The Latest Research on Grapevine Virology

Highlights of the 20th International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG) Meeting in Thessaloniki, Greece

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Meet the Authors: **Judit Monis** provides specialized services to help growers, vineyard managers and nursery personnel avoid the propagation and transmission of disease caused by bacteria, fungi and viruses in their vineyard blocks. Please visit *juditmonis.com* for information or contact *juditmonis@yahoo.com* to request a consulting session at your vineyard or virtually. **Nuredin Habili** is an honorary Fellow at The University of Adelaide and The Australian Wine Research Institute (AWRI). Nuredin would like to thank Affinity Labs of the AWRI for providing funding for the trip to Thessaloniki.

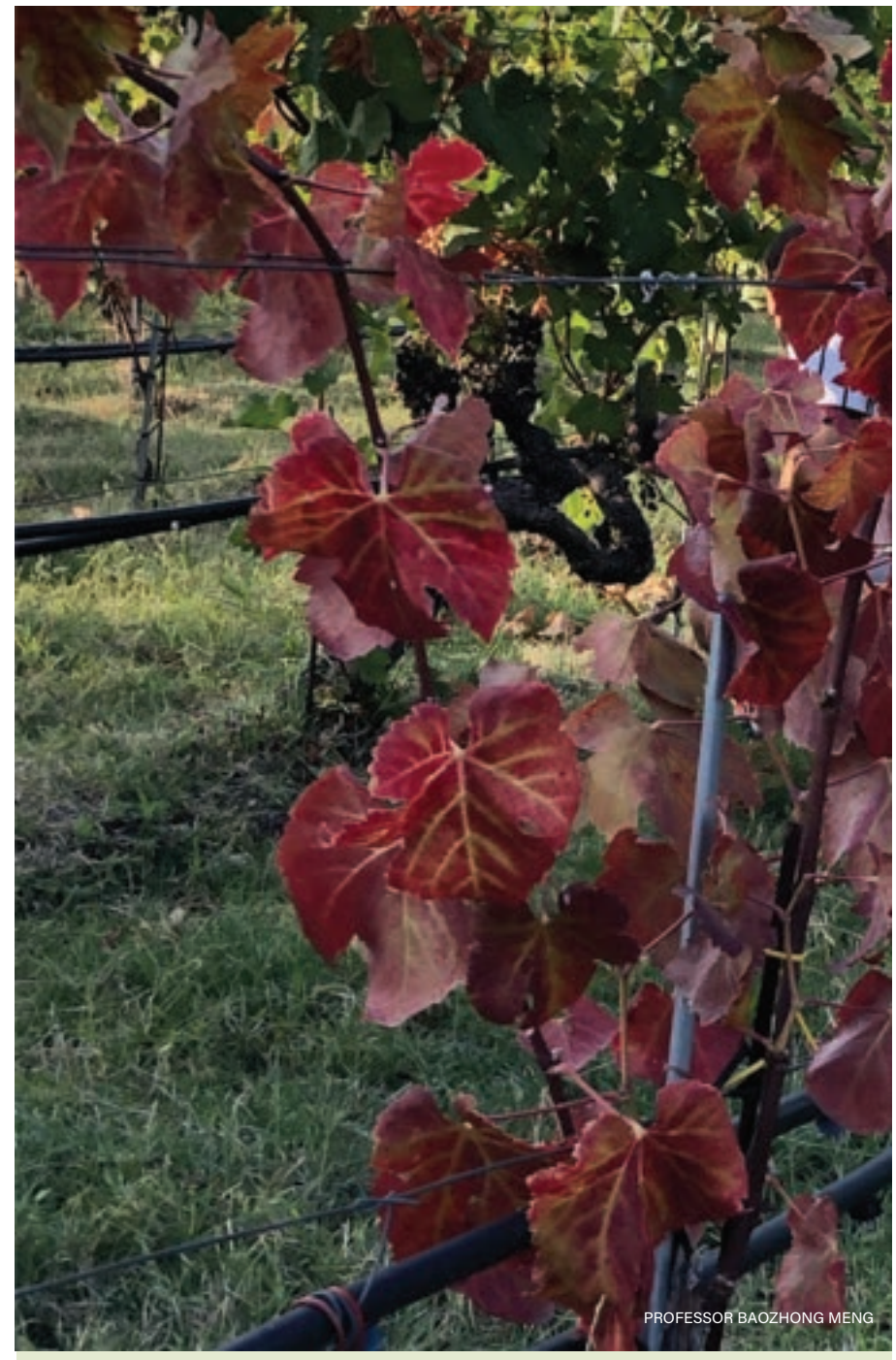

FIGURE 1 Symptoms of leafroll disease on the Greek variety Xinomavro at Kyr Yanni vineyard in Naoussa, Greece.

The 20th meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG) was held in Thessaloniki, Greece, Sept. 25-29, 2023.

The conference included a visit to the Vergina Archaeological site, showcasing the King Philip II Tomb, and a visit to Kyr Yanni Vineyard and Winery. We observed typical leafroll disease symptoms on the red Greek variety, Xinomavro, at Kyr Yanni Vineyard (**FIGURE 1**).

The ICVG meeting is usually held once every three years. The last ICVG meeting was held in Santiago, Chile in 2018, but due to the COVID-19 pandemic, the meeting had to be delayed. The next meeting will be held in New Zealand in 2026. The scope of ICVG is to promote collaboration and interaction between pathologists who specialize in viruses, viroids and phytoplasmas that infect grapevines.

The meeting in Thessaloniki was well attended, with 110 participants and six keynote speakers who highlighted specific research allocated to each session (**FIGURE 2**). The meeting had 44 oral and 48 poster presentations compiled through the efforts of research entities located across different grape growing regions of the world. The presentations were categorized into a total of six sessions: Diagnostics, Disease etiology, epidemiology, vine certification and other management approaches, phytoplasmas, virus characterization and diversity, and plant-virus and vector interactions.

A broad range of research and knowledge was presented. In this article we will describe some of the novel achievements discussed at the meeting. We were not able to cover all the research presented due to print space restraints. The reader is encouraged to find all the articles in the meeting abstract book: *www.icvg.org/proceedings.cfm*

New Findings on Known Viruses and Viroids

The meeting—which started with a moment of silence to honor former ICVG President, Professor Giovanni Martelli, who died in 2020—started with the keynote presentation of ICVG's president, Professor Marc Fuchs (Cornell University, USA). Fuchs presented updates and highlights of grapevine virology since the last meeting in 2018. To date, 101 viruses from 21 families have been reported in the grapevine. This count is higher than the number of viruses found in any other crop, however, not all are responsible for disease development. In the area of diagnostics, he referred to novel approaches, *e.g.,* using surface acoustic wave sensors, to detect fanleaf and leafroll viruses.

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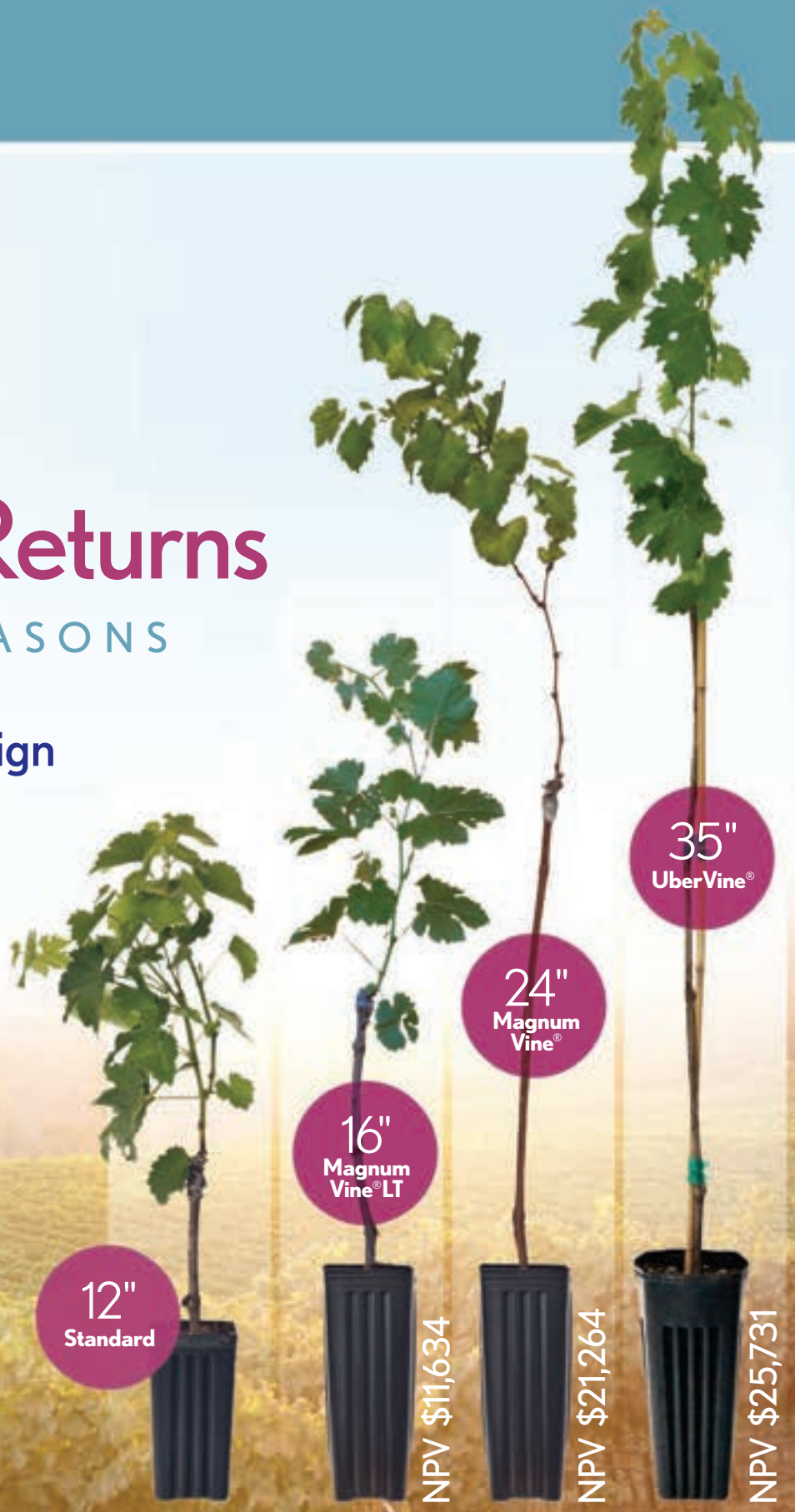

*Source: ASEV Catalyst Report funded by Wonderful Nurseries

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FIGURE 2 Delegates at the 20th ICVG meeting at Thessaloniki, Greece, held September 25-29, 2023.

Another novelty is research aimed to train dogs for the olfactory detection of grapevine leafroll and grapevine red blotch diseases. The second keynote speaker was Professor Stefanos Kounddouras of Aristotle University of Thessaloniki. He emphasized that due to global warming, the harvest in Greece now begins earlier. For instance, Cabernet Sauvignon was harvested 23 days earlier this year. This shift in timing could potentially disrupt vector activity and alter the rate of virus infections.

Disease Etiology and Epidemiology

In 2022 Grapevine Red Blotch Virus (GRBV) was found for the first time in different commercial vineyards in Australia. A survey was completed in an Australian germplasm collection to investigate the presence and potential spread of GRBV. The researchers detected GRBV in three different varieties by using a modified nested PCR. Surprisingly, there was an inconsistency with detecting the virus.

We wonder if the challenge of detecting GRBV (which Judit Monis has not had issues determining in California) might be due to Australian climatic conditions or how the samples are processed. Furthermore, reports from authorities in Australia note that the grapevine infections did not show typical red blotch symptoms.

In contrast, work in British Columbia by Jose Ramon Urbez-Torres' team reported typical symptoms in Canada. The virus was detected in cambial scrapings from dormant wood (fall/winter) or basal leaves collected in the summer. In California, the spread dynamics of GRBV were studied by Madison Fiasco (Cornell University) who performed yearly surveys of the presence of *Spissistilus festinus* (the vector of GRBV) and the distribution of infected plants in different Cabernet vineyard blocks. Despite the high infection rate in a Cabernet Sauvignon block, it was hypothesized that the spread of the virus was low due to a 10 times lower population of the insect vector compared to an adjacent Cabernet Franc block. Syrah decline is a disorder characterized by reddening of leaves with grooving and swelling of the graft union of certain Syrah clones. In the extremes, clone 383 is highly susceptible while clone 473 is reportedly resistant to Syrah decline.

Anne-Sophie Spilmont (IVES, France) presented data to prove that the Syrah decline disorder is caused by genetic characteristics and not by a pathogen. In an elegant study, clones that represented the two extreme Syrah decline sensitivities were self-pollinated. The F1 populations were grafted onto 110R rootstock. By the fifth year after grafting, typical Syrah decline symptoms were observed only in the plants derived from the sensitive clone at a Mendelian segregation rate of 1:3 (suggesting the presence of a dominant gene). In contrast, no symptoms were observed in the population derived from the "resistant" self-pollinated clone. In a poster presented by Darko Voncina (University of Zagreb, Croatia), experiments to transmit Grapevine Leafroll Associated Virus (GLRaV-3) by using the vine mealybug (*Planococus ficus*), as a vector, did not yield any positive infection in the 411 herbaceous and woody plant species tested. Vector transmission was only achieved through *Vitis* to *Vitis* species.

Diagnostics

The diagnostic session kicked off with a lecture by Hano Maree (Stellenbosch University, South Africa). Professor Maree discussed the consequences and responsibilities of using high throughput sequencing (HTS) for plant virus discovery. He noted that in grapevines, HTS has led to the discovery of economically important viruses, such as Grapevine Pinot Gris Virus (GPGV) and GRBV.

However, many viruses have been reported with no association with disease, notably, the discovery of many *Vitiviruses*, (e.g., Grapevine virus A, B, D, E, F, G, H, I, J, K, L, M , N, O), but only Grapevine virus A (GVA), GVB and GVD have been reported to be associated with rugose wood disease.

Clearly, more viruses will continue to be discovered by using this powerful technology, and science will need to provide biological data to help regulators decide which viruses should be kept out of planting material and which ones are considered innocuous.

Related to virus sequencing and discovery, Mamadou Fall (Université de Sherbrooke, Canada) described a modified double-stranded RNA isolation method that would allow the characterization of actively replicating viruses in fungi and plants. Bhadra Vemulapati (Brock University, Canada) presented work on the development of drop digital PCR (ddPCR) for the detection of GPGV that promises to be a sensitive technique to determine the virus copy number without needing a reference sample. Sudarsana Poojari from the same university spoke about the application of an amplification-free assay for the detection of GLRaV-3 by using CRISPER (Clustered Regularly Interspaced Palindromic Repeats) Cas13a. Michel Hilly (Université de Strasbourg France) presented work on the application of datamining to determine the historical

evolution of GPGV. The work showed that GPGV originated in Asia, with China likely being its country of origin.

Robin MacDiarmid (New Zealand Institute for Plant and Food Limited) presented her findings on a field visual detection of GLRaV-3 by using RBG (red, blue, green) photography and machine learning. In the study a detection model was developed by analyzing 26,000 field photographs of healthy and diseased (symptomatic) vines collected during three growing seasons using a phone app. The researchers hope that the technology will allow for the diagnosis of red virus symptoms through the analysis of photos taken from a camera mounted on a tractor or by autonomous robots.

Certification and Disease Management

The certification and other disease management approaches session started with a lecture by Maher Al Rwahnih (University of California, Davis, USA). Rwahnih described the regulatory process that allowed the Foundation Plant Services to replace the woody indexing procedure with the application of HTS in quarantine and certification programs. A series of presentations and posters focused on clonal selection and sanitation.

A study on the application of HTS to determine the virome of six grapevine varieties and subsequent pathogen elimination was reported by Vanja Miljanić (University of Ljubljana, Slovenia). The HTS results (confirmed by RT-PCR and Sanger sequencing) revealed the presence of viruses that belong to the *Nepovirus*, *Ampelovirus*, *Tymovirus* and *Trichovirus* genera, plus two viroid species. The sanitation protocol employed a combination of thermotherapy (36-38°C), along with the micrografting of meristem tips of 0.1-0.2 mm onto in vitro-propagated rootstock seedlings. This process successfully eradicated all viruses but was ineffective against the viroids.

Dunja Leljak-Levanić (University of Zagreb, Croatia) described the successful use of somatic embryogenesis on virus elimination while Eva Varallyay (Hungarian University) reported on the use of chemotherapy. In the Hungarian study, different concentrations and combinations of ribavirin, zidovudine and 2-thiouracil were used in grapevines grown in *vitro*. Based on the preliminary results, the researchers concluded that none of the treatments was able to increase the efficiency of virus elimination. Gábor Jakabpresented the use of BABA (β-amino butyric acid) to induce different plant defense mechanisms in grapevines. Interestingly, the results indicated that the treatment eliminated Arabis Mosaic Virus (ArMV) and GLRaV-1 but had no effect on Grapevine Fanleaf Virus (GFLV) and GVA infections.

Virus Characterization and Diversity

Baozhong Meng (University of Guelph, Canada) presented a lecture on research to elucidate the molecular and cellular biology of GLRaV-3. The construction of a full-length infectious clone, coupled with agro-inoculation of tissue culture-grown grapevine plants, allowed the demonstration of Koch's postulates for this *Ampelovirus*. After regeneration and a dormancy period, the infected grapevines showed typical leafroll symptoms. Consequently, it is proposed to drop the word "associated" as the name of the virus should be Grapevine Leafroll Virus. Qi and colleagues (University of Adelaide) reported

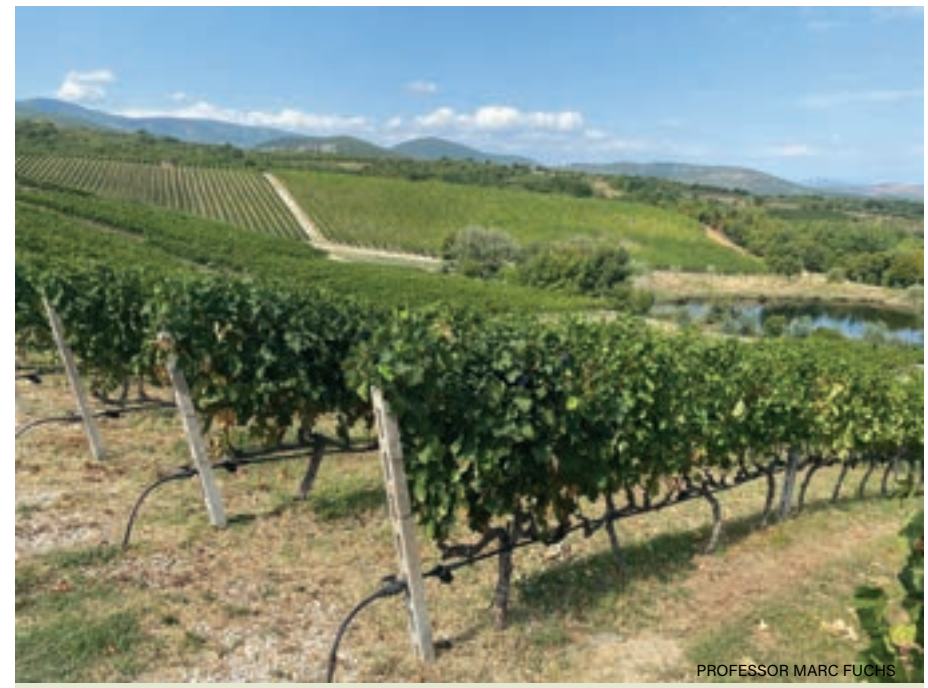
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FIGURE 3 A beautiful view of the Kyr Yanni vineyard. Naoussa, Greece.

on the genetic diversity of Grapevine *Rupestris* Stem Pitting-associated Virus (*GRSPaV*) in 15 varieties grown in South Australia. Phylogenetic analyses indicated that groups 1, 2a, 3 and 4 of GRSPaV are present. Grapevine Pinot Gris Virus is present all over the world, but it does not show symptoms in most grapevines. Complicating matters, Grapevine Leaf Mottling and Deformation (GLMD) symptoms associated with GPGV infection can be confused with those caused by *Nepoviruses* (*e.g.,* ArMV, GFLV).

In France, Anne-Sophie Spilmont reported a 32% infection rate of GPGV when 117 vineyard blocks around France were surveyed. Additionally, a low correlation of GPGV infection with GLDM symptoms was found, questioning the relationship between GPGV and GLMD. In Australia, Nepoviruses are not present in commercial vineyards; therefore, if a GPGV-positive vine would show GLMD symptoms, this would be associated with the virus. However, very few GPGV vines have shown GLMD symptoms in Australia or the USA. Furthermore, when these symptomatic vines were subjected to HTS, a few other viruses were detected (K. Kaur, Agriculture Victoria, Australia, personal communication).

Olufemi Alabi (Texas A & M University, USA) described the molecular characterization of divergent isolates of GRBV in the recently released Pierce's Disease-tolerant, interspecific hybrid Blanc du Soleil. Genetic mutations and recombination events were reportedly responsible for the generation of the divergent isolates of GRBV in the Blanc du Soleil variety. The study points to the need for screening breeding material to avoid the introduction of infected material into production winegrowing areas. Mate Carija and colleagues (Croatia) research showed that the response of different grapevines to GLRaV-3 infection is variety- and virus variant-dependent. In the study, Merlot was the fastest variety to display viral symptoms regardless of virus variant while the Croatian red grape Tribidrag appeared to be more resistant to leafroll infection.

Plant Virus and Vector Interactions

Urbez-Torrez reported that two species of treehoppers, *Stictocephala basali* and *S. bisonia*, were able to transmit GRBV in artificial transmission experiments under laboratory conditions. Greenhouse and field experiments will follow to determine the ability of these treehoppers to transmit the virus in

planta. In the U.S., the alfalfa three-cornered treehopper, *Spissistilus festinus*, is known to transmit *GRBV* in a circulative, non-propagative manner. Victoria Hoyle (Cornell University, USA) presented data on the feeding preferences of *Spissistilus festinus* in the vineyard ecosystem. The analysis of the DNA extracted from the insect's gut indicated that the vector relies on many hosts for feeding and grapevine is not the preferred host. Therefore, even if *Spissistilus festinus* is present, the use of insecticides is not recommended as a disease management practice.

Munir Mawassi (The Volcani Center, Israel) and Emmanuelle Vigne (INRAE, France) presented work on the characterization of isolates of GLRaV-3 and GFLV, respectively, that cause mild or no symptoms in grapevines.

The aim of the research is to use these mild strains on initial cross-protection experiments. Cross-protection is a method that has been used for many decades to control Citrus *tristeza* virus in Citrus crops. Scientists expect that cross-protection would be accepted by consumers as it does not produce genetically modified vines. In theory, a vine already infected with a mild strain of the same virus could be protected against a severe strain that might be later introduced in the vineyard. The HTS methods can be used to identify mild virus strains established in vineyards.

In Clare Valley, South Australia, a symptomless Shiraz (Syrah) infected with GVA (strain I) was found. We expect that this source of Shiraz may be tolerant to future infections by a severe strain of GVA (strain II) that causes Shiraz Disease. Margarida Teixeira-Santos (INIAV, Portugal) reported the involvement of GRSPaV on the mitigation of graft incompatibility of Syrah on the 110R rootstock by silencing the virus with dsRNA prior to grafting. In South Australia, Grapevine Virus A (Strain II) severely affects Shiraz and Merlot varieties. Nuredin Habili et al. have observed that if *Diplodia seriata*, a grapevine trunk disease fungus, is present in the vineyard, the vines start to die back.

However, in the absence of the fungus, symptoms, like leafroll disease, manifest. Related to disease resistance, Olivier Zekri (Novatech, France) and Christophe Ritzenthaler (IBMP-CNRS-Strasbourg, France) presented work on the collaborative project between industry and a research institution to develop transgenic grapevine plants by using nanobodies that confer resistance to ArMV and GFLV. Gérard Demangeat (Université de Strasbourg, France) presented data on the discovery of genetic resistance to GFLV found in two accessions of *Vitis silvestris*. The study showed that the gene(s) confers resistance to the virus infection and has no effect on nematode population. The research shows promise for producing GFLV-resistant plants by using traditional breeding techniques.

Conclusions

The ICVG meetings provide an opportunity for researchers from all over the world to present, share and discuss their discoveries on grapevine viruses. We need to learn more about the biological properties of newly discovered viruses before we decide to reject plant material for propagation as many viruses that have recently been discovered have not been implicated in disease. **WBM**