to 10^{-5} and was systematically greater than ELISA making it possible to consider this technique as a good candidate for replacing it or even certain PCR tests. Our results highlight HTS as an accurate and reliable method to detect and identify viruses in fruit tree species, as well as a technique complementary with the conventional methods for the selection of high value fruit plants required in germplasm, exportation, certification or quarantine programs.

GERMANY-WIDE APPLE HAMMERHEAD VIROID SCREENING IN APPLE REVEALS HIGH AHVd GENOME SEQUENCE VARIABILITY AND LEADS TO AN OPTIMIZED AHVd SPECIFIC REVERSE TRANSCRIPTION REAL-TIME PCR

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Introduction

In 2020, Apple Hammerhead Viroid (AHVd) (family *Avsunviroidae*, genus *Pelamoviroid* (1)) was first detected in Germany in two apple orchards in Baden-Wuerttemberg (JKI, Dossenheim). AHVd RNA was first described in China in 2014 (2). Plant pathogenic viroids are small circular, un-encapsulated, non-coding single stranded RNA molecules. A diverse symptomatology is reported for AHVd infections in apple (*Malus domestica*), varying from no symptoms (latent infections) to fruit quality impairing (mosaic, ringspot symptoms) and destructive symptoms (radial limb cracking, shoot decline, dieback). Symptoms are variety-dependent and mixed infections with other viroids or viruses impede a conclusive association of AHVd with a disease (3). As AHVd is a graft-transmissible agent, the prevalence of AHVd in apple is highly relevant and was investigated in 2022 in a Germany-wide apple screening.

Materials and Methods

The Germany-wide screening comprises over 1100 apple samples originating from 14 federal states and 41 locations, representing more than 200 *Malus domestica* varieties. Samples were received from scattered and commercial orchards, from nurseries, trial and educational fruit growing farms and from variety collections. Shoot samples (phloem) were nucleic acid extracted (CTAB method) and sample pools were analyzed for AHVd presence with different published and unpublished Reverse Transcription (RT)-PCR protocols. RT-PCR products of single samples were sequenced.

Results and Discussion

Sequence analysis revealed a high AHVd genome sequence variability. Observed divergence in detection results for multiple RT-PCR assays might be explained by the occurrence of numerous genome sequence variants. Phylogenetic analysis of AHVd accessions (NCBI Genbank) and of new German isolates visualizes that sequence variations might correspond to accession origin. As a consequence, we developed an AHVd specific RT-Real-Time PCR with the aim of targeting all identified genome sequence variants. All individual screening samples will be analyzed with the developed and optimized AHVd RT-Real-time PCR for a comprehensive AHVd analysis.

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SESSION 3

EMERGING DISEASES

Keynote speaker: Ioannis Tzanetakis

Chair: *Ioannis Tzanetakis & Ni Hong*

BLUEBERRY DECLINE ASSOCIATED WITH NEW AND EMERGING VIRUSES

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The USA leads the worldwide production of blueberries, accounting for nearly 40% of total production. Within the USA, Oregon is one of the largest blueberry suppliers with a production of 150 million pounds in 2021 and a crop value of $172 million (up 43% compared to 2020)[3] (USDA-NASS, 2022). Viral diseases are the main constraint for blueberry production with around 17 distinct viral species reported in blueberry crops to date[1,2] (Martin et al., 2012; Saad et al., 2021). In the Pacific Northwest, blueberry scorch virus (BlScV), a carlavirus, is one of the most damaging viruses on blueberry. Recently another carlavirus, blueberry virus S (BluVS), which is most closely related to BlScV was described in Oregon and Washington[4] (Villamor et al. 2023). We screened more than 1,800 blueberry leaf samples from Washington and Oregon for both viruses. Coat protein sequences of at least 100 positive samples were obtained. Sequence identity ranged between 75-100% to the known carlaviruses in the region, but in a few occasions, sequences shared a nucleotide identity of only 60% to either BlScV or BluVS. These putative new isolates are being characterized by high throughput sequencing (HTS) and long-range PCR. Additionally, in 2022, a sudden decline of blueberry bushes was observed in an isolated field in Oregon. Disease symptoms included: yellowing or reddening of the leaves, ringspot, witches' broom, and necrosis that often progressed into plant death. Preliminary data suggested no fungal pathogens were associated with the decline. Further studies did not detect the presence of *Xylella*, phytoplasma, or other bacterial-like pathogens, except Agrobacterium. A new strain of tomato ringspot virus (ToRSV), two new luteoviruses, and another previously uncharacterized virus were detected by HTS. The luteoviruses are distinct and separate new species. The fourth virus is a new mitovirus. The role of these viruses in causing disease symptoms is completely unknown, except that these viruses were detected in symptomatic plants. In a small-scale field survey, virus incidence was 33%, 53%, and 100% for the new luteovirus, ToRSV, and the mitovirus, respectively. We also observed that these four viral agents occur frequently in mixed infections. The role of these newly emerging blueberry viruses is yet to be determined and studies to collect more genomic data coupled with disease symptoms is ongoing.

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CHARACTERIZATION OF A NEW VIROID ASSOCIATED WITH MARBLING AND CORKY FLESH IN JAPANESE PLUMS

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Introduction

Japanese plums (*Prunus salicina*), are mainly grown in South Africa for fresh fruit export purposes. However, for over 20 years, symptoms of marbling and corky flesh symptoms have been observed in multiple plum cultivars, without any identified causal agent. To investigate this disease, a high-throughput sequencing (HTS) approach was employed to analyse samples from both symptomatic and healthy plum trees to identify a potential causal agent.

Materials and Methods

Total RNA was extracted from leaf material using a modified CTAB extraction protocol (1). Total RNA was assessed for quality and shipped to Macrogen (Korea) for ribo-depletion, library construction and sequencing on an Illumina platform. *De novo* assemblies and BLAST analysis were used to identify a novel viroid, after which an RT-PCR assay was designed, and a survey conducted over three seasons. The identified novel viroid was evaluated for graft transmission. Multiple detection assays were developed to be used in the certification program to ensure propagation material is free of this viroid.

Results and Discussion

The samples analysed by HTS did not reveal any known fruit tree viruses that could explain the observed symptoms. However, all samples that contained viroid contigs displayed either marbling on the fruit skin or corky flesh within the fruit. The nucleotide sequence identity of these viroid contigs was found to be highest with apple dimple fruit viroid and citrus dwarfing viroid, but less than 77%. Through molecular characterization, it was determined that this viroid sequence belonged to a new species in the family *Pospiviroidae*, genus *Apscaviroid*, and was named plum viroid I (PlVd-I). This viroid has recently been accepted by the ICTV as a viroid and named *Apscaviroid plvd-I* (2). In three seasons of surveys the viroid was only present in trees displaying marbling and/or corky flesh in fruit, and not in healthy trees, indicating a clear association with the disease. Additionally, the new viroid was found to be graft transmissible and capable of causing disease symptoms in the first year after transmission. Therefore, it can be concluded that the a new viroid apscaviroid plvd-I can be associated with marbling disease in plums.

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CHARACTERIZATION OF STRAWBERRY VIRUS 1, A NEW STRAWBERRY CYTORHABDOVIRUS, ITS DISTRIBUTION, ANTIBODY PREPARATION AND EFFECT ON FRUIT YIELD IN THE CZECH REPUBLIC

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Introduction

High-throughput sequencing (HTS) is a unique method that allows the detection of viruses without prior knowledge of their genome sequence. Its application is particularly suitable for crops where viruses are present in mixed infections and when it is difficult to isolate viruses from plants by conventional methods. In addition to diagnostic methods, knowledge of the biological properties of newly discovered viruses, their vectors, distribution and economic harmfulness is necessary for effective control. The complete molecular and biological characterization of the recently discovered strawberry virus is presented in this contribution.

Materials and Methods

HTS (Illumina and Ion Proton platform), RT-PCR, molecular cloning, Sanger sequencing were used to obtain and verify sequence data. Virion particles were visualized by transmission electron microscopy. Diverse virus transmission methods were applied, including aphid- and graft-mediated procedures. Viral recombinant nucleoprotein was used for rabbit immunization. DAS-ELISA optimization with the raised IgGs was performed using standard approaches. StrV-1 harmfulness was studied in 'Darselect' and 'Karmen' cultivars following their artificial infection.

Results and Discussion

In 2017, we found a new virus on strawberry, named strawberry virus 1 (StrV-1). The complete sequences of three StrV-1 genotypes (A, B, C) were obtained. StrV-1 has nine protein-coding sequences and is characterized by a unique genomic organization. The genome size of StrV-1 is around 14,000 nt. Phylogenetic analyses clustered StrV-1 into one clade with tomato yellow mottle-associated virus. The finding of bacilliform particles and their localization in the cytoplasm confirmed the classification of the virus within the genus *Cytorhabdovirus*, species *Cytorhabdovirus fragariae*. Using the designed 2f/7r primers, StrV-1 was detected in 49 out of 159 tested strawberry plants using RT-PCR. The virus was transferred to *Nicotiana occidentalis* 37B and *N. benthamiana* by *Aphis ruborum* and *A. forbesi*. Upon StrV-1 graft-inoculation, *F. vesca* indicator clones revealed light green sectors and irregular vein clearing on young leaves (1). The recombinant StrV-1 N protein was produced for a rabbit immunization. A fraction of IgG immunoglobulins was isolated from serum, conjugated with alkaline phosphatase, and used for DAS-ELISA. Artificial inoculation of 'Darselect' and 'Karmen' cultivars in a field experiment showed that StrV-1 caused significant yield losses up to 32 % in infected strawberries (Karmen) that exhibited no distinct symptoms (2). Virus isolates related to StrV-1 genotypes A and B were found recently in China (3).

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Acknowledgements

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SESSION 4

VECTOR TRANSMISSION AND VIRUS-VECTOR-INTERACTION

Keynote speaker: Piotr Trębicki

Chair: *Piotr Trębicki & Francesco Di Serio*

VECTOR TRANSMISSION AND VIRUS-VECTOR-INTERACTION

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The majority of plant viruses are spread by vectors, which can be insects, mites, nematodes or fungi. Hemipteran insects, such as aphids, whiteflies, leafhoppers and planthoppers, are the most important vectors of plant viruses in terms of the number of viruses that they transmit but also the significant impacts of the resulting disease on global food production. Some viruses can be transmitted by multiple insect species and can infect many plant species, others rely on a specific vector to be transmitted to a very limited number of hosts. Regardless of how viruses are transmitted and how complex the mode of transmission, and despite how much effort we put towards disrupting their transmission and reducing their spread, disease outbreaks are common, difficult and costly to manage.

Biotic and abiotic factors have a critical influence on plants, vectors, and therefore virus persistence. Temperature, precipitation and light have a direct effect on plants and insect vectors, affecting host plant resistance, insect fitness and incidence, virus transmission and acquisition efficiency. Environmental changes affect the interactions between host plants, insects and viruses, advantage over the other and influencing epidemiology of vector transmitted viruses. Climate change has the potential to disturb the distribution and severity of plant viruses on a global scale by directly and indirectly affecting vector transmission and virus-vector-plant interactions.

Additionally, viruses can induce changes in the host plant to influence vector host preference. Infected plants can be more attractive to insects, through the volatile compounds that they emit, decreasing host plant resistance and increasing plant quality, which can increase population size and structure. Virus infected hosts, and presence or absence of virus in the insect vector, can also influence vector feeding and behavior, which is intended to benefit the virus through increased spread, and therefore persistence in the environment. This high level of complexity that influence disease epidemiology of vector transmitted viruses is difficult to predict and manage and can be very specific for the location as well as particular plant, virus and vector combination.

PLANTHOPPER *CIXIOSOMA SP.* (HEMIPTERA: CIXIIDAE) TRANSMITS '*FRAGARIA* × *ANANASSA*' PHYLLODY PHYTOPLASMA (16SRXIII) TO PERIWINKLE (*CATHARANTHUS ROSEUS*)

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Introduction

Since 2015, symptoms of phyllody and achenes' hypertrophy have been reported in numerous strawberry orchards from several regions of Chile. The pathogen associated with this disease is a phytoplasma belonging to the 16SrXIII ribosomal group, duly named strawberry phyllody phytoplasma or '*Fragaria* × *ananassa*' phyllody phytoplasma (StrPh) [1]. Phytoplasmas are transmitted by insect vectors in the field in a persistent propagative manner, which is their most efficient mechanism of horizontal transmission [2]. The purpose of this study was to identify insect vectors of StrPh.

Materials and Methods

Insect samples were collected by sweep net in strawberry orchards in Litueche, O'Higgins Region. The collected insects were identified visually to select Hemiptera species for the following assays. Transmission assays were performed by placing living insects collected in the field in contact with healthy periwinkle (*Catharanthus roseus*) plants for nine to 17 days. DNA was extracted from individual insects and plants using CTAB method. Molecular identification of insects was performed by PCR amplification and sequencing of the mitochondrial COI gene. Phytoplasma infection of insects and plants was detected by PCR amplification and sequencing of the 16S rRNA gene of phytoplasma.

Results and Discussion

Among the collected Hemiptera, StrPh was detected from the DNA extract of a group of cixids, which were identified as a new species of the genus *Cixiosoma*. Forty-six to 112 days after the start of the transmission assay, the plants in contact with the infected insects developed symptoms of virescence and phyllody. StrPh was detected from all the symptomatic plants. These results confirmed that this *Cixiosoma sp*. is a vector of the '*Fragaria* × *ananassa*' phyllody phytoplasma and can transmit this pathogen to periwinkle. The ability of this *Cixiosoma sp*.to transmit StrPh to strawberry is currently under evaluation.

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VECTOR MONITORING OF QUARANTINE PESTS AND REGULATED NON-QUARANTINE PESTS IN FRUIT CROPS AND VITICULTURE

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Introduction

Quarantine pests (QPs) such as Flavescence dorée phytoplasmas or the bacterium *Xylella fastidiosa* and regulated non-quarantine pests (RNQPs) are immense threats for fruit crops and viticulture (1, 2). QPs and RNQPs are often distributed by plant-sucking insect vectors such as Auchenorrhyncha, psyllids, scale insects or mealy bugs. European spread of these vectors, often together with the associated QPs or RNQPs, accelerates due to climate change, globalization and travelling. Currently, monitoring is mainly based on visual inspection of symptoms, laboratory diagnostics of single samples and morphological classification of vectors in orchards and vineyards. Increasing risks for spread of new QPs and RNQPs lead to the necessity for highly efficient and sensitive monitoring methods. Here, a new monitoring method based on amplicon sequencing of unsorted arthropod mass catches from orchards and vineyards is presented.

Materials and Methods

Arthropods were collected using a modified leaf vacuum. Auchenorrhyncha were separated from the remaining specimen (by-catch) and morphologically identified up to the lowest possible taxonomic rank as a control for later NGS results. Auchenorrhyncha and by-catch were homogenized separately in liquid nitrogen and 10-20 mg Arthropod powder was used for nucleic acid extraction with a CTAB based protocol. Auchenorrhyncha and by-catch DNA was mixed to mimic an unsorted mass catch. A 340 bp region of the COI marker gene was amplified from the samples and sequenced (Illumina MiSeq300PE). Reads were trimmed, merged, filtered and denoised using trimmomatic and VSEARCH. Afterwards, different databases, including reference sequences generated in the project, were used for insect species identification.

Results and Discussion

Currently, sequencing results for COI from four different locations are available and sequencing of samples from 13 additional locations from southwestern Germany is in progress. The first results show that all Auchenorrhyncha species that were identified with NGS in the Auchenorrhyncha only sample were also identified in the sample mimicking the unsorted mass catch. Therefore, results from the unsorted mass catch are reliable and pre-sorting will not be necessary in the future. However, some species that were morphologically identified but represented by a very low number of individuals were not identified with NGS. This can be due to several reasons and might be solved by further optimization of the bioinformatic pipeline. Additionally, the 16S marker gene is currently amplified and sequenced from the samples to identify associated bacteria and phytoplasmas.

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Spread of Blueberry Mosaic Associated Virus (BlMaV) from cultivated blueberries to wild plantations or vice-versa in natural ecosystems

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Introduction

Bluberry mosaic disease (BMD) was first reported in the 1950s in Michigan but the causal agent was recently characterized and tentatively named as blueberry mosaic associated virus (BlMaV)[1] (Thekke-Veetil et al. 2014). The virus was recently detected in many countries including Turkey[2] (Çağlayan et al. 2015). Although there is not many studies about the natural transmission ways of BlMaV, *Olphidium* spp. stated as potential vector for this virus due to it is the most common vector for *Ophiovirus* species. In this study the ability of *Olpidium spp.* as a potential vector of BlMaV was evaluated both in cultivated blueberry orchards and wild plantations in Turkey.

Materials and Methods

Soil samples were collected from the rhizosphere of BlMaV infected blueberry plants in one commecial orchard and two wild blueberry plantations in Blacksea Region. These soils from each location were distributed equally in 12 pots. Then lettuce, carrot, broccoli and cucumber as trap plants were planted together with an in vitro propagated blueberry plants (cv. Bluecrop) in each pot. The experiment was carried out in 3 replications. Thus, totally 9 seedlings from each trap plant species and 36 blueberry plants were used. All plants were regularly tested both for *Olpidium spp.* and BlMaV by PCR and RT-PCR analysis

Results and Discussion

Eight weeks after planting, resting spores of *Olpidium* species were observed in the roots of 6 lettuce, 1 carrot, 2 broccoli and 6 blueberry plants next to lettuce. Morphological observations were confirmed by PCR analysis and only *Olpidium virulentus* was detected. The sequences were deposited in GenBank (MW483635-640; MW48925-28). When all trap plants and adjacent blueberry saplings were tested by using primers specific to a nucleocapsid protein (NP) gene of BlMaV (Isogai et al. 2016), it was detected only in lettuce plants among trap plants and in blueberry plants planted next to lettuce. Since lettuce is a good host for both *O. virulentus* and BlMaV, the experiment was repeated with 6 replications using only lettuce. Thus, 21 blueberry and 13 lettuce isolates from the experiment and 9 blueberry isolates from the field for RNA3 (MW422205-234), one blueberry and one lettuce isolate for RNA2 (MZ513461-462) and one blueberry isolate for RNA1 (OK040161) of BlMaV were amplified, sequenced and submitted to the GenBank. This study confirmed that *O. virulentus* plays an efficient role in the transmission of BlMaV from cultivated blueberries to wild ones or vice-versa and lettuce is a good host both for *O. virulentus* and BlMaV.

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SESSION 5

VIRUS (PATHOGEN) HOST INTERACTION

Keynote speaker: Carolyn Malstrom

Chair: *Carolyn Malstrom & Hano Maree*

GENERATION OF INFECTIOUS CLONES OF PRUNUS NECROTIC RINGSPOT VIRUS (PNRSV) AND PRUNE DWARF VIRUS (PDV) TO ANALYZE POTENTIAL SYNERGISTIC EFFECTS OF THEIR MIXED INFECTIONS

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Introduction

Prunus necrotic ringspot virus (PNRSV) and prune dwarf virus (PDV) are the most common viruses that infect the almond tree [Prunus dulcis (Mill.) D.A. Webb]. Mixed infections of PNRSV and PDV result in peach stunt disease and are associated with bud failure and rosetting resulting in trees with a bare appearance and reduced fruit set (1, 2). Both viruses belong to the genus *Ilarvirus*, family *Bromoviridae* and have a tripartite genome organization. RNA 1 and RNA 2 encode the two subunits of the RNA polymerase, meanwhile, RNA 3 encodes directly the movement protein and the coat protein is expressed via a subgenomic RNA (1). In this work, we have generated infectious clones for both viruses using different approaches, which included several binary plasmids, sequences carrying ribozymes structures at the 3 termini, or fusions of different cassettes to increment the percentage of infected plants. Finally, the infectious clones were used to analyze in detail the effect of the PNRSV and PDV mixed infections in *Nicotiana benthamiana* and cucumber plants.

Materials and Methods

Total RNA was obtained from almond petals or cucumber plants using the RiboZol™ reagent and used first, to characterize the 5'/3' termini of the three viral RNAs of PNRSV and PDV by RACE analysis using a modified protocol which uses a stem-loop primer designed to target the end of the poly(A) tail. In a second step, the three viral RNAs were amplified by RT-PCR and introduced in expression cassettes under the control of the 35S promoter from cauliflower mosaic virus (CaMV) and the terminator from the potato proteinase inhibitor (PoPit), with or without a ribozyme sequence. The different cassettes, individual or in tandem, were cloned in a binary plasmid, derived from the pGreen (pMOG800 plasmid) or the novel mini binary vectors (3), and introduced in agrobacterium strain C58.

Results and Discussion

-Clear symptoms were observed in 20% of cucumber plants agroinfiltrated with different PNRSV and PDV infectious clones after 10 days post infiltration.

- The presence of symptoms correlated with viral RNA accumulation in northern-blot analysis.
- Mixed PNRSV and PDV infections were performed in *N. benthamiana* and cucumber plants and the accumulation of viral RNAs was compared with single infections.

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GENOME ANALYSIS OF '*CANDIDATUS* PHYTOPLASMA RUBI' HIGHLIGHTS SEPARATED POSITION OF 16SRV PHYTOPLASMAS

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Introduction

Phytoplasmas of the 16SrV-group (syn. elm yellows phytoplasmas) comprise pathogenic bacteria responsible for vector-borne diseases in different woody hosts. They include the economically important diseases in Europe such as *flavescence dorée* of grapevine or *rubus stunt* of raspberry and blackberry. Phytoplasmosis of *Rubus*-cultivars is caused by the pathogen '*Candidatus* Phytoplasma rubi' (1). Typical symptoms beside stunting are proliferation, virescence and phyllody. Recently, we determined the complete genome of '*Ca.* P. rubi' providing an excellent starting point for a comparative genome analysis with other full phytoplasma genomes.

Materials and Methods

Functional reconstruction of '*Ca.* P. rubi' strain RS (accession number CP114006) was performed as previously described (2). Secretome prediction was accomplished by analysing the deduced proteins with respect to signal peptide and transmembrane domains. The unique and shared genetic repertoire of '*Ca.* P. rubi' strain RS, '*Ca.* P. vitis' strain CH (acc. no. CP097583) and '*Ca.* P. ziziphi' strain jwb-nky (acc. no. CP025121) was distinguished through clustering of orthologs. Complementary analysis was performed with respect to the coding in other phytoplasma genomes.

Results and Discussion

Metabolic network and comparative analyses highlight the typical shared repertoire of proteins involved in transport across the membrane and a specialized carbohydrate metabolism typically for all phloem-colonizing phytoplasmas (3) but particularities in utilization of carboxylic acids clearly separate 16SrV-phytoplasmas from other phytoplasma branches showing an advanced level of reductive evolution.

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INVESTIGATION OF MORE LOCI IN LINKAGE GROUP 1 IN APRICOT (*Prunus armeniaca*) AS CANDIDATE RESISTANCE MARKERS AGAINST TO PLUM POX VIRUS

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Introduction

Understanding the mechanism of resistance to pathogens in fruit trees is arguably one of the most important areas of study. A major resistance locus, named *PPVres* and located in the upper part of Linkage Group 1 (LG1) of apricot, accounted for 70% of the resistance to plum pox virus. A cluster of genes of the MATH–TRAF family located in *PPVres* locus on LG1 could be responsible for the resistance to PPV, and particularly a 5 nt deletion on a MATH gene has been hypothesized to result in loss of function and thereby resistance gene silencing in susceptible accessions (Zuriaga et al., 2013, 2018). This 5 nt deletion has been used as the most reliable and easy to use in selecting for the resistant apricots (Decroocq et al., 2014; Gürcan et al., 2019). Besides to MATH–TRAF family, a genome-wide association (GWA) approach in apricot highlighted other potential resistance loci, and resolved each to a limited set of candidate genes for further study (Mariette et a., 2016). Here we also implemented GWA to determine PPV resistance linked loci in LG1.

Materials and Methods

DNA sequence of 32 apricots, including resistant and susceptible breeding material were obtained using high-throughput sequencing. Genomic variants within a region of ~300 kb spanning PPVres locus, flanking the 5 nt deletion were determined. Fifteen indel loci were further investigated together with the 5 nt deletion by segregation analysis. PCR amplification of 800 seedling of F1 mapping populations were performed for the 16 loci.

Results and Discussion

DNA reads of the 32 apricots produced by high-throughput sequencing were mapped to the PPVres locus of the reference apricot genome with minimum 30x coverage. Over 200 genomic variants, including INDELs and SNPs, linked to the 5 nt deletion were determined by GWA. Among the 15 loci, a 15 nt deletion located at 114 054 nt poison of 300 kb depicted over 95 % co-segregation with the 5 nt deletion located at the position 118 674. Further, another 12 nt insertion at the position 266 005 nt exhibited one hundred percent allelic variation with the 5 nt deletion. As Marrette et al., (2016) reported previously, it is likely that more loci linked to the PPV resistance. The new PPV resistance linked loci has potential to be used for marker assisted selection in apricots.

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VIRAL INFECTIONS ENDANGER THE SURVIVAL OF THE WILD APPLE POPULATION

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Introduction

Malus sieversii, progenitor of many apple cultivars throughout the world is an important source of genetic diversity for apple breeding programs. Due to the reduction of the distribution area and the gene pool, *Malus sieversii* is becoming more vulnerable and its protection is important for the conservation of the genetic diversity of the apple tree and the biodiversity of the Tien Shan ecosystem [1]. *Apple chlorotic leaf spot virus (ACLSV), Apple mosaic virus* (ApMV), *Apple stem grooving virus* (ASGV), *Apple stem pitting virus* (ASPV) and *Apple necrotic mosaic virus* (ApNMV) are among the most common viruses which lead to significant harvest losses. The purpose of this work was to assess the spread of viral diseases and determine the infectious status of the wild apple populations of the Northern Tien Shan.

Materials and Methods

Malus sieversii samples collected from 4 sites within the Northern Tien Shan from the Djungarsky Alatau mountain range and the Zailiysky Alatau mountain range. RNA isolation from 230 samples of wild apple was performed by CTAB method. Detection of five viruses was carried out using specific primers by the RT-PCR.

Results and Dıscussıon

In the present study, 45 samples of wild apple were positive for ACLSV, 30 samples were positive for ASPV (29 coinfection). ASGV, ApMV and APMNV were not detected in any of the populations. For the first time, viral infection in the *Malus sieversii* populations in Kazakhstan was characterized. According to the results, the percentage of infected wild-growing apple trees from the Zailiysky Alatau was 88% of all infected apple trees, which was much higher than from the Dzungarsky Range, which was only 12%. It can be assumed that the viruses could have been transferred from a large number of cultivated apple orchards that surround the wild population in Zailiysky Alatau, while in Dzhungarsky Alatau the population of wild-growing apple trees was separated from cultivated ones. The presence of viruses in wild apple populations warrants further research on other pathogens.

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LITTLE CHERRY VIRUS 2 AND "NOT SO LITTLE CHERRY VIRUS 1"; RESULT OF A 8 YEARS FIELD TRIAL.

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Introduction

Little cherry disease is an economically important viral disease that affects sweet and sour cherry species in field stock nurseries and commercial orchards. It is caused by two distinct viruses, little cherry virus 1 (LChV-1) and little cherry virus 2 (LChV-2). Both viruses are members of the *Closteroviridae* family, but LChV-1 is a member of the genus *Velarivirus* while LChV-2 is classified within the genus *Ampelovirus*. Symptoms caused by these two viruses range from asymptomatic plants to severe leaf and fruit symptoms, including the eponymous little cherries. The symptoms are highly variable among cherry cultivars and dependent on the environmental conditions, the cultivar susceptibility and the virus species and isolate (1, 2). The disease is present in many cherry-growing areas of the world, including Switzerland. Through a long-term field trial assay, this study provides useful information on the biological impact of LChV-1 and LChV-2 separately or combined on four different cultivars of sweet cherry (*Prunus avium* L.) in an orchard condition.

Materials and Methods

In 2015 a field trial was put in place in Nyon Switzerland with four cultivars of sweet cherry ('Kordia', 'Régina', 'Merchant', and 'Techlovan') grafted onto 'Maxma 14' rootstock. Four treatments were graft-inoculated: LChV-1, LChV-2, LChV-1 + LChV-2 and a healthy control with three replicates per treatment. The plants were established on a row with each treatment delimited by a two plants buffer (cv 'Summit'). The trees were harvested, the crops assessed and 100 randomly selected fruits were measured individually (weight and size) per tree. Trunk diameter was measured at 35 cm above the ground just above the graft-union beneath the first branch.

Results and Discussion

The 8-year-old field trial was composed of trees in full production. Our study confirmed a strong effect of the cultivar on the sensitivity to LChV-1 and LChV-2 with 'Regina' and 'Merchant' being very susceptible. A milder effect of LChV-1 compared with LChV-2 was also measured. The assessment of vigor (trunk diameter) showed a strong negative effect caused by the presence of LChV-2 in all cultivars tested while LChV-1 had no impact. The *Closteroviridae* family contains severe pathogens of woody plants, yet the velariviruses described on grapevine, apple and pears appear to be comparatively mild (3, 4), a trait is also shared with LChV-1 or "not so Little cherry virus 1".

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DEVELOPMENT OF AN INFECTIOUS CLONE OF BLACKBERRY CHLOROTIC RINGSPOT VIRUS AND ITS DELIVERY TO BLACKBERRY

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Introduction

Blackberry yellow vein disease (BYVD) hinders blackberry production in the Southeastern United States. The disease is caused by a combination of viruses with blackberry chlorotic ringspot virus (BCRV, genus *Ilarvirus*), being one of the most prevalent viruses in the complex[1]. Aiming to understand the virus biology and its contribution to the development of BYVD, we developed a BCRV infectious clone.

Materials and Methods

Genomic RNAs were cloned into a PJL89 Agrobacterium binary vector. The coat protein is hypothesized to have a role in replication, and therefore we developed a construct coding for this protein. The clones were agroinfiltrated to indicator leaves using a needless syringe, with or without the coat protein construct and the tomato bushy stunt virus p19 RNA silencing suppressor. RT-qPCR assays were conducted to determine BCRV infection of systemic tissue. Mechanical transmission of BCRV from agroinfiltrated plants to healthy indicators was performed using infected sap. Dodder seeds, *Cuscuta pentagona*, were germinated and established in BCRV-positive *Nicotiana benthamiana.* The dodder stems were allowed to move and establish onto virus-tested blackberry seedlings to evaluate virus transmission.

Results and Discussion

Both *N. benthamiana* and *N. occidentalis* were found infected using RT-qPCR; likewise, the virus was detected in systemic tissue of the back-inoculated indicator plants. Dodder can be used for virus transmission as several blackberry plants tested positive for BCRV after dodder attachment. These results demonstrate the infectivity and transmissibility of the virus. This work provides the tools to study the biology of BCRV in blackberries that will aid in understanding the role of this virus in the development of BYVD.

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CITRUS CONCAVE GUM-ASSOCIATED VIRUS AND CITRUS VIRUS A: COGUVIRUSES WITH A WIDE HOST RANGE CODING FOR A WEAK RNA SILENCING SUPPRESSOR

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Introduction

Citrus concave gum-associated virus (CCGaV) and citrus virus A (CiVA) are two relatively new viruses classified in the genus *Coguvirus*, family *Phenuviridae.* The bipartite genome of these viruses consists on the negative-stranded RNA 1, encoding the RNA-dependent RNA polymerase (RdRp), and the ambisense RNA 2 coding for the nucleocapsid protein (NP) and a putative movement protein (MP). After their first identification by high-throughput sequencing in citrus, CCGaV and CiVA were reported also in apple and pear trees, respectively, showing that the host range of these viruses is quite wider than initially expected. However whether these coguviruses may also naturally infect stone fruit trees is not known. To address issues related with the epidemiology of CCGaV and CiVA, novel serological and molecular detection methods were developed and here we report data on field surveys performed in the last years in Southern Italy. Moreover, since it is not known whether coguviruses code for protein(s) able to suppress RNA silencing-based plant defenses, experimental trials addressing this question were performed and the results will be presented.

Materials and methods

Detection methods based on serology or multiplex RT-PCR were developed and used in field surveys in Campania region (Southern Italy). The potential role of the NP of CCGaV and CiVA as RNA silencing suppressor (RSS) has been investigated using a transient expression assay based on co-infiltration of two *Agrobacterium tumefaciens* strains, one expressing the GFP (inducer of gene silencing) and the other one expressing the NP genes (candidate suppressor gene) or a control RSS, into transgenic *Nicotiana benthamiana* plants (16c) constitutively expressing the reporter gene GFP.

Results

CCGaV and CiVA were identified in several citrus and pome fruit tree species. In particular, while CCGaV was found in citrus and in several apple cultivars, CiVA was found in citrus and in pear trees. Moreover, these viruses, were never found in samples from stone fruit trees grown in the same area, thus suggesting that these trees species are likely not natural hosts of CCGaV and CiVA. Moreover, observation of GFP fluorescence and Northern blot assays supported that NP proteins of CCGaV and CiVA act as weak suppressors of RNA silencing, thus providing a first evidence of the multifunctionality of a coguvirus-encoded protein.

POSTERS

P1: IDENTIFICATION AND CHARACTERIZATION OF NOVEL VIROIDS IN PEAR

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Introduction

Pear originated in western China and is the third most important fruit after apple and grape in the world, with important economic value. During the long-term evolutionary process, pear trees may be infected with a large number of exogenous circular RNAs (circRNAs) with regulatory effects that affect their growth and metabolism, but the species and the effects on pear of these circRNAs remain less characterized.

Materials and Methods

Thirteen pear leaves with obvious symptoms were collected in Baoding and Beijing, China, and subjected to long-stranded non-coding RNA sequencing (lncRNA).

Results and Discussion

The lncRNA reads were assembled, resulting in 286 circRNA contigs, and 20 contigs were selected for RT-PCR detection using abutted primers. The results showed that seven DNA bands of the expected size were amplified, with sizes ranging from 239 to 523 bp, were cloned and sequenced. As aligned with the sequences registered in NCBI, no identities were detected with the host genome, suggesting that they are most likely exogenous circRNAs, tentatively named pear circular RNAs. Of them, PcRNA1 and PcRNA2 were further characterized, suggesting that they have multi-branched without a central conserved region and ribozymatic activities. Phylogenetic analysis revealed that they were both clustered together *Avsunviroidae* members. As the dimeric RNAs of PcRNA1 and PcRNA2 were synthesized and mechanically inoculated into the leaves of tomato, tobacco, *Chenopodium amaranticolor* and cucumber seedlings, RT-PCR and Dot blot assays at 21 days post inoculation (dpi) revealed that PcRNA1 could infect tomato and tobacco, while PcRNA2 not. Total RNAs were extracted from tomato seedlings and subjected to northern blot in combination with two-dimensional electrophoresis analysis using positive and negative-stranded RNA probes of PcRNA1. It confirmed that PcRNA1 dimeric RNAs were cleaved, ligated into circular forms, and initiated an asymmetric rolling replication cycle in tomato. As measured the tomato seedlings infected by PcRNA1, the results showed that the average height of the seedlings inoculated with PcRNA1 were lower than those of the controls. The further transcriptomic analysis of the infected seedlings together the controls indicated that the genes related to plant hormone transduction were differentially down-regulated, which was further confirmed by RT-qPCR, suggesting that PcRNA1 can inhibit phytohormone synthesis to regulate plant growth. Collectively, these reults suggested that pear plants may harbor many viroid or viroid-like RNAs besides pear blister canker viroid, which most likely contributed to modulate the diverse biological processes of the host plants.

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P2: CHARACTERIZATION OF PYRUS VIRUS A (PYVA), A NOVEL VELARIVIRUS IDENTIFIED IN PEAR TREES

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Introduction

Viruses associated with economically significant pome fruit diseases on commercial cultivars have been thoroughly studied and characterized over the years. Nevertheless, beyond commercial cultivars, a significant genetic diversity is preserved in collections of genetic resources. Therefore, germplasms are essential for conserving plant genetic diversity and as a source of genetic material to introduce new traits for breeding and for direct use for food production. In addition, assessing the viral status of old historical cultivars maintained in these collections will also provide helpful information to plant breeders about possible resistant or tolerant fruit tree cultivars.

Materials and Methods

During a large-scale survey of pear trees (*Pyrus L.*) from the germplasm collection at the CRA-W (Gembloux, Belgium) to characterize their virome, a novel velarivirus was identified in several asymptomatic trees. High-throughput sequencing (HTS) techniques and bioinformatics tools were used to reconstruct the genome of the novel virus, tentatively named Pyrus virus A (PyVA). Further phylogenetic analyses were done to provide a better provisional taxonomic assignation. RT-PCR confirmed the virus's presence, and the presence of viral particles was confirmed via transmission electron microscopy.

Results and Discussion

The RNA-dependent RNA polymerase (RdRp), coat protein (CP), and heat-shock protein 70 homolog (HSP70h) amino acid gene product of PyVA share less than 72% (RdRp), 60%(CP), and 42% (HSP70h) of identity to Malus domestica virus A (MdoVA, *Velarivirus*), which is far from the 75% identity threshold determined by the ICTV for relevant gene products (RdRp, CP, and HSP70h). In addition, phylogenetic analysis using the RdRp nucleotide sequences placed PyVA within the genus *Velarivirus*. Therefore, based on the genomic organization, its phylogenetic relationships, and its genetic relationship with the other members of the genus, we propose PyVA as a novel species of the genus *Velarivirus* within the family *Closteroviridae*. The following steps to better characterize this novel virus are (*i*) test if some proteins can be expressed in *E. coli*, (*ii*) develop a detection test (molecular and/or serological), (*iii*) perform a local scale epidemiological survey, (i*v*) test if the virus is transmitted by grafting to commercial pear varieties, such as Conference and Doyenné du Comice, and finally, (*v*) if symptoms can be observed in different graft inoculated pear varieties.

P3: OCCURRENCE OF VIRUSES INFECTING *FRAGARIA* AND *RUBUS* SPP. IN ECUADOR

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Introduction

Dozens of viruses infecting strawberry (*Fragaria spp.*) and blackberry (*Rubus spp.*) have been reported worldwide [1, 2]. In Ecuador, the production of strawberries and blackberries has increased considerably, but studies on the occurrence of viruses have been scarce. While no viruses have been reported from strawberry fields in Ecuador, *Raspberry bushy dwarf virus* (RBDV), a pollen-borne idaeovirus (*Bromoviridae),* remains the only *Rubus* virus reported from Ecuador [3].

Materials and Methods

In this study, high throughput sequencing (HTS) was used to detect and identify viruses in different fields from Tungurahua province, the most important production area of the country. Strawberry plants exhibiting red leaves and blackberry plants showing leaf yellowing and mosaics were sampled. Total RNA was extracted from strawberry samples following the methodology described in [2], whereas total RNA or double-stranded RNA (dsRNA) was extracted from blackberry [2].

Results and Discussion

Strawberry necrotic shock virus (SNSV) and strawberry mild yellow edge virus (SMYEV) were found, in 10% and 35% respectively in strawberry samples. In blackberry, RBDV was found in 100% of the samples. Furthermore, there was no correlation between SNSV or SMYEV occurrence and the red leaf disorder observed in strawberry; whereas RBDV was associated with the yellowing and mosaic symptoms observed in the field. Only one blackberry sample tested positive for both RBDV and SNSV, but there was no clear exacerbated symptom in this plant. In addition, a new reovirus related to raspberry latent virus, was found in one blackberry dsRNA sample. This is the first HTS based survey on virus occurrence in strawberry and blackberry plants of Ecuador. These findings enable researchers and producers to consolidate propagation schemes of virus-tested material to reduce the affect of viruses in these two important crops.

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P4: HOT WATER TREATMENT FOR THE ELIMINATION OF VIRUSES, PHYTOPLAMAS AND BACTERIA FROM FRUIT TREE BUDWOOD - Results of the THERMOFRUIT project

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Introduction

The ThermoFruit project (2019-2021)[1] assessed the feasibility of hot water treatment - HWT - for the elimination of different pathogens affecting fruit species. Based on the heat sensitivity of pathogens (1) and HWT carried on *Vitis* (2), the project targeted some epidemic pathogens on fruit material including *Plum pox virus (PPV)*, *Ca.* Phytoplasma prunorum associated with European stone fruit yellows (3), *Ca.* Liberibacter spp. associated with the Huanglongbing disease and the agent of fire blight *Erwinia amylovora*. These pathogens pose serious threats on the propagated material in nurseries or genetic resources centers. The target is to assess HWT as a complementary control strategy to implement on the example of the vine sector and improve the overall quality of fruit plant material.

Materials and Methods

Woody bud sticks are used with pre- and post-acclimatization at room temperature. We evaluated the impact of heat and water dipping on plant tissues and on bud sprouting with modalities from 40°C to 60°C with various dipping times ranging from 15’ to 180’. Impact of the treatment on fruit material was studied with 28 pathogen-free varieties including *Prunus*, *Malus*, *Pyrus* and *Citrus*. The same protocol was applied on bud sticks from infected trees with the different pathogens. After treatment, cuttings or grafted plants are monitored for at least one vegetative cycle and the presence of the targeted pathogen is tested either with biological or laboratory tests.

Results and Discussion

The heat sensitivity thresholds of the tested fruit species show disparities according to species as seen on the recovery rate of the grafted material after treatment. The evaluation of HWT on different types of pathogens virus, phytoplasma and bacteria has made possible to identify effective time and temperature couples for the elimination of PPV on plum and apricot trees, of *Ca.* P. prunorum on plum trees and of *E. amylovora* on apple and pear. The results of this work make possible to consider the application of the HWT on a larger scale within professional sectors and research institutes to secure the multiplication, genetic resources or the entry into the territories.

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P5: ASSESSING A GREENHOUSE PROTOCOL TO IDENTIFY SUSCEPTIBILITY OF APRICOT AND PLUM TO EUROPEAN STONE FRUIT YELLOWS PHYTOPLASMA

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Introduction

How to protect *Prunus* orchards against phytoplasmas and particularly against *Ca.* Phytoplasma prunorum ? In the last years many studies have been carried out to provide a better understanding of the epidemiology of the European stone fruit yellows (ESFY) disease (1) but no means of direct control of phytoplasmas is currently available. The main current strategies rely on vector control and secured plant propagation. Even if no genetic resistance has been identified, field observations show various behaviors among *Prunus* species towards the disease. The use of less susceptible species or cultivars (cvs.) seems relevant for the management of the disease at the territorial level. French plum and apricot professional sectors are moreover interested in knowing the susceptibility level of cvs. already established or in development to anticipate possible impacts in orchards.

Materials and Methods

Various studies report the behavior of plum or apricot cvs. on plants observed in the field with or without artificial transmission of the disease (2, 3). Few works have been carried out under controlled conditions. An experiment was carried under greenhouse from 2019 to 2021 on several plums (*Prunus domestica* L. and *Prunus salicina* Lindl.) and one apricot (*Prunus armeniaca* L.). The potted plants are grown at 25°C and artificially inoculated with *Ca.* P. prunorum. The plants where monitored, tested for the presence of the phytoplasma for over three vegetative cycles. On the third cycle, the height of plants from grafting point and chlorophyll levels were also measured.

Results and Discussion

The experimental protocol in greenhouse is promising with symptoms expression of the disease for highly susceptible plum cultivars such as Fortune. On *P. salicina* trees the comparison of the height of the plants from the grafting point between the positive trees and the negative trees shows a smaller size for the positive trees. We observed several limits and some improvements have been identified, mainly for targeting others susceptibility assessment criteria. A new larger experiment began in 2022 on ten apricot cvs. and ten plum cvs. with known behavior to extend the results and work on a scale of susceptibility. Complementary listed criteria include phytoplasma quantification, physiological or morphological observations on positive plants regarding to non-infected.

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P6: TOWARDS THE CHARACTERIZATION OF BIOLOGICAL PROPERTIES AND SYMPTOMATOLOGY OF HTS-IDENTIFIED VIRUSES

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Introduction

In the jungle of HTS viruses and data, biological characterization remain a central goal to bring complementary information on the newly-identified viruses. Only few viruses identified on fruit tree material since the last decade have been associated with symptoms and a huge work remains to describe potential associated biological or physiological disorders and their agronomical impact (1, 2). In this context, we have initiated a multiple-scale and long term approach focusing on biological properties of new viruses. The aim is to provide knowledge to scientific teams, certification regulators and plant health authorities by answering the questions : is there an association between viruses and symptoms ? What is the severity of the symptoms observed ? Is there a potential threat to the fruit material commercialization or fruit production ? Are they present in the territory ?

Materials and Methods

The work is based on complementary approaches like collecting field data and positive woody samples, carrying biological tests on various indicators in greenhouse and in the field and assessing various hosts plants. Field information is collected from professional sectors and technical actors on suspected viral-type organisms. Positive samples are requested from the research teams and institutes involved in the initial sampling and/or HTS testing. Greenhouse and field tests are carried on HTS-tested indicators or varieties. The classical bioassay methodology is extended both on repetitions and duration to reveal unusual symptoms. The protocol is scalable and based on collaborative construction with research teams.

Results and Discussion

These experiments are extended over several years of observations and tests. Ongoing assays are targeting Apple rubbery wood disease and its associated viruses (ARWv-1 and ARWv-2), *Plum bark necrosis stem pitting-associated virus* (PBNSPaV), *Apricot vein-clearing associated virus* (AVCaV), *Nectarine stem pitting-associated virus* (NSPaV), *Peach-associated luteovirus* (PaLV), *Peach-associated luteovirus 2* (PaLV 2). Firsts results carried on the last three Luteoviruses show no associated symptoms on peach-GF305 indicator plant in greenhouse and variable transmission efficiency by grafting (3). Further bioassays are scheduled from 2024 for *Malus* and *Pyrus* on *Citrus virus A* (CiVA), *Citrus concave-gum associated virus* (CCGaV), *Apple Hammerhead viroid* (AHVd) and *Malus* luteoviruses.

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P7: MOLECULAR ANALYSIS OF RUBUS YELLOW NET VIRUS ON RASPBERRY FROM BOSNIA AND HERZEGOVINA

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Introduction

The raspberry is a promising fruit whose production is increasing in Bosnia and Herzegovina (BiH). More than 40 virus species are known to infect Rubus and those associated with the raspberry mosaic disease complex (MD) are the most common (1). One of the components of the MD complex is Rubus yellow net virus (RYNV), a pararetrovirus belonging to the genus *Badnavirus*, that was found in plants as episomal endogenous pararetrovirus (EPRV) (seems to not induce infection) and intactpararetrovirus forms (IPF) (1). Recently the full genome sequence of a RYNV isolate from blackberry was determined from BiH (2). To fill the lack of information on RYNV in BiH, surveys were conducted to verify the presence and distribution of RYNV in the country, and to examine the population structure and genome elements in local raspberries of RYNV.

Materials and Methods

During 2019-2022, 190 leaf samples of different raspberry varieties were collected from 14 locations in BiH. The presence of the RYNV, whether as EPRV or IPF, in the collected samples was checked by PCR and RT-PCR assays, respectively, using RYNV6-F/RYNV6-R primers (1). All PCR-yielded amplicons (463 bp) were Sanger-sequenced. Moreover, one PCR-positive sample to RYNV (V-35/19; Bratunac locality) was subjected to next generation sequencing (NGS) using Illumina TruSeq (Seul, North Corea).

Results and Discussion

The most common symptoms observed in raspberry visited fields were leaf blotching, irregular chloroses leaf mottling and leaf deformations. RT-PCR results showed the presence of RYNV in 37/190 (19,4%) of tested samples, mostly in mixed infections with raspberry leaf blotch virus (RLBV) and raspberry leaf mottle virus (RLMV) (data not showed). However, 4 isolates showed an episomal form in RT-PCR after DNase-treatment, suggesting that the positive amplification before treatment was originated from RYNV DNA and not RNA. Based on the NGS results and using the RYNV reference genome in the GenBank (acc.n°. KF241951), eight primer pairs were designed to amplify the sequence of 2068 (nt) (Acc. No. OP620414). The sequence covers ORF3 and ORF4, showing 100% nt and aa identity with a Canadian 2 isolate (KF241951). This is the first report of RYNV in raspberries in BiH; and the virus was found in commercial orchards and nurseries pointing to a dissemination through the infected propagating material.

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P8: DETECTION OF BLACKCURRANT LEAFROLL ASSOCIATED VIRUS 1 (BCLRAV-1) IN BLACK AND RED CURRANTS IN BOSNIA AND HERZEGOVINA

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Introduction

The closterovirus blackcurrant leafroll associated virus 1 (BcLRaV-1) has been reported in black and red currants from the USA, Czech Republic, Slovenia and Switzerland (1). Sequence comparison of five isolates from Europe and North America revealed high molecular variability. The main aim of the study was to investigate the presence of the virus in Bosnia and Herzegovina, BiH currants.

Materials and Methods

Specific primer pairs targeting the HSP90 gene can detect all BcLRaV-1 isolates described so far. Thirteen (13) red and black currants collected during 2018 from Travnik, Bugojno and Teslić were tested by RT-PCR. Amplicons were sequenced on both directions and raw sequences were assembled with BioEdit 7.0.5.3.

Results and Discussion

Amplicons of the expected 382bp fragment of the HSP90 gene were obtained in three samples; one red currant from Travnik, where black currant reversion virus (BRV) was previously detected and one white and black currants each from Teslić in co-infections with gooseberry vein banding associated virus (GVBaV) (2). All three plants displayed virus-like symptoms. Nucleotide (nt) sequences were 71-85% identical among themselves revealing high genetic variability among the BiH isolates. The BcLRaV-1 isolates from red and white currants showed highest nt similarity 99,8% and 99,2%, respectively with the complete genome sequences of two Czech Republic isolates from red currants (Acc. Nos MH460557 and MH460558). The isolate from black currant was 94.5% identical to a Swiss isolate also from black currant (Acc. Nos MH541840).

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P9: DEVELOPMENT OF A REAL-TIME PCR ASSAY FOR THE DETECTION AND IDENTIFICATION OF RUBUS STUNT PHYTOPLASMA IN RUBUS SPP.

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Introduction

Rubus stunt phytoplasma ('*Candidatus* Phytoplasma rubi', RSP), a member of the 16SrV group, causes a severe disease of *Rubus* spp. (Bertaccini et al. 1995; Van der Meer 1987). Rubus stunt (RS) disease occurs in wild and cultivated *Rubus* in Europe, the former Soviet Union, and the Middle East. Introduction of RS to disease-free areas can have a devastating impact on the *Rubus* cane-fruit industry. Rapid, sensitive, and specific methods are essential for disease detection and control. This study describes a sensitive and specific real-time PCR assay that allows for faster detection and identification of RSP in *Rubus* spp..

Materials and Methods

Synthetic DNA or nucleic acid extracts (DNA or total nucleic acid) from midribs of leaves from healthy or infected plants with phytoplasma species closely related to RSP and other species reported to infect *Rubus* spp. were used as controls to evaluate and verify the performance characteristics of the developed assay. The primers and Locked Nucleic Acid (LNA)-modified probe for the specific detection of RSP's *tuf* gene target were designed using the Realtime PCR Tool (Integrated DNA Technologies) and checked for specificity using NCBI's BLASTn tool.

To determine the absolute sensitivity of the assay, a dilution series of synthetic DNA molecules corresponding to the target was used. The standard curve and assay efficiency were calculated using Light Cycler® 96 (LC96) System software.

The repeatability of the assay was determined by evaluating the intra-assay variation among ten-fold serial dilutions of a RSP-positive analyte. The reproducibility of the assay was evaluated by testing multiple RSP-positive analytes in independent runs, on two different real-time PCR platforms. Percent coefficient of variation (%CV) of Cq values was used to measure the variation around their mean.

Results and Discussion

A sensitive and specific assay for RSP detection was developed, with the LNA-modified probe targeting species-specific single nucleotide polymorphism(s) in a 15 bp region of the *tuf* gene. The assay reliably detected down to 5 copies of the synthetic target. This is the second lowest absolute LOD reported among the published assays. PCR efficiency of the assay was found to be 110%, which is within the acceptable range. Percent CV values for checking the repeatability and reproducibility of the assay were within the acceptable range reported in the literature. The developed assay will aid in screening of *Rubus* propagation material for RSP in a quick and effective manner to mitigate the risks associated with this pathogen.

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P10: THE DOWNREGULATION OF MITOCHONDRIAL ATP SYNTHASE SUBUNIT O FACILITATES APPLE NECROTIC MOSAIC VIRUS INFECTION

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Introduction
Apple (*Malus domestica*) is one of the most economically important fruit crops around the world, however its yield and economic benefits are restricted by biotic and abiotic stress. Apple mosaic disease (AMD) is one of the most serious threats to apple production, and it is widespread in the world's major apple-producing countries (1). Recent studies revealed that apple necrotic mosaic virus (ApNMV) is significantly associated with AMD in China (2). Mitochondrial ATP synthase is the enzyme responsible for the synthesis of ATP from ADP and oxidative phosphorylation. The role of mitochondrial ATP synthase subunits during abiotic stress responses has been analyzed, however, the identity of its response to biotic stress remains to be determined.

Materials and Methods
Y2H, BiFC, Co-IP and LCI assays were performed to identify the interaction between MdATPO and ApNMV CP. The *ATPO* was overexpressed and silenced to explore its role in response to ApNMV. The potential transcription factors in the ABA pathway that were involved in the regulation of *ATPO* were predicted by PlantRegMap.

Results and Discussion
In the present study, we proved that apple mitochondrial ATP synthase subunit O (MdATPO) interacts with ApNMV CP *in vitro* and *in vivo*. It was further verified that overexpression of *MdATPO* in *Nicotiana benthamiana* inhibited viral accumulation. In contrast, silencing of *NbATPO* facilitated viral accumulation, providing evidence that *ATPO* plays a defensive role during ApNMV infection. Further investigation demonstrated that ApNMV infection accelerated abscisic acid (ABA) accumulation, and ABA negatively regulated *ATPO* transcription, which was related to the capacity of ABA insensitive 5 (ABI5) to bind to the ABA-responsive elements (ABREs) of the *ATPO* promoter. Altogether, our results indicate that transcription factor ABI5 negatively regulates *ATPO* transcription by directly binding to its promoter, leading to the sensitivity of host to ApNMV infection. The current presentation facilitates our comprehensive understanding of the intricate responses of these hosts to the ApNMV infection.

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P11: INVESTIGATING THE PRESENCE AND DISTRIBUTION OF PRVF IN HUNGARIAN SOUR AND SWEET CHERRY

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Introduction
Sour cherry (*Prunus cerasus*) and sweet cherry (*Prunus avium*) are economically relevant crops in Hungary, as Hungarian varieties are cultivated not only in the Carpathian basin but are exported to other European countries and to the USA, Canada, and Chile. Plant material has to be certified before exportation to ensure quality and absence of harmful pathogens. The aim of the study was to analyze sweet and sour cherry collection for viral presence through high throughput sequencing (HTS) of small RNAs.

Materials and Methods
95 individual trees were selected from two different locations in Hungary and from their leaves RNA was extracted. RNA was pooled into seven small RNA sequencing libraries according to the location and species. Bioinformatic analysis of sequenced reads was done by using CLC Genomics workbench. Published primers were used to confirm the presence of PrVF in the libraries and in the single samples, amplifying a fragment of 463 bp and 760 bp of RNA1 and RNA2 respectively[2,3] (Tahzima et al. 2019; Villamor et al 2017). To amplify bigger fragment of RNA1 and RNA2 new primers were designed are then tested to better comprehend the variance of PrVF and its distribution in the samples.

Results and Discussion
Bioinformatic analysis suggested the presence of several viruses such as Cherry virus A (CVA), Prunus necrotic ringspot virus (PNRSV) and Prunus virus F (PrVF) a Fabavirus which infection we found for the first time in Hungary. Our result showed and confirmed the presence of PrVF RNA1 and RNA2 in both sweet and sour cherry samples from different locations. More than half of the individual sour cherry varieties were positive to PrVF, while only 20% of the sweet cherries were infected with PrVF. In contrast to the widespread presence, we cannot correlate typical symptoms to the presence of PrVF, suggesting its latent infection as indicated in previous literature[1] (Koloniuk et al. 2018). Further analysis, based upon sequence comparison of bigger part of RNA1 and RNA2 are currently ongoing to help to identify the variability of this highly variable Fabavirus in the sour and sweet cherry samples.
This work was financially supported by NKFIH K127951. FD is a PhD student of the Doctoral School of Biological Sciences at MATE.

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P12: CHARACTERIZATION OF SPANISH OLIVE VIROME BY HIGH THROUGHPUT SEQUENCING

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Introduction

Olive (*Olea europaea*) is a strategic crop in Spain, with more than 309 million productive trees cultivated representing 30.6% of the world's production. Among the 17 viruses described infecting olive, only cucumber mosaic virus, cherry leafroll virus, and strawberry latent ringspot virus had been occasionally described infecting olive in Spain. However, during the summer of 2020, in a regular inspection of the sanitary status of olive trees in the main olive-producing area in Spain, Jaén (Andalucía), an increasing number of trees showing yellow leaf discoloration and extensive defoliation was observed. A similar symptomatology was detected in another Spanish olive-producing area, Castellón (Valencian Community). Trees showing this symptomatology were selected for high throughput sequencing (HTS) analysis.

Materials and Methods

A total of 94 samples from both symptomatic and asymptomatic olive plants (cultivars Picual and Serrana) were collected in 2020 and 2021 during the summer season in the main olive-producing area in Spain, Jaén and in Castellón. Total RNA was purified from 200 µL of plant extract using the Plant/Fungi RNA isolation kit (Norgen Biotek Co.) following the manufacturer's instructions. HTS analysis was performed using total RNA as template and sequencing was performed at Macrogen Inc. Library construction was carried out using the Illumina TruSeq Stranded Total RNA Ribo-Zero Plant Kit (2 × 150 bp paired-end reads) in a NovaSeq 6000 platform. CLC Genomics Workbench v.10.1.1 (Qiagen) and Geneious Prime v2020.2.5 (Biomatters Ltd.) were employed for bioinformatic analyses. Specific PCR-based assays were performed for the viruses detected by HTS to confirm their presence by Sanger sequencing.

Results and Discussion

The results of the study revealed the presence for the first time in olive Spanish orchards of olive leaf yellowing associated virus and olea europaea geminivirus for which the complete genomic sequences were determined (MW056495, MW316657, MW316658, OK475023, OK475024). Partial sequence of olive latent virus 3 was also reported (OK475025). In addition the complete sequence of olive viral satellite RNA (OK484515) was described. The characterization of these findings increases the knowledge of the Spanish olive virome (1).

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P13: APPLE STEM PITTING VIRUS, APPLE STEM GROOVING VIRUS, APPLE CHLOROTIC LEAF SPOT VIRUS, AND APPLE HAMMERHEAD VIROID IN LOQUAT IN SPAIN

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Introduction

Loquat (*Eriobotrya japonica*) is an important crop in Spain with an annual production of 40,000 tones. Only apple chlorotic leafspot virus (ACLSV), apple stem grooving virus (ASGV), and loquat virus A (LoVA) had been previously described infecting loquat in China, there was no information about the virome of this crop in EU countries. With the aim of preventing the eventual emergence of viral diseases, trees from growing areas in the eastern region of Spain were analyzed by high-throughput sequencing (HTS), RT-PCR, and DAS-ELISA.

Materials and Methods

A total of 91 leaf samples from different cultivars (Andrés, Algerie, Xirlero, Juliana, Crisanto Amadeo, and Toni Tomaca) were randomly collected in loquat orchards in Callosa d'en Sarrià, and in Sagunto in Spain. Total RNA was purified from 200 µL of plant extract using the Plant/Fungi RNA isolation kit (Norgen Biotek Co.) following the manufacturer's instructions. Libraries were sequenced (2 × 150 bp paired-end reads) on a NovaSeq 6000 platform (Illumina). Bioinformatic analysis of HTS raw data was performed by CLC Genomics Workbench v.10.1.1 (Qiagen Bioinformatics) and Geneious Prime v2020.2.5 (Biomatters, Inc.). RT-PCR reactions for confirmation purposes were performed using a OneStep PrimeScript RT-PCR kit (Takara Bio Ink) using specific primers and specific ELISA tests were performed according manufacturers's instrucitions (Bioreba). Sequence alignments were carried out using ClustalW implemented in MEGA X. Secondary structure prediction was performed by Vienna RNAfold (v2.14.3) implemented in Geneious Prime software

Results and Discussion

The results of the study revealed the presence of ASPV (MW045213) for the first time in this crop and showed that loquat is a natural host of ASPV. In addition, ASGV (OK272504), ACLSV (KX579123) but not LoVA were identified infecting trees in Spanish loquat orchards. These are the first reports of the presence of ASPV, ASGV and ACLSV in loquat in Spain. Near full-length sequences of their genomes were characterized and phylogenetic relationships studied. The identification for the first time of apple hammerhead viroid (AHVd) is reported, a viroid thought to be limited to apple infection to date. The complete circular genome of the Spanish loquat isolate AHVd-SL73.6 was determined (OK272503), showing high level of sequence similarity with the Chinese reference isolate, but 58 nt shorter than the reference genome due to the presence of two deletions. This study can contribute to improve the sanitary status of Spanish loquat orchards, and thus help to protect them (1,2).

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P14: APPLE DIMPLE FRUIT VIROID IS A NATURAL HOST IN POMEGRANATE

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Introduction

In summer 2019, during a routine inspection of pomegranate trees cv. Mollar de Elche, in Elche, fruits showing bright yellow rings were observed. There were no internal symptoms in those fruits nor leaf symptoms on the trees. A fruit with similar symptoms and coming from the same production area had previously been identified in a French supermarket. To investigate the etiology of these symptoms, samples were analyzed by high-throughput sequencing (HTS).

Materials and Methods

Total RNA was purified from 200 mg of leaves and fruit pedicels from symptomatic field samples using the Plant/Fungi total RNA purification kit (Norgen) according to the manufacturer's protocol. The library was prepared using TruSeq Stranded Total RNA LT Sample Prep Kit and sequencing using NextSeq 500 platform. In the case of the fruit from the French supermarket, small RNAs were purified using mirPremier microRNA isolation kit (Sigma-Aldrich) from 200 mg of fruit pedicel according to the manufacturer's protocol. The library was prepared using TruSeq Small RNA Library Kit and sequencing using a HiSeq 2500 platform. De novo assembly was performed with CLC Genomics 10.1 or Vague 1.0.5 software and recovered contigs were annotated by Blast analysis (BlastN/X) against local and Genbank virus, viroids and nt/nr databases. Contigs were extended by rounds of mapping of residual unassembled reads using Geneious Prime 2020. Specific primer pairs were designed for detection purposes and phylogenetic trees were reconstructed using the Maximum Likelihood algorithm implemented in MEGA X applying Kimura 2-parameters. Dataming using a similar approach was performed on pomegranate RNASeq data publicly available as GenBank SRAs.

Results and Discussion

Multiple apple dimple fruit viroid (ADFVd) detections from HTS data from Spanish pomegranate samples and from RNASeq SRAs from Israel confirm ADFVd infection in a range of pomegranate samples. This was confirmed by RT-PCR detection followed by Sanger sequencing of the amplicons obtained from the fruit obtained from a French supermarket. Phylogenetic analysis of multiple alignment of ADFVd complete genome sequences revealed that pomegranate and fig ADFVd isolates are closely related, suggesting that they may possibly be exchanged between these two crops that can frequently be found growing close to each other in Mediterranean countries. The detection of only ADFVd in some symptomatic samples suggests the pathogenicity of ADFVd. The datamining efforts in pomegranate SRAs unexpectedly provided evidence suggesting that, in addition, to hop stunt viroid and ADFVd, plum viroid I might naturally infect pomegranate (1).

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P15: INTRA- AND INTER-VARIETAL GENETIC DIVERSITY OF ILARVIRUSES PRESENT IN AUTOCHTHONOUS ALMOND VARIETIES [PRUNUS DULCIS (MILLER) WEBB] IN THE SPANISH MEDITERRANEAN REGION

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Introduction

The most common viruses that infect the almond tree [Prunus dulcis (Mill.) D.A. Webb] worldwide are Prunus necrotic ring virus (PNRSV) and plum dwarf virus (PDV). Mixed PNRSV and PDV infections are associated with bud failure and rosetting resulting in trees with a bare appearance and reduced fruit set (1, 2). In this work we have analyzed the incidence of the two viruses in varieties from the germplasm banks of CEBAS-CSIC of Murcia (30 samples of 30 different varieties), IRTA of Reus (15 samples of 15 different varieties) and commercial plantations from Requena, Valencia (52 samples of a single variety). The complete sequence of RNA 3 of 51 isolates for PNRSV and 35 for PDV and their phylogenetic relationships were determined.

Materials and Methods

Total RNA was obtained from almond petals using the RiboZol™ reagent and used to detect the presence of PNRSV and PDV by RT-PCR and molecular hybridization using primers and probes specific to the CP gene. Several samples were selected to determine the RNA 3 sequence of both viruses that would allow us to perform phylogenetic analyses.

Results and Discussion

- PNRSV was the most prevalent virus in almond trees in all the collections analyzed. - Intra-varietal genetic diversity was slightly lower than inter-varietal.
- The percentage of co-infection of PNRSV and PDV was higher in monovarietal populations than between varieties.
- A clear synergistic effect was observed in the accumulation of RNA 3 of the two viruses in co-infections with respect to simple infections.

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P16: NEW EMARAVIRUS FOUND IN *THEOBROMA CACAO* L. IN AMAZONAS, PERÚ.

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Introduction

Theobroma cacao is an important crop in Peru, which exports more than 130,000 tons of fruit in 2018, reaching the sixth place of cacao exporters worldwide. In addition, Amazonas region of Perú and Ecuador is known as the center of origin for Cacao trees. Despite its economic and historical significance, there is no information about viruses affecting cacao in Peru. Therefore, we proposed to identify viral species that can be affecting cacao in Peruvian Amazonas using RNA sequencing approach.

Materials and Methods

A small survey was carried out in the cacao germplasm bank in Chachapoyas, Perú. This bank maintains more than 100 accessions collected from the Amazonas Rainforest. We selected 10 plants showing symptoms resembling viral infections and one asymptomatic plant. Total RNA was extracted using Sigma Spectrum Total RNA extraction kit (Sigma-Aldrich, Germany) and processed using TruSeq stranded total RNA with RiboZero for Plants (Illumina, CA, USA) for library construction and Illumina NovaSeq 6000 for sequencing. Trimming and *de novo* assembly was performed using CLC Genomics Workbench v 24.0.1. Identification of viral contigs was performed using BlastX approach against a custom database including viral species of all the genera known to infect plants. Primers were designed from the obtained contigs for PCR validation and field survey.

Results and Discussion

From two plants of the collection, we obtained four contigs showing amino acid sequence identity with several species of *Emaravirus*. BlastX comparison retrieved the following results for the predicted ORFs: the first contig (7222 nt) showed 45.9% of identity with RNA1 from EMARaV, the second contig (2236 bp) showed 33.9% of identity with RNA2 from AsMaV, the third contig (1275 bp) showed 35.45% of identity with RNA3 from PaEV and the fourth contig (1011 bp) showed 32.9% of identity with RNA4 from AsMaV. The leaves of the plants infected with the new *Emaravirus* showed mild mosaic and yellow spots that became necrotic and were later detached from the leaves. Later, we collected 60 plants including cultured and wild samples, and detected all the four RNAs from eight samples. All the eight samples showed the same symptoms observed in the NGS-analyzed plants. This is the first report of an *Emaravirus* infecting cacao around the world. Further studies are under development to detect the virus in commercial clones and also in different mite species that can be involved in the spread of the pathogen.

P17: THE NATURAL TRANSMISSION AND GENE FUNCTION OF PEAR CHLOROTIC LEAF SPOT-ASSOCIATED VIRUS

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Introduction

Pear chlorotic leaf spot-associated virus (PCLSaV) was firstly discovered in Asia pear (*Pyrus pyrifolia*) trees and associated with chlorotic leaf spot (CLS) disease in China (2). Later, the virus was reported to infect pear trees grown in Japan (1). The virus belongs to the genus Emaravirus in the family *Fimoviridae* of the order *Bunyavirales* (3) and possesses a genome composed of five negative-sense single-stranded RNA (-ssRNA) segments coding for five proteins. The viral disease has been prevalent in some pear fields in China, indicating the presence of natural transmission vectors. This study revealed the natural transmission of the virus by a vector and the potential function of the protein P5 encoded in the viral RNA5.

Materials and Methods

A binary vector pCNY containing an eYFP gene was used for subcellular localization experiments. For BiFC assays, the target gene was constructed into binary vectors pEarleygate201-YN and pEarleygate202-YC by using Gateway Technology (Invitrogen, Carlsbad, CA, USA). Viral RSS activity was evaluated in GFP-expressing *Nicotiana benthamiana* 16c plants.

Results and Discussion

The damage of eriophyid mites on pear trees have been noticed for a long time in China. Field investigation revealed that eriophyid mite infection in pear trees are very common with high density in newly developed young leaves, which commonly showed PCLSaV induced symptoms. The mites were identified as *Phyllocoptes pyrivagrans* basing on their morphological and molecular features. By using *P. pyrivagrans* from PCLSaV-infected leaves as a inoculum, the virus was transmitted to *P. betulifolia* seedlings without inducing visible symptom, and *N. benthamiana* plants with mild chlorotic leaf spots. The amplification and sequencing of virus genomic RNAs confirmed the virus infection in these plants. Moreover, the virus could be transmitted among *N. benthamiana* plants by mechanical inoculation. The viral P5 is an RNA silencing suppressor, showing the activities to suppress local and systemic RNA silencing. The protein also can trigger ROS accumulation and PCD in *N. benthamiana* leaves. The P5 is self-interactable and shows multiple subcellular locations in *N. benthamiana* leaf cells and accumulates in the nucleus as bodies, plasmodesmata and ER. In BiFC tests, it interacts with the viral movement protein P4 with signal located at PD. The results indicated that *P. pyrivagrans* is responsible for the prevalence of CLS disease in pear fields and the viral P5 is an important pathogenic factor, playing roles in the virus infection and cell-cell movement.

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P18: *CLEMATIS VITALBA* IS AN ALTERNATIVE HOST PLANT OF PRUNUS VIRUS I.

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Introduction

Clematis vitalba is a perennial climbing shrub, native to Europe. The perennial lifespan and invasive behavior of the plant suggests its potential pathogen reservoir role, as it was shown to host phytoplasma species. Some years ago, bright yellow line pattern and ringspot leaf symptoms, suggesting the presence of a virus were observed near Budapest in Hungary.

Materials and Methods

From the symptomatic leaves RNA was purified. From the RNA small RNA libraries were prepared and sequenced in parallel with ribodepleted RNA sequencing. HTS generated reads were analyzed by bioinformatic methods using CLC Genomic Workbench. Result of the bioinformatics analysis were validated by RT-PCR using virus specific primers.

Results and Discussion

Bioinformatic analysis indicated the presence of a putative Ilarvirus. RT-PCR validation using Ilarvirus specific primers were designed according to the contigs, and sRNA reads. RT-PCR using Ilavirus specific primers enabled us to amplify part of the three genome segments of an Ilarvirus. Sanger sequencing of the amplified part showed the presence of a Prunus virus I (PrVI), a new virus described recently infecting cherry in Greece (Orfanidou et al, 2021). Since then, PrVI was also detected in Slovenia in *Picris echoides* (Rivarez et al., 2022), suggesting its broader natural host range and widest geographical distribution. The virus strain detected in the *Clematis* is 96-98%nt identical to the Greek reference genome. *Clematis* can climb on native *Prunus* species making possible the transfer of viruses between them. However, to find out if the virus is present in native *Prunus* species, is there any animal vector playing role in its spread and which plant could be its original host further studies are needed.

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P19: SURVEY OF RASPBERRY VIRUSES, ASSOCIATED ARTHROPODS AND RECOVERY OF *IN VITRO* RASPBERRY CULTURES IN THE CZECH REPUBLIC AND NORWAY

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Introduction

High-quality pathogen-free seedlings, protection from arthropod vectors and proper nutrition are main prerequisites for production of planting material for an effective raspberry production. Cultivated *Rubus* spp. are susceptible to multiple viruses causing yield losses (1). In the Czech Republic and Norway, until recently, only some nepoviruses (2) and raspberry bushy dwarf virus (RBDV) were known to occur on raspberry. Here we present results of our joint project on the raspberry virome and potential arthropod vectors of raspberry viruses (3). Special interest was paid to cryopreservation methods of healthy raspberry clones as well as virus isolates in cryopreserved shoot tips.

Materials and Methods

Sequence data were obtained using high-throughput sequencing (HTS) and Sanger sequencing. RT-PCR and RT-qPCR were used for virus diagnostics. The "cryoknife" for virus eradication and cryopreservation for safe conservation of healthy plants and viral isolates was examined.

Results and Discussion

In 2021-2022, 49 raspberry plants were tested using HTS. The results verified by Sanger sequencing showed the presence of six known viruses (black raspberry necrosis virus, BRNV; RBDV; raspberry leaf blotch virus; raspberry leaf mottle virus, RLMV; raspberry vein chlorosis virus; Rubus yellow net virus) and two new viruses preliminarily named raspberry enamovirus (RaspEV) and raspberry rubodvirus (RaspRV). Complete genome sequences of RaspEV and RaspRV were obtained. Screening of about 650 plants and 465 batches of arthropods showed the highest abundance of either RBDV (in CR) or BRNV and RLMV (in both CR and Norway). Simultaneously we developed a new multiplex real-time PCR kit for the detection of BRNV, RBDV, raspberry ringspot virus and strawberry latent ringspot virus. Finally, the "cryoknife" for BRNV eradication tested on raspberry *in vitro* cultures was highly effective, indicating the suitability of this method for healthy plant material production.

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Acknowledgements

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P20: TARGETING TO APOPLAST IMPROVES THE ACCUMULATION AND STABILITY OF C-TERMINAL HALF OF SARS-CoV-2 NUCLEOPROTEIN EXPRESSED IN NICOTIANA BENTHAMIANA BY PLUM POX VIRUS-BASED VECTOR

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Introduction

Proteolytic degradation of heterologously expressed proteins in plant cells may negatively affect overall production yields. Our recently published results demonstrated that our plum pox virus (PPV)-based expression vector pAD-agro enabled the production of foreign proteins of different size, structure and origin (1). Nevertheless, their accumulation was in several cases significantly reduced due to their low stability *in planta*. Current strategies to increase product yields rely on the co-expression of plant protease inhibitors, silencing of protease-encoding genes, or targeting of proteins to the subcellular compartments with a lower activity of proteolytic enzymes (2). In our work, we aimed to increase the yields of foreign proteins expressed in plants using the pAD-agro vector by targeting their transport to the apoplast. The C-terminal fragment of SARS-CoV-2 nucleoprotein (designated CoN3) was used as a model polypeptide, as its detectable levels, yet significant proteolytic degradation was observed in previous experiments.

Materials and Methods

The sequence of SARS-CoV-2 nucleoprotein comprising amino acids 187-420 was designed to include a native *Nicotiana benthamiana* extensin secretory signal (designated SSext) on its N-terminus. The corresponding gene was *de novo* synthesized (Eurofins Genomics) and cloned into the pAD-agro by restriction digestion and ligation to generate recombinant construct pAD-agro_CoN3-SSext. Leaves of *N. benthamiana* plants were syringe-infiltrated with *Agrobacterium tumefaciens* EHA105 harboring pAD-agro_CoN3 and pAD-agro_CoN3-SSext constructs. Top leaves of inoculated plants were analyzed 14 days post-infection by Western blot using polyclonal antibodies against PPV capsid protein and SARS-CoV-2 nucleoprotein.

Results and Discussion

Targeting foreign proteins to the apoplast has been proven to be an efficient way to improve their stability and accumulation in plant tissues. Previous studies demonstrated successful trafficking of a protein expressed by tobacco etch potyvirus-based vector to the nucleus, chloroplasts and mitochonodria of infected cells, depending on its insertion position within viral polyprotein (3). However, the possibility of secretion into the apoplast or its effect on protein stability has not yet been studied. Our preliminary results show that the fusion of SSext to the N-terminus of CoN3 indeed significantly increased its accumulation in *N. benthamiana* leaves compared to CoN3 alone. Moreover, enhanced stability enabled its purification by immobilized metal affinity chromatography. Thus, this approach may help to improve the production of foreign polypeptides using potyvirus-based vectors.

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P21: ARGENTINE TANGO OF TWO HARMONIZED HOP STUNT VIROID GENETIC GROUPS IN GRAPEVINE (*VITIS VINIFERA* L.) – FIRST REPORT FROM SLOVAKIA.

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Introduction

Viroids are categorized as subviral infectious agents due to their overall structural simplicity. Each viroid species is represented by only one single-stranded circular RNA molecule with covalently bound ends. In contrast to their very simple molecular composition, lacking also any own ORFs, viroids are widespread and successful phytopathogens, posing as significant agronomical threat. Hop stunt viroid (HSVd) is one such very common pest of several crops (hop, cucumber) or trees (plum, peach). Despite its omnipresence, mainly in grapevine (*Vitis vinifera* L.) (1; 2), molecular data characterizing HSVd population and prevalence were missing from Slovakia.

Materials and Methods

Total RNAs from each grapevine plant were extracted from cortical scrapings or leaf petioles using a Spectrum^{TM} Plant Total RNA Kit. High-throughput sequencing (HTS) via the Illumina MiSeq platform was performed on three of these samples, previously ribo-depleted using Ribo-Zero rRNA Removal kit. Simultaneously, whole genome amplification was performed via two-step RT-PCR using HSVd-specific overlapping primers (1) and the prediction of RNA secondary structures was performed using the online web tool Mfold (http://www.unafold.org/mfold/applications/rna-folding-form.php).

Results and Discussion

Initial RT-PCR tests revealed 73.08% occurrence of HSVd in our samples (38/52). Analyses of the complete nucleotide sequences of selected 19 grapevine HSVd variants revealed the existence of two distinct genetic groups, reported for the first time in Slovakia. The groups were internally named "6A" and "7A" based on the particular stretch of adenines at nucleotide positions 39-44/45, respectively. Both groups discriminated by seven specific and jointly shared mutations mapped in the positions consistent with the data obtained in previous HSVd grapevine adaptation and evolution studies (2). Low intra-group divergence between the 6A and 7A group (~ 0.3 and 0.2%, respectively) was also a common denominator. Additionally, beside their co-existence within the same geographical location, the mixed co-infection by both groups was also identified within single grapevine plant. Analyses on the level of the RNA secondary structures revealed these seven mutations could have a potential compensatory explanation reflecting a host-specific adaptability (3). These data could open a wider door into a more in-depth viroid research in Slovakia, mainly regarding the study of its host-specific adaptations via site-specific mutations.

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P22: MOLECULAR CARACHTERIZATION OF CARLAVIRUS ISOLATES INFECTING HOP IN ITALY

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Introduction

In recent years, hop (*Humulus lupulus* L.) cultivation is increasing worldwide including Italy (1). In 2017 a survey carried out in Italian hopyards highlighted a high percentage (about 65%) of virus infected plants (2). Since most of the viruses found belong to the *Carlavirus* genus, a comprehensive study aimed at the species identification and molecular characterization was performed to explore their features and variability.

Materials and Methods

Total RNA of leaf samples collected from 51 hop plants (50 cultivated belonging to 20 cultivar and 1 wild hop) infected by carlaviruses (2) were analyzed by conventional RT-PCR using specific primer pairs targeted to the coat protein (CP) gene of hop latent virus (HpLV), American hop latent virus (AHLV) and hop mosaic virus (HpMV). CP fragments from twelve positive samples belonging to different cultivars and from different locations were cloned, sequenced, and phylogenetically analysed. Moreover, six samples were further characterized by high throughput sequencing (HTS) using Illumina platform and the assembled genomes compared with other carlavirus isolates.

Results and Discussion

Most of the samples positive for carlaviruses were infected by HpLV (49 out of 51; 96.1%), three samples by HpMV (5.9 %) and two by AHLV (3.9 %); three samples had a mixed infection: one HpLV + HpMV and two HpLV + AHLV. The ORF 5 coding for the CP was characterized for the twelve selected samples (1 AHLV, 9 HpLV and 2 HpMV). All the sequences were phylogenetically closely related to isolates of both European and non-European origin, according with the different origin of the plant material used for establishing the hopyard. HTS analysis confirmed the presence of the viruses previously detected and identified by classical diagnostic methods. Seven complete genomes (1 AHLV, 4 HpLV and 2 HpMV) were assembled and the six constitutive ORFs were retrieved in frame and without premature stop codons as for other characterized genomes from different countries, available in Genbank. Based on these results and considering that the hop plants are obtained mainly by vegetative propagation, we believe that the establishment of a certification program for cultivated hop cultivars, especially nuclear stocks, is a crucial issue. Finally, to our knowledge, this is the first report of AHLV, HpLV and HpMV in Italy.

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P23: BOTH *PRUNUS* AND NON-*PRUNUS* PLANTS CAN HOST A NOVEL ILARVIRUS – PRUNUS VIRUS I.

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Introduction

New emerging viral infections are still a present threat concerning also the agricultural and food industry. Implementation of high-throughput sequencing (HTS) into the field of plant virology has significantly sped up the discovery of new viruses and thus enabled the early diagnosis of potential pathogens. Recently, new plant viral species belonging into the *Ilarvirus* genus was discovered naturally infecting sweet cherry (*Prunus avium*) and peach (*P. persica*) trees, resulting into its denotation as prunus virus I (PrVI) (1). A study showing its presence in different plant host (*Clematis vitalba* L.) was recently also published (2). In this study we bring further evidence of the PrVI spread in *Prunus* and non-*Prunus* hosts.

Materials and Methods

Total RNAs from clematis (*C. vitalba*) and myrobalan (*P. cerasifera*) leaves were extracted using a Spectrum™ Plant Total RNA Kit. Ribosome-depleted RNA preparations were used for cDNA library synthesis (Nextera XT, Illumina) followed by HTS on an Illumina MiSeq platform. Additional detection was performed via two-step RT-PCR using in-house designed PrVI-specific primers spanning each of the five ORFs in all the three viral genomic RNAs. The specificity of RT-PCR products was assured by Sanger dideoxy sequencing.

Results and Discussion

As a part of viral monitoring in the agro-ecological interface, we have identified PrVI in two clematis samples (PlCv5 and PL622), from which the nearly complete PrVI genome sequences were obtained. Both isolates revealed a relatively high mutual nucleotide identity (95.5-98.0%), while the identity with the reference Greek PrVI isolate (MW579753-5) reached 94.5-97.5%. Three viral genomic segments were also successfully detected by RT-PCR using in-house designed primers, thus effective for further monitoring studies. Interestingly, one plum sample (*P. cerasifera*), growing near the PlCv5 clematis, tested PrVI-positive via RT-PCR. This finding expands the known *Prunus* host range reported previously (1), and shows clematis plants as a natural reservoir of PrVI, as recently reported in an independent study (2). However, the epidemiology of the virus, having apparently a broad host range, remains still to be elucidated.

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P24: INSIGHTS INTO THE VIROME OF GREEK POME FRUIT ORCHARDS AS REVEALED BY RNA-SEQ

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Introduction

The leading technologies of high-throughput sequencing have contributed undisputedly to plant virus diagnosis (1) and have also facilitated and accelerated the comprehensive identification of the virome of various crops (2), including pome fruits. Numerous, previously unknown, viral agents infecting apple have been characterized in the last few years (3). In the frame of these efforts to reveal and study mainly the apple virome, RNA-Seq was used to assess the virus status of apple trees in three different apple producing regions of Greece.

Materials and Methods

A small-scale survey was conducted in three different apple-producing regions in Greece. Samples were collected from trees (some pear, quince and wild pear trees were also included) with virus disease symptoms but also from asymptomatic ones and from different orchards. Multiplex RT-qPCR assays and ELISA were used to detect known viruses and viroids in the samples. Five samples from each region were selected and total RNA was extracted and pooled in three composite samples to be analyzed by RNA-Seq (Illumina NextSeq Sequencing System). Ribosomal RNA was removed and library was prepared omitting poly-A selection step. The depth of the sequencing was set to fifty million reads per pooled sample. The presence of viruses and viroids identified by HTS analysis was verified in all samples by RT-PCR amplification and sequencing of PCR products.

Results and Discussion

The presence of the most commonly found and recently discovered viruses and viroids, such as apple luteovirus 1 (ALV1), apple ruberry wood virus 1 and 2 (ARWV1, ARWV2), citrus concave gum-associated virus (CCGaV) and apple hammerhead viroid (AHVd), was identified in mixed infections in Greek orchards. ARWV 1, ARWV 2, CCGaV and AHVd were for the first time reported in apple trees and ARWV2 in pear trees in Greece. Apple stem pitting virus (ASPV) was the most prevalent virus. The few pear, quince and wild pear samples were found infected with significantly less viruses than apple trees. Phylogenetic analyses of the almost full genomes obtained for all viruses and viroids allowed determination of their genetic diversity and further estimation of the efficiency of current detection molecular methods. A putative novel plant virus of the genus *Cytorhabdovirus* was also identified. Furthermore, the richness of the virome differed among the three regions reflecting in a great extent the age of the trees and the phytosanitary status of the propagation material used along with the different agricultural practices and cultivation systems applied.

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P25: COMPLETE GENOME SEQUENCES OF DIVERGENT RASPBERRY BUSHY DWARF VIRUS ISOLATES FROM *RUBUS* SPP. IN TURKEY

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Introduction

Raspberry bushy dwarf virus (RBDV), recently renamed to *Idaeovirus rubi*, is one of the most common viruses infecting *Rubus* species worldwide (Aboughanem-Sabanadzovic et al. 2021). However, there is still limited number of genome sequences available in the GenBank database and majority of the sequences include partial sequences of RNA-1 and RNA-2. Therefore, whole genome analysis of 14 RBDV isolates were obtained in this study and comparative analysis were performed with other world isolates. The results confirm the complete genome sequence of RBDV and its molecular detection strategy in Turkey. This study is beneficial for the management strategy of the virus and for protecting an important plant species in the nation.

Materials and Methods

Rubus spp. samples some of which were exhibiting virus-like symptoms but mostly symptomless were collected from 5 different provinces located on different geographical regions in Turkey. Among RBDV positive samples by RT-PCR, nine divergent isolates were selected for Virion-associated nucleic acids (VANA) method for RNA isolation and the extract was subjected to high-throughput sequencing (HTS). After the assembly of HTS data, several overlapping primer pairs were designed to validate deep sequencing results by Sanger sequencing. Nine raspberry, five blackberry RBDV positive samples were selected for whole genome analysis by using these primers.

Results and Discussion

This study revealed that two contigs (Contig 3 and4) have the highest similarity with raspberry bushy dwarf virus (RBDV)- RNA1 (93.3%) and RBDV-RNA2 (82.9%) with a typical genome organisation of RBDV containing 4 ORFs. Whole nucleotide sequences of RNA1 among Turkish isolates shared 94% identities while they shared 93% identity to the non-Turkish isolates. Most of the RBDV isolates from different geographical regions in Turkey clustered together with other RBDV world isolates, while one divergent blackberry isolate from Hatay province (TS46) were placed adjacent to grapevine isolate of RBDV from Canada (12G412) sharing 95.52% identity. Phylogenetic analysis of available RBDV isolates based on RNA2 sequences resulted in similar grouping of Turkish isolates to that of RNA1. RNA2 nucleotide sequences of Turkish isolates shared 87.5% nucleotide sequence identity with each other whereas they shared 84% identity to the other isolates available in the GenBank. Most divergent blackberry-Hatay isolate (TS46) was grouping together with RBDV-grapevine and cherry isolates is challenging the previous host based grouping of RBDV isolates. This study has utilised RT-PCR as a complementary molecular technique to the RNA-Seq data, to successfully confirm the full genome analysis of divergent RBDV isolates from Turkey.

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P26: THE APPLICATION OF HTS TECHNOLOGY TO THE CASE STUDY OF SLRSV-INFECTED RAGGIOLA OLIVE CULTIVAR

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Introduction

Strawberry latent ringspot virus (SLRSV, family *Secoviridae*, genus *Stralarivirus*) is a bipartite RNA virus able to infect a wide range of plant hosts species, including olive. In 2002, the role of the SLRSV was highlighted as determining factor of the phenological and agronomical differences between the two genetically indistinguishable cultivars Raggiola (SLRSV-infected) and Frantoio (SLRSV-free) (1). In the last years, High-throughput sequencing (HTS) technology has been recognized as a powerful tool to investigate the plant virome thus potentially effective for the assessment of the phytosanitary status of a plant. In the present work HTS technology was applied to provide further evidence and data on this peculiar plant-pathogen interaction.

Materials and Methods

Genomes from five 'Frantoio' and five 'Raggiola' olive trees were analyzed by fingerprinting considering eleven microsatellites. Total RNA extracted from the same plants was also sequenced by Illumina, (Genartis S.R.L., Verona, Italy); raw data were analyzed using CZ ID (https://czid.org/) and Geneiuos Prime® ver. 2022.1.1. Assembled SLRSV genomes were analyzed with MEGA 11 and Clustal W and compared to the others SLRSV genomes retrieved from NCBI database.

Results and Discussion

The fingerprinting analysis confirmed the genomic identity between 'Frantoio' and 'Raggiola'. As expected, HTS analysis did not detect SLRSV in the five 'Frantoio' trees whereas in all the five 'Raggiola' trees tested, contigs mapping on SLRSV genomes were retrieved. No other viruses were detected in both 'Frantoio' and 'Raggiola' samples confirming the role of SLRSV in determining the phenological and agronomical traits of cv. Raggiola. RNA 1 and RNA 2 from all the 'Raggiola' samples, comprehensive of 5'-UTR, polyprotein and 3'-UTR, were assembled from the HTS data. As already reported for SLRSV (2), the nucleotide sequences showed a high variability; the ORFs had 78 % (RNA 1) and 70 % (RNA 2) of homology with the others SLRSV sequences corresponding, respectively, to 89 % and 75 % similarity, when aminoacidic sequences were considered. Phylogenetic analysis on the RNA 2 showed that the olive isolates clustered together in a branch of the B group (2). To our knowledges, this is the first genome characterization of SLRSV isolates from olive. Using HTS technology we were able to verify and definitively confirm the previously results highlighting how this technology is a powerful and effective tool for a deep screening of the phytosanitary status of plant material and, thus, candidate to play an ever more important role in the certification and quarantine schemes.

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P27: VALIDATION OF A DUPLEX REAL TIME RT-PCR FOR THE DETECTION OF PLMVD AND COMPARISON OF DIFFERENT NUCLEIC ACID EXTRACTION PROTOCOLS.

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Introduction

In 2019, with the Implementing Regulation (EU) 2019/2072, the European Commission included peach latent mosaic viroid (PLMVd) in the list of pests for which visual inspection, and, in particular cases, sampling and testing are required for *Prunus persica* (Annex IV, part J). Detection and identification of viroids generally poses many different issues among which high mutational rate and difficulties in massive analysis; to overcome them, a duplex reverse transcriptase real time PCR (dRT-qPCR) designed by merging two already published protocols (1,2) was developed, validated and tested with different nucleic acid rapid extraction protocols.

Materials and Methods

The dRT-qPCR was developed setting the optimal primers and probes concentrations, the reaction conditions and evaluating the amplification efficiency. The test was fully validated according to the EPPO standard PM 7/98 (3). Different extraction protocols were examined: three 'classics' using ready-to-use kits and twelve 'rapid' with different combinations of membranes and solutions. The best combinations were subjected to a test performance study (TPS) conducted in five European laboratories (CAV – Italy, CREA-DC – Italy, ILVO – Belgium, IVIA – Spain, UNI-MI – Italy).

Results and Discussion

As expected, the dRT-qPCR test was able to detect PLMVd with an efficiency comparable to the single tests (1,2). In the *in-house* validation, good performance was shown considering the analytical sensitivity, selectivity, repeatability and reproducibility. Concerning analytical specificity, the inclusivity highlighted an improvement since the test was able to detect isolates that were detected by only one of the original tests (1,2), while the exclusivity was not affected. Generally, the test gave positive results when RNAs from all the classic extraction kits were used, but it was able to detect PLMVd also when rapid extraction test were used. These results were confirmed also in the TPS, where optimal values for accuracy (96-100%), diagnostic sensitivity (92-100%), diagnostic specificity (100%), and reproducibility (88-100%) were achieved. Based on these results, the dRT-qPCR test has proven to be a diagnostic tool able to detect different isolates of PLMVd and will be very useful in massive screening, thus facilitating a wider, earlier and economic control of the viroid, preventing its spread both in propagation material and in the field.

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P28: *ELUCIDATING THE VIROME OF THE MAIN PRUNUS SPECIES GENOTYPES CULTIVATED IN GREECE*

Orfanidou, C.G.[1], Katsiani, A.[1], Sassalou, C.L.[1], Beta, C.[1], Panailidou, P.[1], Drogoudi, P.[2], Katis, N.I.[1], Maliogka, V.I.[1]

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Introduction

Due to their vegetative propagation and grafting, stone fruit trees are infected with a variety of plant viruses and viroids. The effective management of these pathogens is based on the production and use of certified plant material and the virome characterization of fruit tree genotypes is crucial in this process. The purpose of this study was to unveil the phytosanitary status of a wide range of *Prunus* sp. genotypes cultivated in Greece with an ultimate goal to assist their certification process.

Materials and Methods

During 2019-2022, large-scale surveys were conducted collecting a total of 484 samples from a range of different genotypes belonging to 5 prunus species (37 from almond, 30 from sweet cherry, 138 from apricot, 96 from plum and 183 from peach). All samples were mainly molecularly tested for the presence of viruses and viroids included in the EU regulation 2019/2072. Finally, total RNAs extracted mainly from sweet cherry trees and 1 plum tree were subjected to HTS analysis based on the pipeline described by Orfanidou and colleagues (1).

Results and Discussion

High prevalence of viruses/viroids was revealed in the tested material, which differed between the *Prunus* species. In almond genotypes, a 56.8% of the samples were infected by PNRSV, followed by PDV (35.1%). A similar pattern was observed in plum trees, in which PDV (41.7%), PNRSV (37.5%) and PLMVd (38.5%) prevailed whereas PPV and ACLSV were detected in lower percentages. In apricot, PNRSV was prevalent (39.1%), followed by ACLSV (13.8%), PPV (8.7%) and PDV (2.9%). Peach genotypes were mainly infected by PLMVd (65%), PPV (47%), PNRSV (45.9%) and ACLSV (35%). Lastly, LChV-1 was the most common virus (53.3%) in sweet cherry followed by PDV (20%), PNRSV (20%), ACLSV (13.3%) and CGRMV (6.7%). In a second step, HTS was used to further characterize the phytosanitary status of selected *Prunus* genotypes. Results confirmed the presence of the widely spread LChV-1 or ACLSV in the sweet cherry genotypes tested while also newly emerged viruses were identified such as PrVF, CVF, PrVI, PrVT and a group of divergent isolates of CLV-1. Moreover, NSPaV and PBNSPaV were detected in a pooled sweet cherry/plum sample. Taken together, our results highlight the frequent occurence of a high number of viruses in the cultivated *Prunus* genotypes and underline the need for production and use of certified propagating material for upgrading the stone fruit industry in Greece.

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P29: ASSESSMENT OF ONT SEQUENCING (MINION) FOR PLANT VIRUS DETECTION AND COMPARISON WITH ILLUMINA-BASED SEQUENCING

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Introduction

To minimize the risks associated with virus transmission through vegetative propagation the use of virus-tested propagation materials is strongly encouraged. HTS-based untargeted detection replacing specific molecular methods, or even biological indexing, is extremely promising for this purpose: the complete virome can be assessed without the need for prior knowledge. Illumina sequencing has already proven to be a reliable and sensitive method for virus detection, yet also the ONT technology has gained interest as a promising tool over the last years. Nanopore sequencing generates long reads, can be performed in every lab and decreases the costs.

Materials and Methods

For MinION sequencing a protocol based on Liefting et al. (2021) was used. Total RNA extraction from leaf samples was followed by ribosomal RNA depletion and random primed double stranded cDNA synthesis. Barcodes and adapters were added and samples were sequenced for 72 hours on a MinION Flow Cell. Reads were classified by comparing against the Genbank non-redundant nucleotide database with Kraken2 and mapped to virus reference genomes with Minimap2.

Results and Discussion

Five plant samples previously screened with Illumina sequencing, infected with multiple viruses/viroids from different genera, were selected for this study. Overall, most of the viruses could be detected and were identified correctly with this MinION sequencing-based protocol. Viruses detected below 50 rpm with Illumina sequencing could only be detected with poor genome coverage or failed to be detected. It can be concluded that correct virus detection and identification is possible with this MinION protocol but sensitivity is still lower compared with Illumina sequencing.

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P30: RAPID DEVELOPMENT OF AFFORDABLE, VIRUS-MIMICKING ARTIFICIAL POSITIVE CONTROLS (VIMAPC) THAT MIMIC TITER AND TROPISM

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Introduction

A major challenge in developing detection assays is the lack of positive controls. Without them, negative results cannot be verified, rendering assays void. The transportation of positive control materials for detection assays can also pose difficulties, particularly when dealing with pathogens that are not endemic to a country. Importing infected material or nucleic acids is a time-consuming process that requires specialized permits and, in some cases, may even be prohibited. In order to addresses these issues we have developed a novel method of creating artificial positive controls that can be used for any virus. It is simple and can be used by any laboratory that conducts PCR diagnostics. Moreover, it is more cost-effective than alternative methods and has a much faster turnaround time Additionally, it generates a control that accurately reflects the targeted organism's characteristics, such as titer and tropism.

Materials and Methods

The proposed method involves designing chimeric reverse transcription primers. These have 5' ends containing the sequence of the target organism and the 3' ends containing the sequence of an available template organism, ideally belonging to the same genus as the target. The resulting DNA fragment contains the template sequence and a region of the target that can be used for PCR amplification. This chimeric molecule is then used in a PCR reaction with primers specific to the target, in exactly the same manner as the samples being tested. As the artificial control contains sequence of the template organism, it can be designed to produce an amplicon size different from that of a natural infection, making identification of contamination simple.

The source material for this method is infected tissue with a virus belonging to the same genus as the target. Using this approach, the artificial control can mimic the natural titer of the target. While this method targets RNA transcripts, this approach also works for DNA viruses as targeting transcripts for detection has previously been shown to be more sensitive than using genomic DNA[1,2] (Laney et al., 2012; Shahid et al., 2017); simply put RNA transcripts are found in greater abundance.

Results and Discussion

In order to demonstrate the feasibility of the approach, we have developed artificial controls for several RNA and DNA virus combinations, with a number of genera represented.

This approach enables the rapid development of a realistic positive control that mimics the attributes of the target and can be implemented in as short as a week. Additionally, false positives are immediately identifiable as these positive controls are designed to have a different size than the natural target amplicons.

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P31: BLUEBERRY VIRUS L – A NEW LUTEOVIRUS WHICH IS HIGHLY PREVALENT IN THE UNITED STATES

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Introduction
In current times it is unusual to find a new virus present in ~80% of samples collected across a continent and within an agriculturally important crop. However, this is the case for the novel blueberry virus described here.

Materials and Methods
High throughput sequencing and full genome characterization resulted in the identification of a new virus species present in blueberry. An additional six, near full genomes were sequenced. Screening primers were designed to investigate the prevalence of this new virus across five states, with over 600 samples tested. The screening region of over 250 samples were sequenced with percentage pairwise nucleotide identity calculated.

Results and Discussion
Blueberry virus L (BlVL) is a new luteovirus (Family *Tombusviridae*). While the majority of luteoviruses encode six or seven proteins, with two involved in movement, a small number including BlVL, have no readily identifiable movement proteins. The BlVL genomes share >93% genome pairwise nucleotide identity, with no isolates containing these two movement proteins. Of the over 600 samples from five US states, 79% tested positive. The sequenced screening region showed 84.8-100% pairwise identity across the 250 samples. BlVL is the most widespread blueberry virus present in the United States.

P32: SURVEY FOR OTHER BLACKCURRANT REVERSION VIRUS VECTORS

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Introduction

Blackcurrant reversion disease is the most damaging viral disease that affecting *Ribes* plants. Besides other plant viruses detected in reverted leaves of *Ribes* plants (1, 2), as the only one infectious agent has proved blackcurrant reversion virus (BRV) (3) and its vector — blackcurrant gall mite *Cecidophyopsis ribis* (4), which is also serious pest for currants. BRV has been detected in wild currants and ornamental *Ribes* plants, also in *Ribes* plants which has been considered as immune to BRV infection and also are free from gall mites, suggesting that not only mites could be involved in transmission of the virus but also exists another vector.

Materials and Methods

To detect presence of BRV, *Ribes* plants and plant-sucking insects infesting *Ribes* plants was collected and tested in our study. Phire Animal Tissue Direct PCR kit (Thermo Fisher Scientific) was used for extraction of total genomic DNA/RNA mixture from individual gall mites, spider mites, other eriophyid mites as well as aphids were used for BRV detection and identification of insect species. For BRV detection was used Qiagen OneStep RT-PCR kit. The RT-PCR was performed with two primer pairs P1/P2 (5) and primers Mites4/ CeF4 complementary to mite ITS genome was used as internal RT-PCR quality control. Totally were tested 1126 gall mites, 162 aphids and other mite species picked from redcurrants, blackcurrants, and mountain currants (*Ribes alpinum*).

Results and Discussion

57.2 % mites (644/1126), 18.5% aphids (30/162) and some from tested spider mites and eriophyid mites gave positive test results with primer pair P1/P2. Based on FGA data analysis, most of all tested gall mites correspond to specie *Cecidophyopsis spicata* and 61.3% of them were infected with BRV, whereas *C. ribis* constitute only 4% from tested gall mites, but 95.6% of them were infected with BRV. Results demonstrates that many mite and aphid species which feed on infected plants contain the virus and could be involved in BRV transmission, but it is necessary to clarify which acts as vectors in the field condition.

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P33: INVESTIGATIONS INTO THE VIRUS STATUS OF BLUEBERRIES IN GERMANY WITH A SPECIAL FOCUS ON THE SO CALLED "OFF TYPE"

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Introduction

Blueberry is a high-value crop that is cultivated in Germany on approx. 3000 ha and which economic importance has increased significantly. In recent years, blueberry plants with an untypical growth behavior have been noticed more and more frequently in plantations. This so-called "off type" has developed into a problem that is threatening the existence of the farmers due to dramatic yield losses. The disease is mainly characterized by stunted growth, leaf reddening and reduced fruit set with smaller fruits. Numerous attempts to clarify the cause were unsuccessful.

Materials and Methods

Conspicuous samples were taken in various German growing regions in the years 2020-2022. Selected samples were analyzed by Illumina high-throughput sequencing of total RNA extracts, and the sequence data were analyzed for known and previously unknown viruses. Specific primers were designed for the newly discovered viruses and individual plants again sampled and tested by RT-PCR to confirm the presence of the viruses.

Results and Discussion

About a dozen viruses have been reported infecting blueberry and most were originally found in North America[1] (Martin et al. 2012). Initial investigations into the viral status applying Illumina throughput sequencing have led to the identification of two novel viruses in diseased plants, a mitovirus and a luteovirus, the latter of which may well be involved in disease expression. A presumably low virus titer and an uneven distribution in the shrubs still make a reliable diagnosis by RT-PCR difficult, so that a clear correlation could not yet be established. In addition, the Blueberry mosaic associated virus associated with mosaic symptoms has been identified for the first time in Germany[2] (Menzel et al. 2021). In order to safeguard blueberry production, further investigations are absolutely necessary and ongoing.

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P34: DEVELOPMENT OF AN IMPROVED LITTLE CHERRY VIRUS 2 (LChV-2) REVERSE TRANSCRIPTION-PCR DETECTION METHOD

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Introduction

Little cherry virus 2 (LChV-2) as plus single stranded RNA virus belongs to the genus *Ampelovirus* (family *Closteroviridae*) and infects sweet and sour cherry. Little Cherry Virus Disease complex (LChV-2 and LChV-1) causes huge yield losses in cherry production (1). Typical symptoms like reduced fruit size and flavor as well as early leaf reddening are caused mainly by LChV-2. Transmission by insect vectors and by grafting promotes a fast spread of LChV-2. The disease is of worldwide distribution. High sequence variability within the LChV-2 isolates impedes reliable Reverse Transcription (RT)-PCR testing. In order to improve LChV-2 diagnosis, we developed two new primer sets based on sequence alignments of current database entries and own sequences.

Materials and Methods

Based on an alignment of LChV-2 genome sequences available from NCBI database (Dec. 2019) and JKI internal isolate sequences two LChV-2 detection primer pairs were designed. The degenerated primers were developed in two different genome regions, in the RNA-dependent RNA polymerase (RdRP) gene and coat protein (CP) gene, and evaluated using a set of more than 100 available samples (LChV-2 sources maintained at JKI institute, Dossenheim and external samples send to JKI for testing). Two different sampling time points (summer and autumn) were analyzed. Total nucleic acids from the midrib of leaves were extracted using a modified silica extraction method (2). All samples were analyzed for LChV-2 in RT-PCR using the two developed and three additional published LChV-2 detection primer sets.

Results and Discussion

Evaluation of the developed LChV-2 detection systems revealed high sensitivity and good discrimination against all virus sources available as well as seasonal differences. Sampling is recommended in autumn and spring, since it is more reliable than testing in summer. Typical early leaf reddening symptoms provide a good indication for LChV-2 infection.

For a reliable LChV-2 RT-PCR analysis we advise testing with the newly designed two primer sets in combination with the primer pair LChV2-CP544RTF/R (3).

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P35: DETECTION AND CHARACTERIZATION OF THE FIRST STRAWBERRY POLEROVIRUS-1 ISOLATE IN STRAWBERRY IN GERMANY WITH HTS AND VECTOR TRANSMISSION BY STRAWBERRY APHID

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Introduction

Strawberry polerovirus-1 (SPV-1) was first described in strawberry (*Fragaria* x *ananassa*) in eastern Canada (1) as phloem-limited positive sense ssRNA virus (family *Solemoviridae*, genus *Polerovirus*). Members of the *Solemoviridae* are transmitted by specific aphid vectors. SPV-1 probably is part of mixed virus infections causing strawberry decline (SD). Symptoms of SD like stunted foliage, reduced fruit size and brittle roots lead to a significant yield reduction in strawberry plantations. SPV-1 was first reported in the United States and in Argentina in 2016, subsequently it was detected in Czech Republic (2, 3). To our knowledge, this is the first report of SPV-1 in Germany.

Materials and Methods

SPV-1 was detected in an asymptomatic field strawberry plant of the variety Darselect, originating from a propagation site in the administrative district of Karlsruhe, Baden-Wuerttemberg, in 2017. Total nucleic acids were extracted from strawberry plant sample (isolate 07/17) via CTAB extraction. SPV-1 was amplified in reverse transcription (RT)-PCR with specific primers (pol411 F/R, JKI, Dossenheim). In a transmission study, the German SPV-1 isolate 07/17 was successfully transmitted by strawberry aphid (*Chaetosiphon fragaefolii,* family *Aphididae*) from SPV-1 positive strawberry plant to healthy strawberry plants (*F. vesca* accession UC4). For high-throughput sequencing (HTS) analysis (RNA Seq) leaves from a freshly vector transmitted SPV-1 positive plant sample were taken. Total RNA was used for library preparation in HTS pipeline. Sequencing run was performed using the NextSeq 500/550 high output kit, 2x150bp paired-end (Illumina). HTS pipeline including sequencing run was conducted at the DSMZ Plant Virus Department (Leibniz-Institute, Braunschweig, Germany). Bioinformatic analysis of raw data was performed at our institute with Geneious Prime 2020.04 (Biomatters, Auckland, New Zealand).

Results and Discussion

In NextSeq run 2 x 4,759,792 reads were obtained. After quality trimming and removal of duplicates, the quality filtered reads were paired. After host genome subtraction paired reads were de novo assembled into 148,288 contigs. 6 contigs mapping to SPV-1 Canadian reference sequence (NC_025435) were assembled. The German SPV-1 isolate 07/17 possesses six predicted open reading frames comparable to the Canadian sequence. The German isolate and the Canadian accession share over 98% nucleotide identity. The retrieved sequence was verified and completed at the 5' and 3' ends by RT-PCR and RACE-PCR to determine the complete virus genome. The results obtained so far as well as phylogenetic analyses of open reading frame sequences (ORFs) of the German SPV-1 isolate 07/17 and representative species in the family *Solemoviridae* are presented.

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P36: UNCOVERING THE WORLDWIDE DIVERSITY OF LITTLE CHERRY VIRUS 1 AND 2 AND THE ASSOCIATED VIRUSES IN *PRUNUS SPP.*

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Introduction

Two Closteroviridae members, little cherry virus 1 (LChV-1, *Velarivirus*), and little cherry virus 2 (LChV-2, *Ampelovirus*) are associated with Little cherry disease (LChD) (1). LChD is an economically significant viral disease affecting worldwide sweet cherry (*Prunus avium*) and sour cherry (*P. cerasus*) production. Genetically, LChV-1 and -2 isolates are highly variable and, as a result, some may escape existing molecular detection tests. There is therefore an interest in generating whole genome sequences for both viruses to better understand their worldwide genetic diversity, allowing us to develop inclusive protocols for their detection.

Materials and Methods

During the present study, 78 *Prunus* samples from 20 countries and six continents were collected. Most of them exhibited or had exhibited putative symptoms of little cherry disease. Genetic material from the samples was analyzed using three different nucleic acids targets methods, total RNA, double-stranded RNA (dsRNA), and virion-associated nucleic acids (VANA). The resulting extracts were subjected to high-throughput sequencing (HTS) using Illumina technology. Bioinformatics analyses aimed at detecting the viruses infecting each sample and, whenever possible, at reconstructing their genome. The genetic and evolutionary variability of LChV-1 and -2 were analyzed in depth at both consensus sequence and single nucleotide polymorphisms (SNPs) levels.

Results and Discussion

In total, 15 viruses were identified in the samples, the most prevalent were LChV-1, LChV-2, cherry virus A (CVA), prune dwarf virus (PDV), apricot pseudo-chlorotic leaf spot virus (APCLSV), and american plum line pattern virus (APLPV). Thanks to genome reconstructions, this study almost doubled the number of genomes available for LChV1 (29 new genomes compared to 39 already available), LChV2 (22 new genomes compared to 15 available), PDV (14 new genomes compared to 30 RNA 1, 26 RNA 2, and 19 RNA 3 sequences) and APLPV (8 new genomes compared to 3). The present study constitutes the first report of LChV-1 in the Netherlands, Austria, and Slovenia and of LChV-2 in the Netherlands and Switzerland. It also identified for the first time apricot pseudo-chlorotic leaf spot virus (APCLSV) in Japan. A higher richness of viral species was observed, from Europe and Asia, with some viruses only detected from those regions. The generated genomes will facilitate the development and adaptation of robust molecular detection systems able to cope with the high genetic variability of little cherry viruses and will help improve our understanding of drivers of viral genomic diversity and multiple infections in *Prunus* sp.

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P37: OCCURRENCE OF TWO LITTLE CHERRY VIRUSES IN STONE FRUITS IN SERBIA

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Introduction

Little cherry virus 1 (LChV-1, genus *Velarivirus*) and little cherry virus 2 (LChV-2, genus *Ampelovirus*), members of the family *Closteroviridae*, are considered important cherry pathogens worldwide. In susceptible sweet and sour cherry cultivars, LChV-1 and LChV-2 can cause severe yield losses and reduce fruit quality. Beside cherries, both viruses were found in other stone fruit species: plums, apricots, peaches, and almonds (1). The aim of our study was to access the presence of LChV-1 and LChV-2 in major *Prunus* species cultivated in Serbia.

Materials and Methods

During 2017−2020 202 stone fruit samples (67 sweet cherries, 17 sour cherries, 53 apricots, 38 plums, and 27 peaches) were collected from several regions of Serbia. All samples were analyzed with reverse transcription polymerase chain reaction (RT-PCR). TNA was extracted from fresh leaves with a 2% CTAB buffer. Reverse transcription was performed using random hexamer primers. PCR reactions were performed using the LCV1U/LCV1L primer pair that amplify a 419 bp fragment of the LChV-1 3' non-translated region and LCV2UP2/LCV2LO2 for the amplification of the 438-bp fragment of the LChV-2 methyltransferase gene, respectively (2). PCR products were analyzed by agarose gel electrophoresis. Selected isolates of both viruses (10 LChV-1, and one LChV-2) were sequenced and phylogenetically analyzed.

Results and Discussion

The presence of LChV-1 was confirmed in 18 analyzed samples (8.9%), and LChV-2 in only one sweet cherry sample (0.5%). LChV-1 was detected in 10 sweet cherries, five apricots, and three plums. Analyzed viruses were not detected in any of the tested sour cherry and peach samples. Serbian LChV-1 isolates showed 76.10–100% nucleotide (nt) identity. A phylogenetic analysis identified Serbian LChV-1 isolates from plums and sweet cherries as members of the G5 phylogenetic group. Two apricot isolates belong to the G3 phylogenetic group and one isolate to the G5 group.

This report describes the first report of little cherry virus 1 infecting plums in Serbia and confirms the presence of isolates from G3 and G5 phylogroups in Serbian stone fruit orchards. Out of all tested isolates, little cherry virus 2 was confirmed in only one sweet cherry tree.

Acknowledgement

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P38: MOLECULAR DETECTION AND CHARACTERIZATION OF CHERRY VIRUS A IN *PRUNUS* SPECIES IN SERBIA

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Introduction

Stone fruits (plums, apricots, peaches, and cherries) are economically important species for Serbian agriculture. These fruits harbor numerous viruses that can decrease yield and fruit quality. Cherry virus A (CVA), a member of the genus *Capillovirus*, was discovered during the cloning of the little cherry agent (1). CVA is widely distributed in more than 10 countries in sweet and sour cherries (2), but also in other *Prunus* hosts. So far, CVA has been confirmed in Serbia in sweet and sour cherries.

Materials and Methods

A total of 106 leaf samples from 31 orchards in 22 locations were collected and examined to determine the prevalence of CVA in stone fruit orchards in Serbia. Great majority of leaf samples were symptomless, but some exhibited symptoms of chlorotic mottling, necrotic spots, and vein necrosis. Samples were tested by reverse transcription polymerase chain reaction (RT-PCR) using a virus-specific primer pair CVA-fw1/CVA-rev1 that amplify a fragment of the movement protein (MP) gene (3). PCR products were analyzed by electrophoresis in 1.5% agarose gel and stained with ethidium-bromide. To further access diversity of the detected isolates, we sequenced a partial MP of four detected isolates.

Results and Discussion

The results of the RT-PCR revealed that 13 samples (12.3%) were infected with CVA. An expected 302 bp fragment was obtained in eight tested samples of sweet cherry, three apricot, and two plums. No positive amplification was evidenced in analyzed sour cherry and peach samples. CVA was detected in both asymptomatic and symptomatic samples. In all positive samples with symptoms, mixed infection with other viruses (prune dwarf virus, little cherry virus 1, and cherry green ring mottle virus) was detected. It is still unclear whether CVA is associated with any of the observed symptoms. The nucleotide sequences of the isolates showed 89.7–99.6% identity. To the best of our knowledge this is the first report of cherry virus A in apricots and plums in Serbia.

Acknowledgement

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P39: ROLE OF LONG NONCODING RNAS INVOLVED IN RESISTANCE TO *BOTRYOSPHAERIA DOTHIDEA* INFECTION IN PEAR

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Introduction

Pear ring rot disease caused by *Botrytosphaeria dothidea* is a severe hazard to healthy development of the pear industry [1]. Long noncoding RNAs (lncRNAs) are emerging to be functional in diverse biological process of plants including defense response. Several researches of identifications and function verification of lncRNAs in fruit trees were reported recently [2], but the role of lncRNAs involved in pear defense response remains unknown. This study aims to investigate the potential regulatory function and mechanism of lncRNAs in pear defense to *B. dothidea* infection.

Materials and Methods

The stem tissues from pear plantlets infected with *B. dothidea* or mock inoculated with ddH_2O were collected for high throughput transcriptome sequencing. The raw data were filtered and the clean reads were assembled to screen for lncRNA. The bioinformatics analysis for expression and function prediction were conducted as previously report [3]. The transgenic Arabidopsis obtained by floral dip method were used for function verification of MSTRG.32189.

Results and Discussion

Among 3555 identified long non-coding RNAs, 2466 lncRNAs predicted to be cis-functional and 995 lncRNAs predicted to be trans-functional. Cis-functional lncRNAs mainly regulate the neighboring genes through positive way. KEGG enrichment analysis showed that the correlated cis-regulated target genes were enriched in several disease-resistant related pathways. There are 286 differentially expressed lncRNAs of which target genes were enriched in multiple disease-resistant related pathways by GO enrichment analysis. More specifically, we identified 650 long non-coding natural antisense transcripts (lncNAT) and 3 endogenous target mimic (eTM) of which MSTRG.32189 was significantly down-regulated after pathogen infection. MSTRG.32189 could inhibit the cleavage effect of miR399 on *PcUBC24* and was specifically expressed in the stem tissue. Transgenic Arabidopsis of OE-MSTRG.32189 showed lower free Pi content and weaker disease resistance compared with WT. In summary, the obtained results demonstrated the regulatory role of lncRNAs in pear defense response to *B. dothidea* and revealed that MSTRG.32189 could function as eTM to regulate disease resistance in plant.

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P40: SANITARY DIAGNOSIS FOR THE QUALITATIVE DEVELOPMENT OF THE CITRUS SECTOR OF ALULA

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Introduction
The establishment of a high value-added citrus production chain is the goal of High Value Citrus for AlUla (HVCA), a research and sustainable development project for the region of AlUla (Saudi Arabia), supported by the French Agency for AlUla Development (AFALULA) in collaboration with the Royal Commission for AlUla (RCU). A sanitary diagnosis was organized and carried out for both the orchards and nurseries as well as for potential initial mother plants (IMPs) to commit to a disease-free production.

Materials and Methods
In 2021 and 2022, more than 260 symptomatic or asymptomatic trees were selected and sampled among 6 microclimates of AlUla and 2 culture types (oases or monocultures) in proportion to their production area, plus in 2 nurseries. Relevant plant samples were pooled either by 2 (asymptomatic trees) or by 5 (symptomatic trees) before analysis. Budsticks of 11 asymptomatic trees constituting interesting genetic resources were sent to the French quarantine station of Anses to undergo a quarantine process as IMPs candidates. All sample pools and IMPs were tested against 20 pests impacting on citrus production, including European Union regulated quarantine and non-quarantine pests, using targeted tests. HTS analyses are ongoing to complete the sanitary status.

Results and Discussion
All plants sampled in AlUla citrus orchards were found to be free from the most serious citrus plant pathogens in the world such as those responsible for tristeza and citrus greening. 8 asymptomatic pools and 11 individual asymptomatic trees tested negative for all tested pests including European Union-regulated quarantine ones. For the non-quarantine ones, symptomatic pools and the majority of the asymptomatic pools tested positive for at least one pest: ophiovirus citri in 2 microclimates; *Spiroplasma citri* in 2 microclimates; citrus bark cracking viroid, hop stunt viroid and citrus exocortis viroid in all microclimates and in the nurseries for 2 of them. Regarding ophiovirus citri and *Spiroplasma citri*, the health status of current AlUla orchards could be controlled by monitoring. For future citrus sector organization, cultural practices management will be critical to avoid viroid contamination of newly planted orchards. Among the 5 first IMPs ready to be released from quarantine, 2 out of 5 were found to be disease free which is qualifying them to be used to initiate a disease-free production.

P41: GENETIC POLYMORPHISM OF *CANDIDATUS* LIBERIBACTER ASIATICUS BASED ON A SEC-DEPENDENT SECRETED EFFECTOR PROTEIN

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Introduction

Citrus Huanglongbing (HLB), previously known as citrus greening, caused by a phloem-limited fastidious *α*-proteobacteria *Candidatus* Liberibacter (1), is one of the most destructive diseases with a global distribution in more than 50 countries worldwide (2,3). Effector proteins secreted by plant pathogenic bacteria are associated with virulence and play important roles during host -pathogen interactions. Yet, up to now, there's no report on genetic polymophism based on *C*las effector proteins. The aim of this study was to to investigate the molecular genetic diversity of *C*Las based on a Sec-dependent effector protein *C*Las04560 to shed light on the correlation beteen phenotype and bacteria population.

Materials and Methods

The present study was performed with 1125 samples mainly collected from 8 different geographic areas in China and 60 samples from USA.

Results and Discussion

Four genotypes of *C*Las04560-ORF588, *C*Las04560-ORF531, *C*Las04560-ORF518, *C*Las04560-ORF474 of a single gene locus in *C*Las were amplified from most of the Chinese isolates. However, in USA isolates, 3 genotypes not including *C*Las04560-ORF518 were identified. Among all the genotypes, *C*Las04560-ORF531 was reported for the first time, which was frequently detected in Yunnan (58.8%) and Zhejiang (52.0%) isolates showing much higher proportion than those from other geographic areas (2.2%-23.1%). While, *C*Las04560-ORF588 was the most predominant genotype both in China (77.2%) and USA (82.2%), which was frequently detected from all *C*las hosts including citrus, periwinkle, dodder, *Diaphorina citri* and *Cacopsylla citrisuga*. Further analysis based on the samples with different symptoms showed genotypic proportional combination may contribute to the development of diverse symptoms. Phylogenetic analysis revealed the genotypes of CLIBASIA_04560 were correlated to geographic origin, more diversity was observed in higher altitude localities. This study demonstrated the genomic polymorphism of *C*Las resulted from effector proteins and the association with phenotype.

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P42: BASELINE SURVEILLANCE OF RUBUS VIRUSES IN THE UK

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Introduction

A number of viruses are known to be present in both wild and cultivated rubus in the UK. High throughput sequencing (HTS) can potentially detect any viruses within a sample, including novel viruses. Rubus samples have been sent to Fera Science Ltd with symptoms consistent with virus infection including mottle/line pattern and distortion. When samples tested negative by conventional methods, they were then tested by HTS to identify viruses present.

Materials and Methods

Samples were tested using HTS by a ribosomal RNA-depleted total RNA approach. Initially samples were extracted by Qiagen RNeasy mini kit with a DNase step. HTS was performed using a MiSeq v 2, using either ScriptSeq complete plant leaf or TruSeq complete plant libraries. Resulting sequences were analysed by the "ANGUA" pipeline (1) (2).

Results and Discussion

Black raspberry necrosis virus, raspberry leaf blotch virus and rubus yellow net virus have been identified by HTS testing. All three of these viruses are known to be present in the UK.

Additional novel viruses were detected. Presented here is a partial genome of a novel blunervirus identified in a rubus from 2015. Testing by HTS identified both this novel blunervirus and raspberry leaf blotch virus in this sample.

Baseline testing of both wild and cultivated rubus species could improve understanding of the virus baseline. However, difficulties in sequencing viruses in fruit such as rubus mean that only fragments of virus were identified. This can cause challenges in understanding which viruses are present and confidence that the virus is present.

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P43: GENETIC POLYMORPHISM OF CANDIDATUS LIBERIBACTER ASIATICUS BASED ON A SEC-DEPENDENT SECRETED EFFECTOR PROTEIN

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Introduction

Citrus Huanglongbing (HLB), previously known as citrus greening, caused by a phloem-limited fastidious α-proteobacteria *Candidatus* Liberibacter (1), is one of the most destructive diseases with a global distribution in more than 50 countries worldwide (2,3). Effector proteins secreted by plant pathogenic bacteria are associated with virulence and play important roles during host -pathogen interactions. Yet, up to now, there's no report on genetic polymophism based on *C*las effector proteins. The aim of this study was to to investigate the molecular genetic diversity of *C*Las based on a Sec-dependent effector protein *C*Las04560 to shed light on the correlation beteen phenotype and bacteria population.

Materials and Methods

The present study was performed with 1125 samples mainly collected from 8 different geographic areas in China and 60 samples from USA.

Results and Discussion

Four genotypes of *C*Las04560-ORF588, *C*Las04560-ORF531, *C*Las04560-ORF518, *C*Las04560-ORF474 of a single gene locus in *C*Las were amplified from most of the Chinese isolates. However, in USA isolates, 3 genotypes not including *C*Las04560-ORF518 were identified. Among all the genotypes, *C*Las04560-ORF531 was reported for the first time, which was frequently detected in Yunnan (58.8%) and Zhejiang (52.0%) isolates showing much higher proportion than those from other geographic areas (2.2%-23.1%). While, *C*Las04560-ORF588 was the most predominant genotype both in China (77.2%) and USA (82.2%), which was frequently detected from all *C*las hosts including citrus, periwinkle, dodder, *Diaphorina citri* and *Cacopsylla citrisuga*. Further analysis based on the samples with different symptoms showed genotypic proportional combination may contribute to the development of diverse symptoms. Phylogenetic analysis revealed the genotypes of CLIBASIA_04560 were correlated to geographic origin, more diversity was observed in higher altitude localities. This study demonstrated the genomic polymorphism of *C*Las resulted from effector proteins and the association with phenotype.

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