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SATELLITE EVENT: HIGH-THROUGHPUT SEQUENCING IN PLANT VIROLOGY: FROM DISCOVERY TO DIAGNOSTICS

(Sunday, 20 August 2023 – all day)

Room: **Rhone 2**

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High-throughput sequencing (HTS) technologies have revolutionized plant virus research and diagnostics by accelerating the discovery of new viruses and by providing a sensitive untargeted approach for the detection of viruses. The latter, together with high data-generation potential of HTS, enables discovery of new and emerging viruses from diverse hosts, archived or ancient samples, and untargeted virus detection in diverse matrices, as well as research on a broad range of topics, such as plant virus epidemiology, diversity, and evolution. Many new plant virus discoveries, increased availability of sequence data, and a lagging biological characterization of HTS-based findings call for a broad consideration on harmonization of sequencing and data analysis approaches, as well as the interpretation of the results from the scientific and regulatory perspective. During this satellite meeting, different aspects of applying HTS in plant virology will be addressed and discussed. Topics will include: discovery and detection of new and emerging viruses; virus diversity, epidemiology, and evolution studies; development of virus detection and identification protocols and validation of HTS-based tests for plant virus diagnostics.

Keynote speakers:

François Maclot
Maja Ravnikar
Yazmin Rivera
Maher Al Rwahnih

SCHEDULE OF THE EVENT

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10:10-10:25	*Maria Mariduena-Zavala*: TRANSCRIPTOMIC AND BISULFITE SEQUENCING IN PAPAYA PLANTS INFECTED WITH BABACO MOSAIC VIRUS (BABMV)
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	Session II **Chaired by Maja Ravnikar and Marleen Botermans**
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12:05-12:25 (Keynote)	*Maher Al Rwahnih*: USE OF HIGH THROUGHPUT SEQUENCING FOR PLANT MATERIAL CERTIFICATION AND RELEASE OF QUARANTINED PROPAGATIVE PLANT MATERIAL AT FOUNDATION PLANT SERVICES IN DAVIS, CALIFORNIA, USA
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13:45-14:00	*Nataša Mehle*: NANOPORE SEQUENCING FOR THE ANALYSIS OF OFFICIAL SAMPLES FOR THE PRESENCE OF (QUARANTINE) PLANT VIRUSES
14:00-14:15	*Julie Pattemore*: HIGH THROUGHPUT SEQUENCING: RESEARCH TO REALITY – THE AUSTRALIAN POST ENTRY QUARANTINE JOURNEY
14:15-14:30	*Hans Maree*: APPLICATION OF HIGH-THROUGHPUT SEQUENCING (HTS) FOR ROUTINE PLANT VIRUS DETECTION IN THE SOUTH AFRICAN CITRUS IMPROVEMENT SCHEME
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15:15-15:30	*Françoise Petter*: THE OUTLOOK FOR HTS IN REGULATORY APPLICATIONS
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WHAT CAN VIRAL METAGENOMICS BRING TO OUR UNDERSTANDING OF THE DIVERSITY AND ECOLOGY OF PLANT VIRUSES IN AGRO-ECOLOGICAL LANDSCAPES?

Maclot F.[1]

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High throughput sequencing (HTS) gave access for the first time to the viral metagenome or virome, by allowing to characterize, without a priori, all or nearly all viruses in a given sample. This broad-spectrum capability of HTS is raising a growing interest to study the diversity and ecology of plant viruses, in particular the richness and composition of viral communities at the agro-ecosystem scale, as well as the virus circulation among host reservoirs and the discovery of new and emerging viruses.
Recent viromics studies revealed diversified and largely unknown phytoviromes in natural ecosystems, with high rates of co-infection and the abundance of so-called persistent (or cryptic) viruses representing more than half of the viruses identified in wild plants. The influence of plant traits (e.g., lifespan, height, occurrence) on the virome richness, and the relationships between host-pathogen richness in cultivated and non-cultivated plant communities were also unravelled. In particular, the richness and diversity of plant communities appeared as influencing the richness and composition of phytoviromes, especially the distribution of persistent and acute viruses. Other results demonstrated the stability of virome richness over time but the large viral intraspecific variability within and among plant communities. Thus, HTS technologies have highlighted and will continue to serve the exploration of the complex network of viral communities in nature for the times to come...

ADVANCES IN HIGH-THROUGHPUT SEQUENCING GIVE NEW OPPORTUNITIES IN DISCOVERY, DIAGNOSTICS AND BIOLOGY OF PLANT VIROMES; RESULTS GENERATED WITHIN INEXTVIR PROJECT

Ravnikar M.[1], Adams I.[9], Aranda M.[12], Babalola B.[6], Boonham N.[2], Candresse T.[3], Curk T.[4], Daoud Hiri K.[8], Devos M.[5], Donaire L.[7], Fontdevilla N.[10], Fox A.[9], Frewer L.[2], Garcia-Arenal F.[6], Gutierrez Aguirre I.[1], Hernando Y.[7], Hilaire J.[2], Hren M.[8], Jones G.[9], Khalili M.[3], Kutnjak D.[1], Maachi A.[7], Maksimovic O.[1], Marais A.[3], Massart S.[10], Nikolski M.11, Rivarez M.1, Rollin J.[5], Salavert F.[2], Schönegger D.[3], Sukhorukov G.[11], Temple C.[10], Tindale S.[2], Zamfir A.[6]

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High-throughput sequencing (HTS) based methods have revolutionised plant virus discovery and virome studies. To facilitate a wide employment of the methods, build capacity and bring new advances to the field we joined forces within a Marie Sklodowska-Curie Innovative Training Network (MSCA-ITN) called INEXTVIR (Innovative Network for Next Generation Training and Sequencing of Virome). Fourteen PhD students, from 11 countries, were trained within the project over 12 European academic and industrial partners from 5 countries. Within INEXTVIR we aimed to generate a better understanding of viral communities and their role in agricultural ecosystems by using the latest advances in HTS technologies coupled with modern big data analytical approaches and socioeconomic analysis. The outputs of the project, which will be presented bring new advances overarching several natural and social science fields, including: comprehensive virome studies with detection and discovery of numerous new viruses in diverse crops (e.g., tomato, carrots, cucurbits, fruit trees); characterization of newly discovered viruses; insights into the interactions between virome and the environment (e.g., effects of habitat biodiversity); development of novel approaches for bioinformatics analysis of HTS-derived virome data; and societal perceptions of the risks and benefits related to the virome in agriculture.

FULL-LENGTH SEQUENCING OF CONCATENATED CIRCULAR DNA VIRUS GENOMES USING ROLLING CIRCLE AMPLIFICATION AND OXFORD NANOPORE SEQUENCING

van de Rhee M.[1], Arunothayanan H.[2], van Bers N.[1], van Dam P.[1]

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Geminiviruses are plant-infecting viruses with a mono- or bipartite single-stranded DNA genome, approximately 2.5-3.1 kb in size. Reliable and accurate detection of virus species and subspecies level sequence types can be performed by modern sequencing methodologies, however the percentage of virus-derived reads is often very low. Here we present a species-independent method to enrich viral sequence data and to generate a consensus sequence from Oxford Nanopore Technologies sequencing reads. Rolling Circle Amplification based on the phi29 polymerase was used to generate concatemers of the circular virus genomes in DNA samples from various infected crop plants, including melon, pepper, eggplant, tomato and okra. Multiplexed sequencing of debranched and linearized amplification products displayed an enrichment of the viral sequence content. Some samples showed up to 83% of the read data derived from Begomoviruses, far more than the values found in non-enriched samples: typically 0-2% of the read data. Moreover, the concatenated DNA molecules generated during the Rolling Circle Amplification step resulted in concatemer reads, ranging from 3 to 20+ fold, that allowed generation of a reliable consensus sequence of the viral genome from a single Nanopore read. Cost-effective and reliable detection, description and sequence typing of geminiviruses and other pathogenic viruses with a circular ssDNA genome can be performed very efficiently and reliably with this method.

TRANSCRIPTOMIC AND BISULFITE SEQUENCING IN PAPAYA PLANTS INFECTED WITH BABACO MOSAIC VIRUS (BABMV)

Mariduena- Zavala M.[1,2], Cornejo J.[1], Quito D.[1], Abdelgawad H.[2], van-Dijk J.[2], Cevallos J.[1], Noceda C.[3,4,5], Beemster G.[2]

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Babaco mosaic virus (BabMV) infects babaco plants (Carica pentagona). Due to genetic similarities with babaco, papaya plants (Carica papaya) were used to identify the transcriptomic and methylation responses occurring after BabMV infection. We performed RNA and Bisulfite sequencing of 32 samples of papaya leaves from plants infected with the BabMV evaluated at -2, -10, 15- and 30-days post-infection. Illumina reads quality for each sample was checked using FASTQC in Galaxy and mapping to papaya genome was performed with HiSat2 for transcriptomic reads and Bissmark tool for mapping and global cytosine methylation level for DNA sequences. Statistical analysis was carried out by multi-contrast test using Deseq2 for the transcriptomic analysis and logistic regression using the Methylkit package in Rstudio for the DNA reads, For the differential analysis FDR corrected p value <0.05 and log2 fold change > 0.5 criteria were used to select the differentially expressed genes and regions per CpG sites. In addition QTC clusters were constructed to identified the gene patterns in both analyses. 1585 genes were differentially expressed in the RNA and 508 methylation sites in the DNA samples, GO overrepresentation analysis indicate changes in protein synthesis, oxidative stress and polysaccharide metabolism at 15- and 30-dpi. The use of both next generation sequencing techniques represents an important contribution to understanding the response of the plant to the virus infection.

AUTOMATED AND REAL-TIME PROFILING OF PLANT VIRUS INFECTIONS TO SUPPORT DIAGNOSTICS AND QUICK RESPONSE IN OUTBREAK EVENTS

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High-throughput sequencing (HTS) technologies are transforming our means to detect pathogens and perform disease diagnosis. Recent advances in genomic sequencing, as the ones pioneered by Oxford Nanopore Technologies (ONT), are enabling extremely rapid and effective detection, and quick transition from research to adoption in diagnostics settings is already occurring. The pocket-size of the sequencer, ease of library preparation, low sequencing costs and (long) read data generated in a short turnaround time are key characteristics of the ONT platforms, with substantial benefits for point-of-care testing and on-time reactions in response to a disease. Here we present the application of ONT for rapid, semi-automated profiling of (multiple) virus infections in plant samples. An amplicon-sequencing based approach was established by combining VolTRAX, a compact USB-powered device for automated sample and library preparation, with MinION sequencing and subsequent real-time analysis of the sequence reads. We demonstrate that the workflow – from RT-PCR amplification of total RNA to taxonomic assessment of pathogen sequences – can be accomplished in less than 5 hours, with a minimal need of human intervention, enabling a sensitive and rapid identification of viruses in diseased plant material.

REVISITING HIGH THROUGHPUT SEQUENCING DATA USED FOR PLANT VIRUS DETECTION IN ORDER TO FIND EVIDENCE OF NON-VIRAL PLANT PATHOGENS AND PESTS

Haegeman A.[1], Foucart Y.[1], De Jonghe K.[1], Goedefroit T.[1], Al Rwahnih M.[2], Boonham N.[3], Candresse T.[4], Gaafar Y.[5], Hurtado-Gonzales O.[6], Kogej Zwitter Z.[7,8], Kutnjak D.[7], Lamovšek J.[9], Lefebvre M.[4], Malapi M.[10], Mavric Pleško I.[9], Onder S.[11], Reynard J.[12], Salavert Pamblanco F.[3], Schumpp O.[12], Stevens K.[2], Pal C.[13], Tamisier L.[14], Ulubas Serçe Î[15], Van Duivenbode I.[16], Waite D.[17], Hu X.[6], Ziebell H.[18], Massart S.[19]

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High-throughput sequencing (HTS), more specifically RNA-seq of plant tissue, has become an indispensable tool for plant virologists to detect and identify plant viruses. During the data analysis step, plant virologists typically compare the obtained sequences to reference virus databases only, which lead to our hypothesis that they might be missing possible traces of other pathogens in the data. In this study, we set up a community effort to re-analyze existing RNA-seq datasets used for virus detection to check for the potential presence of non-viral pathogens or pests. In total 101 datasets from 15 participants derived from 51 different plant species were re-analyzed, of which 37 were selected for subsequent in-depth analyses. In 29 of the 37 selected samples (78%), we found convincing traces of non-viral plant pathogens or pests (>100 reads per million). The most observed organism categories were fungi (15/37 datasets), insects (13/37) and mites (9/37). Nematodes were not observed and only a few samples showed the presence of plant pathogenic phytoplasmas (1/37), bacteria (3/37) and oomycetes (4/37).
In conclusion, we were able to show that it is possible to detect non-viral pathogens or pests in these metatranscriptomics datasets, in this case primarily fungi, insects and mites. With this study, we hope to raise awareness among plant virologists that their data might be useful for fellow plant pathologists in other disciplines (bacteriology, mycology, entomology) as well.

TOWARDS THE INCORPORATION OF HTS IN THE CONFIRMATORY DIAGNOSTICS PROCESS FOR QUARANTINE PLANT VIRUSES IN THE US

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High-throughput sequencing (HTS) has advanced from research use to a powerful diagnostic tool for plant viruses. Efforts to develop and improve validation metrics, interlaboratory comparability studies, and quality measures established in the diagnostics workflow have facilitated its acceptance in diagnostics. The USDA APHIS PPQ, Plant Pathogen Confirmatory Diagnostics Laboratory (PPCDL), an ISO17025 certified facility, provides the official federal diagnostic determination for regulated plant pathogens in the United States. PPCDL relies on various molecular technologies for the detection and identification of plant pathogens. PPCDL scientists have joined national and international efforts to optimize and validate HTS processes for diagnostics and incorporate this technology in the diagnostics workflow. The PPCDL has used HTS to confirm the presence of quarantine viruses in plant samples, examine the genome-wide diversity of emerging plant viruses and detect novel viruses. This presentation will discuss the progress to incorporate HTS as a diagnostic tool that include optimization and validation of processes, and the results of its incorporation for the detection of emerging plant viruses in the United States.

USE OF HIGH THROUGHPUT SEQUENCING FOR PLANT MATERIAL CERTIFICATION AND RELEASE OF QUARANTINED PROPAGATIVE PLANT MATERIAL AT FOUNDATION PLANT SERVICES IN DAVIS, CALIFORNIA, USA

Al Rwahnih M.[1]

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High throughput sequencing (HTS) is an important component of routine testing procedures at Foundation Plant Services (FPS) at the University of California, Davis for plant material certification and quarantine import programs. The US Department of Agriculture-Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-PPQ) and California Department of Food and Agriculture (CDFA) have approved the use of diagnostic testing protocol that replaces biological indexing with a combination of HTS and polymerase chain reaction (PCR) testing for release of plant material. Research conducted at FPS indicates that this protocol results in more accurate test results compared to biological indexing using woody and herbaceous indicators for virus detection. FPS scientists conducted side-by-side studies comparing the efficacy of indexing to PCR and HTS testing in grapevine, Prunus, and rose. The results of these studies indicate that biological indicators give false negative results a significant percentage of the time and do not provide sufficient sensitivity in detecting target viruses or unknown viral pathogens. Similar results were obtained in studies conducted on Prunus and pome fruits by other clean plant centers. The streamlined testing methods yield the most accurate information about the phytosanitary status of material, expedite release times, and reduce potential risks from the transmission of vector-mediated viruses in the field.

VALIDATION OF A HIGH THROUGHPUT SEQUENCING TEST WITHIN AN ISO17025 ACCREDITED PLANT HEALTH LABORATORY

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The use of High Throughput Sequencing (HTS) for the detection and identification of plant viruses and viroids is widely used. So far, validation of HTS mainly focussed on the bioinformatics pipeline. We described and validated an HTS test from biological material till the reporting of the identified viruses and viroids within an ISO 17025 diagnostic framework. This includes the analysis of multiple first line controls and visual assessment of assemblies to further substantiate the reliability of the results. Performance criteria analytical sensitivity, repeatability and reproducibility were determined similarly as for traditional molecular methods using tomato brown rugose fruit virus in infected tomato leaves as a model system. Analytical specificity, selectivity and robustness were assessed in a more generic way. This allowed addition of the detection and identification of viruses by HTS to the scope of accreditation. Using this validation approach, 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. 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. We will present challenges and practical solutions in implementing and validating an HTS test within an ISO 17025 accredited plant health laboratory.

NANOPORE SEQUENCING FOR THE ANALYSIS OF OFFICIAL SAMPLES FOR THE PRESENCE OF (QUARANTINE) PLANT VIRUSES

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Climate changes and increased international trade are accelerating the spread of plant viruses, increasing the chance of introduction of new pathogens into new areas, and their persistence in new host species. Accurate identification of plant viruses is crucial for planning an effective prevention of new disease spread and its eradication. Targeted diagnostic tests are not available for many viruses, including some on quarantine lists. Development and validation of individual targeted tests could be time-consuming and costly. In this case, the use of the generic high-throughput sequencing (HTS) approach is sensible. HTS, using Illumina platform, has been used in our laboratory for selected official samples since 2017, resulting in detection of several new viruses for Slovenia. Recently, we introduced nanopore sequencing for the same aim, and shown that it gives comparable results to Illumina sequencing. Comparative studies were performed for detection of plant viruses in bulk plant samples, detection of various begomoviruses, and detection of unexpected plant viruses. Nanopore sequencing has been found to provide comparable results to Illumina sequencing, while being faster and better suited for small laboratories. Thus, nanopore technology was selected in our laboratory for a full validation according to EPPO standard PM 7/98, considering the specific guidelines of EPPO PM 7/151. The output of this process and our experiences obtained during it will be presented.

HIGH THROUGHPUT SEQUENCING: RESEARCH TO REALITY – THE AUSTRALIAN POST ENTRY QUARANTINE JOURNEY

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The ability to detect all viruses and viroids present in plants undergoing post-entry quarantine is the 'holy grail' for both plant health scientists and regulators alike. For the Australian Government Post Entry Quarantine (PEQ) facility, the aspiration has become a reality after a near-decade long journey from proof-of-concept and validation through to operationalisation. In December 2022, small RNA sequencing and VirReport bioinformatics were deployed as the primary screening tool for virus and viroid detection in imported Prunus, Rubus, Fragaria and clonal grass species at PEQ. The outcomes from this deployment include the potential to reduce quarantine lag times, thousands fewer PCR tests per year, reduced use of biological indexing, and increased capacity for higher volumes of plant imports as a result of increased availability of glasshouse bench space. We anticipate that adopting high throughput sequencing (HTS) will enable plant industries to remain competitive and access more rapidly emerging high-value market opportunities.

APPLICATION OF HIGH-THROUGHPUT SEQUENCING (HTS) FOR ROUTINE PLANT VIRUS DETECTION IN THE SOUTH AFRICAN CITRUS IMPROVEMENT SCHEME

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The South African Citrus Improvement Scheme (CIS) is responsible for the production of pathogen-free propagation material for the southern African citrus industry. The CIS provides certified budwood and seed to local and international nurseries to produce trees. Budwood are produced under strict phytosanitary guidelines and multiplication trees are regularly re-tested. Before admission into the CIS new accessions are subjected to pathogen elimination (heat therapy and shoot-tip grafting) and rigorous testing for viruses and viroids. To reduce the timeline without an increase in risk, a high-throughput sequencing (HTS) detection assay was validated for implementation in the CIS. The credibility of HTS as pathogen detection assay was measured using specific parameters, including repeatability, specificity, sensitivity, and reproducibility. The sensitivity of the HTS assay was compared to routinely used RT-PCR assays in a time course experiment. Controls were introduced at sampling and data analyses levels. Expectedly, both extraction method and sequencing platform resulted in significant differences between the data sets. However, even though the limit of detection of HTS was influenced by pathogen concentration, sample processing method and sequencing depth, HTS detection in this study was found to be equivalent or more sensitive than RT-PCR.

THE ADDED VALUE OF PREPUBLICATION HTS DATA SHARING FOR THE DISCOVERY AND CHARACTERIZATION OF A NEW POTATO TORRADOVIRUS

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High throughput sequencing (HTS) is a powerful technology for the detection of plant viruses. In 2014, the U.S. Customs and Border Protection intercepted potato tubers originating from South America. In 2016 the Netherlands Food and Consumer Product Safety Authority also intercepted potatoes from S. American origin. In both cases these samples were subjected to HTS and viral contigs most similar to torradoviruses were identified. These contigs showed high similarity to an isolate of an undescribed isometric virus, coded SB26/29, which has been previously associated with a disease named potato rugose stunting in southern Peru. Tentatively the virus was named potato rugose stunting virus (PotRSV). Additional sequences obtained from cultivated potatoes in Peru, as part of the CIP potato virome project confirmed that all of these isolates from US, Peru, and the Netherlands belong to PotRSV. PotRSV like other torradoviruses has two polyadenylated RNA segments. RNA1 ranges between 7,086-7,089 nt and RNA2 from 5,228 to 5230 nt. The closest torradovirus species to PotRSV is squash chlorotic leaf spot virus sharing 41% (query coverage 48%) identity at the amino acid level. The prepublication data sharing enabled us to combine our efforts and with compiled data we tracked potential high risk virus movement out of its exotic range. The benefit is a better understanding of the virus genomic diversity and the ability to develop reliable detection assays for this new virus.

Abstracts of posters (numbered)

P1: HIGH-THROUGHPUT SEQUENCING IDENTIFIES CO-INFECTION OF TURNIP YELLOWS VIRUS AND ASSOCIATED RNAS IN SWEDISH OILSEED RAPE

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In autumn 2018, the presence of green peach aphids (Myzus persicae) in southern Sweden presented a risk for infection of winter oilseed rape (OSR; Brassica napus) with turnip yellows virus (TuYV; genus Polerovirus, family Solemoviridae). Therefore, a survey was carried out in spring 2019 with random leaf samples from 46 OSR fields in southern and central Sweden using DAS-ELISA. TuYV was detected in all fields except one, and in the counties of Skåne, Kalmar and Östergötland, the average incidence of TuYV-infected plants was 75% and reached 100% for nine fields. Analyses of the CP gene of nine Swedish TuYV isolates revealed highest identity to TuYV isolates from the UK. One OSR sample was selected for high-throughput sequencing using rRNA-depleted total RNA and Illumina technology. The assembled TuYV sequence of 5661 nt covered the complete genome except for the terminal ends. A phylogenetic analysis showed a close relationship between the Swedish TuYV isolate from OSR and European isolates from pea. In addition, near full-length sequences were obtained for TuYV-associated RNA (TuYVaRNA; 2841 nt) and TuYVaRNA2 (2795 nt), which both shared highest nt identity with German pea isolates of TuYVaRNAs. Similar to other polerovirus-associated RNAs, the two TuYVaRNAs had two ORFs encoding proteins for replication. Potentially, co-infection with TuYVaRNAs could increase the severity of disease and more studies are required to study their incidence and effect on crop plants.

P2: METATRANSCRIPTOMICS APPROACH TO STUDY THE VIROME OF ECONOMICALLY IMPORTANT CROPS

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The use of high throughput sequencing (HTS) has expanded our perspective on the distribution and diversity of plant viruses. Furthermore, due to the increasing number of versatile and improved HTS technologies and the decrease in the cost per sample, implementing HTS has facilitated the diagnosis and discovery of novel viruses. This study aimed to examine the putative virome of economically important crops. Leaf samples were collected from vegetables, ornamentals, and row crops. Information on different nucleic acid extraction methods for HTS, including double-stranded RNA and total RNA, will be presented. Library preparation was performed from pooled samples before sequencing in an Illumina platform. The sequenced libraries were mapped to the host's reference genome, and the resulting sequences were de novo assembled. Both nucleic acid extraction methods successfully yielded sequences of good quality. A metatranscriptomics analysis revealed complete genome contigs of a variety of known and unidentified putative RNA and DNA plant viruses co-infecting the same host. The information obtained in this investigation will help develop a broader perspective on other viruses present in the tested plant species to determine whether co-infections with other viruses are a factor that might influence (negatively or positively) plant physiology, product quality, and yield.

P3: DATA MINING-BASED DISCOVERY OF NOVEL TOBAMOVIRUSES AND VIRUSES ASSOCIATED WITH MACROPHYTES

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The search for new viral sequences provides an opportunity to improve early detection of pathogens, and to predict viral hosts and environmental reservoirs. Accelerated use of high-throughput sequencing has led to an increased amount of publically available sequencing data, which often remains unexplored. Here we present two distinct examples of how data mining can be used either to search for sequences belonging to a group of viruses in various samples or to identify novel viruses associated with a certain group of host organisms. Firstly, we searched for known and novel tobamoviral sequences in a set of published sequence datasets, selected based on Serratus infrastructure. Preliminary results show that novel tobamovirus-like sequences can be found in diverse sets of environments. For novel tobamovirus-like sequences, we presented associations with source environments (e.g., wastewater, human gut) and viral hosts (e.g., sugarcane) and performed phylogenetic analyses. Secondly, we mined publically available transcriptomes of aquatic plants (macrophytes) for viral sequences, utilizing data from the 1000 Plant Transcriptomes Initiative (1KP). Similar experiments have previously revealed the presence of new viral species in a water moss, and a perennial creeping herb. Likewise, we have found that many of the analysed macrophyte transcriptome data sets contain known and novel plant viral sequences, including crop pathogens.

P4: INCIDENCE OF VIRUS INFECTING TOMATO AND PEPPER PLANTS AND SEEDS IN SOUTHEAST ASIAN COUNTRIES BY METATRANSCRIPTOMICS

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Virus infection caused by insect vector from Southeast to East Asia is one of important constraints to the production of solanaceous crops, such as tomato (*Solanum lycopersicum*) and peppers (*Capsicum* spp.). However, limited information is available for incidences of viruses in vegetable crops in many Southeast Asian countries. Here, we report metatranscriptomics using RNA sequencing followed by bioinformatics analyses to determine viruses and viroids in solanaceous plants and/or seeds from Asian countries, including Vietnam, Cambodia, Laos, Thailand, and Indonesia. We prepared a total of 21 libraries from virus infected pepper and tomato plants derived from different geographical regions in Vietnam, Lao PDR, and Cambodia. Also, we collected commercial or informal tomato and pepper seeds from several Asian countries and prepared 17 libraries in this study. We identified a total of 1,008 virus-associated contigs, which were assigned to 33 different virus species belonging to 13 different viral genera. In commercial pepper seeds, pepper cryptic virus 2 and pepper vein yellows virus were frequently detected. Multiple viral infections were detected in common in both plants, moreover, geographical region and host plant were two major factors to determine viral populations in Vietnam samples. Thus, our results provide the comprehensive overview of viral pathogens infecting two economically important plants in the family *Solanaceae* grown in Asian countries.

P5: BIOVALON: A BIOINFORMATICS PIPELINE ADAPTATION AND VALIDATION FOR PLANT VIRUS DETECTION USING HTS METHOD.

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Due to important and rather frequent EU and French regulation evolution, the Anses Quarantine Unit needs to develop targeted and non-targeted tests to detect plants pests. The BioValON project aims to develop a bioinformatics pipeline, part of a HTS test, to detect simultaneously all viruses and viroids in plants in quarantine.
Datasets obtained from total ribodepleted RNAs using Illumina sequencers were used: serial dilutions of virus-infected potatoes (analyzed with another pipeline in 2018) and potentially healthy or known to be infected Citrus, Prunus and Vitis.
The pipeline, resulting from the PrepMedVet project (Anses- Ploufragan), is divided into five steps: quality control, plant's sequences subtraction, regulated pests detection, other pests detection and unassigned reads identification. To validate the pipeline, five objectives were foreseen: successive and correct execution of the different steps, similar detection level obtained with data processed in 2018, negligible plant host remaining reads, validated interpretation criteria for viruses and viroids and identification confirmation of all expected pests.
The three first objectives have been achieved. For the analyzed samples, the expected pests (viruses and viroids) were detected and identified reliably. The interpretation criteria establishment and validation is still underway, notably to evaluate cross contaminations. The results are promising and give hope in the pipeline validation.

P6: DISCOVERY OF THREE NOVEL SPECIES OF CARLAVIRUS AND ONE POTEXVIRUS IN MIXED INFECTION IN HIBISCUS ROSA-SINENSIS IN COLOMBIA USING HIGH THROUGHPUT SEQUENCING

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The genus Hibiscus consists of numerous species of herbs, shrubs, and trees. Although hibiscus is not native to Colombia, it is well suited to its arid soil and dry climates which makes it ideal for cultivating in gardens and grows wild in tropical rainforests. Plant viruses in the genera Betacarmovirus, Begomovirus, Cilevirus, Higrevirus, Ilarvirus, Soymovirus, and Tobamovirus have been reported to infect hibiscus. Hibiscus is a well-known host for Brevipalpus transmitted viruses (BTVs) and, during surveys in the citrus growing regions in Colombia, several hibiscus samples were collected for virus testing. One of the hibiscus leaf samples from Risaralda, showing black spots on upper and lower sides, was selected for virome analysis using High-throughput sequencing (HTS) followed by bioinformatic analysis. BLASTn/BLASTx searches of assembled contigs revealed three novel sequences resembling carlaviruses and one presumed potexvirus. In addition, several contigs of Betacarmovirus, Cilevirus, Nepovirus, and Tobamovirus were also identified. All four novel viruses shared less than 70% nucleotide and 50% amino acid identities with each other and virus sequences available in the GenBank. To confirm the presence of Nepovirus and novel species of carla- and potexviruses in the same sample, RT-PCR specific primers were designed, and amplified products were sequenced. To our knowledge, this is the first report of carla-, nepo-, and potexvirus infection in hibiscus.

P7: A NOVEL AUTOMATED WORKFLOW FOR PLANT VIRUS DIAGNOSTICS FROM HIGH-THROUGHPUT SEQUENCING DATA

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Several workflows have been developed for the diagnostic testing of plant viruses using high-throughput sequencing methods. Most of these workflows require considerable expertise and input from the analyst to perform and interpret the data when deciding on a plant's disease status. The most common detection methods use workflows based on de novo assembly and/or read mapping. Existing virus detection software mainly uses simple deterministic rules for decision-making, requiring a certain level of understanding of virology when interpreting the results. This can also result in inconsistencies in data interpretation between analysts which can have serious ramifications. To combat these challenges, we developed an automated workflow using machine-learning methods, which can decrease human interaction while increasing sensitivity, specificity, and consistency. Our workflow involves three steps: sequence data mapping, feature extraction, and machine learning model training. Using real data, we compared performance of our method with other popular approaches, and show our approach increases sensitivity and specificity while decreasing the detection time for most types of sequencing data.

P8: IDENTIFICATION AND CHARACTERIZATION OF RESISTANCE-BREAKING BARLEY YELLOW MOSAIC VIRUS AND BARELY MILD MOSAIC VIRUS ISOLATES IN GERMANY

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Barley yellow mosaic virus and barley mild mosaic virus cause the yellowing disease in barley and lead to yield losses of 50% in infested fields. The soil-borne vector *Polymyxa graminis* transmits both viral species and forms resting spores containing infectious virus particles. Noteworthy, against *P. graminis* no environmental or economical useful treatment is known. To avoid crop losses due to the two virus species, resistant barley cultivars are used by farmers. Interestingly, even by using cultivars carrying known resistant genes, plants can be found to show virus symptoms and are tested positive in ELISA for either one or both viruses. The aim of this newly started project is to identify resistance-breaking virus-isolates by using field trials and then to characterize the virus isolates by high throughput sequencing. We hope to find characteristic changes in the virus-isolates and want to use this information to further monitor the presence of these resistant breaking virus-isolates in fields in Germany.

P9: EXPLORING PRACTICAL APPLICATIONS OF METABARCODING WITH MINION TO SUPPORT THE SURVEILLANCE OF THE PHLOEM BACTERIA "CANDIDATUS PHYTOPLASMA" AND "CANDIDATUS LIBERIBACTER"

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"*Candidatus* Phytoplasma" and "*Candidatus* Liberibacter" are non-culturable, wall-less phloem bacteria linked to various plant diseases. Some species are quarantine pathogens while others are regulated non-quarantine pathogens (RNQPs). Hence, a fast detection and correct identification is key for both farmers and authorities. In currently available methods, after detection, additional molecular analyses are needed to identify the correct species and its haplotype or (sub)group. Moreover, both pathogens are known to occur together sometimes making detection and identification dependent on several PCR assays.
In the METAMINSURV project, MinION metabarcoding targeting multiple loci will be evaluated to combine broad detection and identification of both types of phloem bacteria in one test. In silico analyses will be done to evaluate and select barcode(s) and primers to be able to target and distinguish as many species, haplotypes and/or (sub)groups as possible. Mock and spike communities will be prepared by mixing DNA, plant or insect samples with gBLOCKs from known species in different concentrations. MinION metabarcoding protocols and data analysis pipelines will be optimized. Besides phloem bacteria, another case study on fungal forest pathogens from spore traps or seeds will be evaluated. METAMINSURV will hence provide insights in the usefulness of MinION metabarcoding for the detection and identification of these pathogens in terms of cost, speed, sensitivity and specificity.

P10: APPLICATION OF NANOPORE SEQUENCING AS A VIRAL DIAGNOSTIC TOOL: THREE ACCESSIONS, THREE OUTCOMES

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Traded plants for planting must be free from harmful systemic pathogens which may determine economic damages in the considered species or behave as a reservoir for further host change and crop infection.
In this context, we evaluated the sanitary status of a *Jasminum officinalis*, a *Ficus carica* and a *Solanum lycopersicum* using the nanopore MinION device (Oxford Nanopore Technologies). A cDNA-PCR, cDNA and RNA-direct sequencing protocols were used to sequence dsRNA or rRNA-depleted total RNA extracted from leaf tissues from *Jasminum* and fig. In the case of tomato, a targeted approach, with the use of virus complementary primers for the cDNA synthesis was tested. The ONT produced long-reads allowing the identification of a carlavirus (Jasmine virus C), two closteroviruses (fig virus A and fig virus B) for *Jasminum* and fig, respectively, while the full-length genome reconstruction of tomato brown rugose fruit virus and pepino mosaic virus was possible for tomato. As per other high-throughput sequencing technology, the ONT demonstrated to be suitable as an early and generic diagnostic tool before symptom onset or in symptomless plants. However, the different approaches tested produced non-comparable results in terms of coverage and number of viral reads, suggesting the need of further improving the available protocols to be more suitable for plant specimens.

P11: DEVELOPMENT OF PCR-BASED ASSAYS FOR GRAPEVINE BADNAVIRUS 1 USING HTS DATA OBTAINED FROM INFECTED GRAPEVINES

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Grapevine badnavirus 1 (GBV-1; family *Calimoviridae*, genus *Badnavirus*) was detected for the first time in 2018 by high-throughput sequencing (HTS) from Croatian autochthonous grapevine varieties 'Ljutun' and 'Vlaška'. Recently, a screening of the grapevine virus collection (University of Zagreb Faculty of Agriculture) by HTS confirmed the presence of GBV-1 in four additional grapevine accessions. The collected HTS data were used to select the genomic region for primers and probe construction and to develope robust detection assays based on end-point polymerase chain reaction (PCR) and real-time PCR (qPCR). The qPCR showed 100-fold higher sensitivity compared to end-point PCR, regardless of the method of nucleic acids isolation (column-based from Qiagen or GES). Both detection methods (qPCR detectability down to a dilution of 1:10,000,000 and end-point PCR of 1:10,000) were more sensitive with nucleic acids isolation by column-based method than with GES (1:100,000 and 1:100, respectively). Using GES GBV-1 was efficiently detected by qPCR throughout dormancy and most of the growing season, with false negatives at the beginning of vegetation (April/May). The survey, conducted in 93 commercial vineyards and five grapevine collections on 4,327 vines, found that GBV-1 infections occurred only in autochthonous grapevine varieties with an overall infection rate of 13.4% and infections at specific sites/vineyards ranging from 1.9% to 96%.

P12: QUADS INTER-LABORATORY EVALUATION OF HIGH THROUGHPUT SEQUENCING METHODS FOR PLANT VIRUS DETECTION

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Members of the Quadrilateral (QUAD) Working Group for Diagnostic Tools Collaboration, organized an inter-laboratory exchange to evaluate high throughput sequencing (HTS) methods for plant virus detection. A set of Prunus and Malus freeze-dried leaf samples were sent to each participating laboratory. Participants performed multiple nucleic acid extractions and sequencing of each sample using method(s) of their choice. Total, small and double stranded RNA extraction methods were represented. The datasets were evaluated for reproducibility and accuracy using several bioinformatic workflows. The individual laboratories analysed the datasets using their own bioinformatic workflows, then all four country data sets were analysed using an independent bioinformatic workflow. While each sequencing method was able to detect the viruses and viroids present, indicating that HTS is an effective screening method for plant virus diagnostics, some differences were observed and noted. While the amount of sample background or contamination was not fully evaluated, it was present to various degrees in many of the data sets and the patterns were unique to each laboratory. The exception, however, was a consistent low level of contamination in one sample detected across all the labs. These results indicate the importance of appropriate QC standards (control) and protocol validation and confirmation of HTS virus detections with a secondary test method.

P13: TRANSCRIPTOME SEQUENCING ANALYSIS AND FUNCTIONAL VERIFICATION REVEALED THE ROLE OF EXOGENOUS MAGNESIUM IN TOBACCO ANTI-PVY INFECTION

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Potato virus Y (PVY) infection causes necrosis and curling of leaves, which seriously affect the yield and quality of Solanaceous crops. The role of nutrient elements in the regulation of plant resistance to virus infection has been widely reported. Previous studies in our laboratory have demonstrated that foliar spraying of MgSO4 could induce Nicotiana tabacum resistance to PVY infection by increasing the activity of defense-related enzymes. Consistent with the results, we found that exogenous magnesium (Mg) had a certain effect on Nicotiana tabacum anti-PVY infection. Meanwhile, Illumina RNA sequencing revealed that Mg induced resistance to PVY infection mainly by regulating carbohydrate metabolism and transportation, nitrogen metabolism, Ca2+ signal transduction and oxidative phosphorylation. Furthermore, we used TRV vector to verify the function of homologs of five Nicotiana tabacum genes involved in above pathways in Nicotiana benthamiana. The results showed that NtTPS and NtGBE were the key genes related to PVY infection, NtPPases and NtNiR were related to resistance to PVY infection, while NtCML36 did not play a role in PVY infection. Our transcriptome database and candidate genes functional verification suggested a novel strategy for resistance to PVY infection and provided a theoretical basis for virus-resistance breeding.

P14: CHARACTERIZATION AND FUNCTION ANALYSIS OF SCMV-DERIVED VSIRNAS IN MAIZE RESISTANT AND SUSCEPTIBLE INBRED LINES

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RNA silencing plays an important role in plant antiviral responses, which triggered production of virus-derived small interfering RNAs (vsiRNAs). Sugarcane mosaic virus (SCMV) infection causes serious economic losses worldwide in maize (*Zea mays* L.). In this study, the maize inbred lines Chang7-2 (resistant to SCMV) and Mo17 (susceptible to SCMV) were inoculated with SCMV (SC, SM) and phosphate buffer (MC, MM), respectively. The systemically infected leaves were harvested to perform whole-transcriptome RNA-seq and degradome sequencing. The results showed that the distribution of vsiRNAs on the sense strand of SCMV genome was higher than that on the antisense strand in both SC and SM libraries. The accumulation level of 21-nt vsiRNAs was higher in SM libraries, while more 22-nt vsiRNAs were accumulated in SC libraries. Through degradome sequencing, we identified 706 transcripts targeted by 204 vsiRNAs. The competing endogenous RNA (ceRNA) networks in SCMV-infected Chang7-2 and Mo17 were constructed and verified. Our results also showed that the transcripts of *DCLs*, *AGOs* and *RDRs* were differentially accumulated in resistant and susceptible maize plants after SCMV infection, which were associated with the production and function of vsiRNAs. These findings provide novel insights into the relationship between SCMV-derived vsiRNAs and potential ceRNA networks in resistant and susceptible maize materials.