



Charting the course of plant virology: innovations in diagnostics and beyond—reports from the DPG meeting

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Abstract

Plant viruses pose a significant threat to global agriculture, resulting in economic losses and threatening food security. High-throughput sequencing and molecular techniques are advancing our understanding of these viruses, allowing for their detection, characterization, and control. The 55th meeting of the DPG Working Group “Viruskrankheiten der Pflanzen” showcased these advancements, encouraging international collaboration and idea exchange to tackle plant virus challenges. The perspectives paper we present summarizes this conference’s findings, highlighting the potential of these technologies in revolutionizing plant virus research.

Keywords Plant virology · Plant virus

Introduction

Plant viruses are a significant threat to global agriculture, causing substantial yield losses and economic impacts. These pathogens can infect a wide range of economically important crops, resulting in reduced productivity, compromised food security, and detrimental effects on trade. To effectively manage and mitigate the impact of plant viruses, it is crucial to develop innovative strategies for their

detection, characterization, and control (Jones and Janssen 2021; Jones and Naidu 2019).

High-throughput sequencing and molecular techniques have emerged as powerful tools in the quest to tackle plant virus-related challenges (Massart et al. 2017). These methods offer unparalleled insights into the discovery of previously unknown viral species, the genetic and molecular basis of plant-virus interactions, enabling the identification of novel resistance genes and the elucidation of the mechanisms underlying viral pathogenicity. Moreover, these technologies allow for the rapid and comprehensive analysis of complex plant-virus communities (Rumbou et al. 2021), providing valuable information for the development of targeted and effective disease management strategies. The 55th meeting of the German Phytomedical Society/Deutsche Phytomedizinische Gesellschaft (DPG) Working Group “Viruskrankheiten der Pflanzen” marked a significant milestone in the field of European plant virus research, bringing together approximately 100 experts in person and an additional 25–30 participants online. The meeting was planned as joint meeting with the colleagues from the Netherlands but also welcomed participants from other European countries and counted participants from Spain, France, and Italy. The event showcased cutting-edge innovations and the latest findings in the rapidly evolving world of plant virology. Conducted in English to foster international communication and collaboration, the conference provided an excellent platform for researchers to share their knowledge, exchange ideas, and

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discuss new approaches to address the challenges posed by plant viruses.

The keynote speakers at the conference, Richard Kormelink (Siskos et al. 2023; van Grinsven et al. 2022; Xu et al. 2022) from the University of Wageningen, the Netherlands and Friedrich (Fritz) Kragler (Kehr et al. 2022; Yang et al. 2019; Yang et al. 2023a, b) from the Max Planck Institute of Molecular Plant Physiology in Potsdam, Germany, provided valuable insights into the molecular mechanisms behind plant-virus interactions and the systemic responses of plants to viral infection. Kormelink's presentation focused on the molecular basis of plant-virus interactions, with specific emphasis on the mode of action of different resistance genes.

Fritz Kragler discussed his research on the intercellular and long-distance transport of viral and plant endogenous macromolecules, contributing to the understanding of how plants coordinate systemic responses to viral infection. Plant virologists can learn from the presentations of these keynote speakers in terms of new strategies for managing plant viruses, novel mechanisms for engineering resistance to viral infection, and the development of more effective and sustainable approaches to agriculture.

This perspectives paper will discuss the recent advancements and applications of next-generation sequencing and molecular techniques in plant virus research, highlighting their potential to revolutionize our understanding of plant-virus interactions and transform the way we approach plant virus detection, discovery, and control. By showcasing the latest findings and innovations from the 55th meeting of the DPG Working Group "Viruskrankheiten der Pflanzen," we aim to provide a comprehensive overview of the current state of the field and to identify future research directions and opportunities for collaboration. As we continue to confront the challenges posed by emerging plant viruses, events like the 55th meeting of the DPG Working Group "Viruskrankheiten der Pflanzen" will play a crucial role in shaping the future of agriculture and plant health. By leveraging the power of high-throughput sequencing and molecular techniques, we can work together to improve the resilience of our agricultural systems, safeguard global food security, and promote a more sustainable future for our planet. The conference featured a diverse range of presentations and discussions that highlighted the advancements in high-throughput sequencing and other molecular techniques in plant virus research. The talks covered a wide range of topics related to plant virus research, such as the discovery of novel viral species, the development of serological test methods for detecting plant viruses, and the epidemiology and control of emerging plant viruses. There were also discussions on the importance of interdisciplinary collaboration and data sharing in developing effective strategies for managing plant viruses. The speakers highlighted the need for open and transparent communication among researchers, industry,

and policymakers to effectively tackle emerging plant virus threats. The conference provided an excellent platform for researchers from different parts of Germany and Europe to share their experiences and collaborate on developing new approaches to managing plant viruses.

Plant virus diagnostics—a new era

Plant virus diagnostics have evolved significantly, resulting in more accurate and faster detection. The gold standard for virus detection has traditionally involved comprehensive molecular techniques, however, their cost and complex requirements underscore the necessity for more accessible and affordable alternatives. Traditional diagnostic tools like enzyme-linked immunosorbent assays (ELISA) and lateral flow devices, together with PCR-based molecular approaches such as real-time quantitative PCR and loop-mediated isothermal amplification, remain central to routine screening and field diagnosis (Mehetre et al. 2021; Wang et al. 2022). Their performance is continuously refined, and newer versions of these techniques are constantly being developed for increased automation and field applicability. Metagenomic approaches hold promise for unveiling the complexity of viral interactions, suggesting the need for further in-depth exploration and data analysis. High-throughput and emerging technologies, particularly nanopore technology, which allows sequencing of single nucleic acid molecules, are gaining prominence, pushing us toward real-time, high-throughput virus detection (Sun et al. 2022). The scope of these new technologies in decoding copious data, predicting virus spread, and devising effective strategies is immensely promising. The future of plant virology will heavily rely on the integration and collaboration of diverse diagnostic methods. Fostering interdisciplinary collaboration and unifying different diagnostic approaches can lead to the development of advanced disease management strategies. This evolving trend was highlighted at the 55th DPG Working Group meeting, showing how we are progressing toward a new era in plant virology.

High-throughput sequencing and "omics" techniques: pioneering the future of plant virus discovery and resistance

High-throughput sequencing (HTS) and "omics" techniques, encompassing RNA-seq transcriptomics, metagenomics, and proteomics, have catalyzed a paradigm shift in plant virus research. These cutting-edge technologies empower researchers to investigate plant-virus interactions with unparalleled depth and resolution. They have facilitated the discovery of novel resistance genes, previously unidentified viral species, and molecular mechanisms that govern plant-virus interactions, thereby transforming our understanding

of plant virology. Here, we highlight recent advances and case studies from the 55th meeting of the DPG Working Group “Viruskrankheiten der Pflanzen”, featuring talks by Paolo Margaria, Ali Pasha, Michael Hagemann, Khalid Amari, and Jessica Lilienthal.

The importance of HTS and “omics” techniques in both basic and applied plant virus research cannot be overstated. HTS is not only currently cutting-edge but continues to provide foundations for forward-looking research. By providing deeper insights into the biology, evolution, and molecular mechanisms of plant-virus interactions, these techniques enable researchers to develop more targeted, effective, and sustainable strategies for the prevention, management, and control of plant viruses. For example, understanding the molecular basis of plant resistance to viruses can help breeders develop new crop varieties with improved resistance to viral diseases. Additionally, the identification of novel viral species allows better knowledge of virus threats and the timely development of adequate diagnostic tools, which in turn can help prevent the spread of viral pathogens and reduce the economic impact of plant virus outbreaks. Paolo Margaria’s work underscores both the challenges and opportunities associated with employing HTS for virus discovery in plant materials from diverse provenances. His research emphasizes the necessity of optimizing workflows—from sample and library preparation to bioinformatics analysis—to guarantee reliable and efficient virus detection. Putting into place workflows and pipelines for virus discovery using HTS, empowers scientists to easily adapt this new technology and progress in their respective research fields (Kutnjak et al. 2021).

Ali Pasha’s investigation of the German hop virome uses high-throughput sequencing to explore virus diversity in various hop-growing sites, uncovering the presence of common hop viruses/viroids and of non-hop viruses/viroids in German hops. One focus of the study is on citrus bark cracking viroid, a new threat to hop plants in Germany (<https://gd.eppo.int/reporting/article-7300>). The generated knowledge can be applied to develop targeted disease management strategies and informed breeding programs for virus-resistant hop cultivars. Michael Hagemann’s research on rapid in silico pathogen detection in high-throughput sequencing data for hop pathogenic viroids showcases the potential of web-based tools, such as MiFi®, for swift and user-friendly pathogen identification. This is particularly important for applied research, as early detection of viral pathogens can help farmers to implement control measures to minimize crop losses. However, before fast in silico detection pipelines can become a standard in pathogen detection, they need to be established for several pathogens and require further drops in sequencing costs, higher computing capacities, and safe data upload. Khalid Amari’s work combines laser-assisted microdissection with RNA-seq to delve into the early events

during plant-virus interaction in turnip mosaic virus-infected *Arabidopsis thaliana* plants, demonstrating the potential of integrating state-of-the-art techniques to gain a comprehensive understanding of plant-virus dynamics. The obtained data in unprecedented resolution will reveal processes important during early virus infection and identify novel candidates limiting virus infection. This knowledge can be applied to develop new antiviral strategies and inform breeding programs for virus-resistant crops. Lastly, Jessica Lilienthal’s transcriptome analysis of cassava brown streak virus-infected cassava plants underscores the power of transcriptomic studies to pinpoint potential resistance genes and elucidate the molecular mechanisms underlying plant-virus interactions. This information can be used to guide breeding efforts and develop targeted control strategies to combat cassava brown streak virus, a major threat to cassava production in Africa. These innovative approaches, along with numerous others presented at the conference, will pave the way for more effective, sustainable, and environmentally friendly strategies for the prevention, management, and control of plant viruses. Ultimately, this will ensure the health and productivity of our global agricultural systems, safeguarding food security and promoting a more sustainable future for our planet.

Molecular mechanisms of plant-virus interactions: implications for disease management

Recent research has offered valuable insights into the molecular mechanisms that underlie plant resistance to viruses, as well as how viruses manipulate host machinery to facilitate infection. Such insights are essential for both fundamental and applied research in plant virology, as they can inform the design of targeted and durable disease management strategies. Richard Kormelink’s research on tomato yellow leaf curl geminivirus (TYLCV) resistance genes underscores the importance of understanding the different types of resistance genes and their mechanisms. His presentation explores dominant resistance genes like Ty-2, which belong to the nucleotide-binding site leucine-rich repeat (NLR) class, and recessive resistance genes like ty-5, which code for *pelota*, an mRNA surveillance factor. While dominant NLR resistance genes are pathogen-specific and can be easily overcome by a new pathogen variant, atypical dominant and recessive resistance genes often provide more durable and broad resistance. This knowledge can be harnessed to develop more robust disease management strategies against geminiviruses. Delphine Pott’s research on geminiviruses and their manipulation of the plant RNA splicing machinery highlights the significance of alternative splicing in viral infection. Geminiviruses associate with the host splicing machinery and require a functional spliceosome for successful infection (Wang and Lozano-Durán 2023). By

understanding how geminiviruses manipulate host RNA processing mechanisms, researchers can identify novel targets for disease management strategies. In a similar vein, Omid Eini et al. investigated the splicing of complementary-sense transcripts of beet curly top Iran virus (BCTIV) and discovered that splicing is essential for the virus's pathogenicity and hypersensitive response (HR) in *Nicotiana benthamiana* plants (Ebrahimi et al. 2022). This finding suggests that understanding the role of splicing in geminivirus infections may offer new opportunities for controlling viral diseases. Jessica Gehrke and colleagues analyzed splicing in bipartite begomoviruses, such as Abutilon mosaic virus (AbMV), to explore their potential for increased coding capacity. They detected a deleted CP ORF and possibly a second DNA circle, which could have implications for understanding the viral replication process and identifying novel strategies to combat begomoviruses. Despite these advances in elucidating the molecular mechanisms of plant-virus interactions, challenges remain in translating this knowledge into practical applications. For instance, the diversity and complexity of plant-virus interactions can make it difficult to develop broadly effective strategies. Additionally, the rapid evolution of viruses and their ability to adapt to new resistance mechanisms presents a constant challenge to maintaining effective disease management.

Detection and control of emerging plant viruses using molecular techniques

The rapid emergence of new plant viruses poses a significant challenge to the agricultural industry. Early detection and control of these pathogens are essential for minimizing their impact on crop yield and quality. Molecular techniques have become increasingly important in detecting and controlling plant viruses, as evidenced by the presentations of Marius Rehanek, Christoph Kartheuser, Roxanna Hossain, and Pier de Koning during the conference.

Marius Rehanek and his team focused on the development of serological test methods for detecting novel emaraviruses in oak and ash trees (Rehanek et al. 2022). Emaraviruses are a group of plant pathogens that have been recently identified in various deciduous tree species worldwide. The team aimed to develop diagnostic ELISAs for the detection of common oak ringspot-associated virus and the ash shoestring-associated virus, two economically significant emaraviruses in oak and ash trees, respectively. They presented their initial results on the heterologous expression and purification of viral nucleocapsid proteins for immunization and obtaining polyclonal antibodies. Christoph Kartheuser from the DSMZ in Braunschweig investigated the presence of legume-infecting viruses on chickpea crops in Germany. Chickpea, a drought-tolerant legume, is gaining popularity in the country due to its nutritional benefits

and nitrogen-fixing abilities. Various RNA viruses were detected, including turnip yellows virus (TuYV) and pea enation mosaic virus-1 (PEMV-1) by reverse transcription-PCR (RT-PCR). Pea necrotic yellow dwarf virus (PNYDV, a nanovirus) was detected for the first time in symptomatic chickpea plants using a combination of rolling circle amplification (RCA) and downstream restriction fragment length pattern (RFLP) analysis.

These molecular techniques, however, also face challenges in the context of emerging plant viruses. For instance, the sensitivity and specificity of the tests may be affected by the rapid evolution of viral pathogens, which can lead to false negatives or false positives. Additionally, the availability of resources and the technical expertise required to perform these tests may be limited in certain regions. Despite these challenges, the conference presentations also highlighted potential novel solutions and approaches to improve the detection and control of emerging plant viruses. These include the development of new diagnostic tools, such as more sensitive and specific ELISAs, as well as the use of resistant cultivars to minimize the impact of viral infections on crop yield and quality.

In addition to the aforementioned molecular techniques, Roxanna Hossain and colleagues presented their work on the construction of a turnip yellows virus (TuYV) cDNA full-length clone. This clone originated from an isolate found in sugar beet plants, a previously considered non-host for TuYV. The cDNA clone was successfully constructed, and its infectivity in *Nicotiana benthamiana*, *Pisum sativum*, and *Brassica napus* was demonstrated. Results on the clone's ability to infect sugar beet plants are still pending. The cDNA clone and its complete genomic sequence can be utilized as valuable molecular tools for a better understanding of virus-host interactions, potentially helping in the development of disease control solutions and the identification of resistance sources against this emerging virus in sugar beet. Pier de Koning and his colleagues discussed their epidemiological insights using Nextstrain, focusing on the tomato brown rugose fruit virus (ToBRFV) first identified in the Netherlands in 2019. Real-time RT-PCR was employed for virus detection and confirmation, while whole genomes were assembled using Illumina RNA sequencing data. The whole-genome phylogenetics analysis, integrated with metadata such as host, variety, seed batch, and geographic location, suggested that multiple introductions might have caused the outbreaks in the Netherlands. The National Plant Protection Organization of the Netherlands maintains a publicly available interactive ToBRFV Nextstrain webpage that showcases data from the tracing research, along with genome sequences retrieved from NCBI GenBank or shared by international partners. This dataset can facilitate a better understanding of the global diversity and spread of ToBRFV. The integration of these additional findings emphasizes the

importance of molecular techniques in understanding and controlling emerging plant viruses. The development of full-length cDNA clones, as well as the use of Nextstrain for epidemiological insights, can contribute to the early detection, prevention, and control of these viruses, ultimately benefiting the agricultural industry and global food security.

Hygiene measurements and phytosanitary aspects

Disease management by hygiene measures and risks of mechanical infection through contamination were also discussed at the meeting. Referring to enormous yield losses caused by ToBRV, phytosanitary aspects were illuminated by Jens Ehlers, who presented the results of a study analyzing numerous samples taken on a ToBRFV-infected production site in Germany in 2022. The study showed that various surfaces of different areas including cultivation and packing as well as common and private rooms of workers were contaminated with the virus. Swap samples were analyzed in bioassays and ELISA. The proportion of ToBRFV contaminated surfaces varied among locations from 48.7% in greenhouses to 0% in offices with limited access to staff. Samples from shared accommodation and private accommodation were 18.4% and 3.6% ToBRFV positive, respectively. Clothing and protective items were found to be highly contaminated with ToBRFV. This study provides evidence for the first time to how and where infectious ToBRFV can be spread by humans beyond the production area. Hygiene protocols, including the use of disinfectants, can help to avoid further dissemination. In general, different methods are available to measure the efficacy of a disinfectant (Nourinejad Zarghani et al. 2023). Shaheen Nourinejad Zarghani presented a case study, in which MENNO Florades, an authorized plant protectant in Germany was tested and showed very promising results in the deactivation of ToBRFV on different surfaces. He investigated the applicability of quantitative ELISA, RT-qPCR, and bioassay to determine and confirm the virucidal efficacy of the disinfectant.

Virus monitoring is crucial to keep plants healthy. In Germany, the bulk of the phytosanitary work is conducted by the plant protection services of the federal states. As an example for the work of the plant protection services of the federal states, Marco Riedel from the LELF presented his work on phytosanitary studies in Brandenburg with specific focus on the testing of planting potatoes in 2022. With respect to a changing climate, Kevin Gauthier presented his research on the influence of environmental conditions on furovirus infection and the effectivity of resistance genes, which is crucial for the estimation of virus threat in the future.

Overall, the 55th meeting on plant viruses showcased a broad range of scientific research, from transcriptome analysis to epidemiological studies and the development of new diagnostic tools presented by enthusiastic speakers, and

their abstracts can be found here: https://plant-protection.net/fileadmin/user_upload/55_DPG_AK_Virologie_2023_Programmheft.pdf. Moreover, the meeting was enriched by an array of exceptional posters, insightful flash talks, and engaging online discussions, covering diverse topics such as plant virus detection, research on the virus status of ash trees, and innovative virulence strategies of the tomato yellow leaf virus, among others.

Conclusion

In conclusion, the 55th meeting of the DPG Working Group “Viruskrankheiten der Pflanzen” offered a comprehensive overview of the current state of plant virology and the future trends that are poised to shape the field. The presentations illuminated the importance of molecular mechanisms of plant-virus interactions, current and future diagnostic trends, and strategies for the detection and control of emerging plant viruses. Advanced techniques are crucial for accurate virus detection, with developments such as nanopore technology promising faster, in-field diagnosis. The role of artificial intelligence (AI) in our area will become increasingly important. From analyzing vast volumes of sequencing data to predict viral spread and designing novel antiviral strategies, AI is rapidly transforming our approach to plant virology. As we move forward, the potential for AI-driven diagnostic tools and the use of AI to predict and respond to emerging viral threats will be an exciting area to explore. In the wake of this wealth of knowledge and the increasing production of data, it becomes imperative to implement robust data management strategies. Ensuring the availability and accessibility of this data to the broader scientific community will foster enhanced collaborations and accelerate advancements in plant virology. Furthermore, harnessing the potential of existing data repositories can unearth new avenues for research, thereby contributing significantly to our understanding and control of plant viruses.

However, as the field evolves, we must not lose sight of its applied nature. The science we pursue in the laboratory must continue to find relevance in agricultural and horticultural practices. Therefore, it is critical to focus on current problems in practice, ensure the interplay between fundamental and applied research, and include scientists connected with practical aspects to a greater extent. Giving more prominence to reports from the field will provide invaluable insights that can guide future research endeavors. As we reflect on the 55th meeting, it is clear that plant virology is on the edge of a new era, one that blends cutting-edge technology with practical, solution-oriented science. This multi-disciplinary approach will be crucial in addressing the challenges posed by plant viral diseases and safeguarding

global food security. In the years ahead, we look forward to seeing the exciting developments this approach will bring.

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Informed consent Not applicable as this work did not involve human subjects.

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