Recent Progress in Phytoplasma Research in Croatian Vineyards

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SUMMARY

Phytoplasmas (formerly called mycoplasma-like organisms) are the causal agents of several hundreds plant diseases including yellows diseases of the grapevine (*Vitis vinifera* L.). A system for the monitoring for grapevine yellows diseases in Croatian vineyards using polymerase chain reaction amplification and restriction fragment length polymorphism analyses has been employed since 1997. Bois Noir (stolbur) phytoplasmas were detected in vineyards of the eastern and north-western Croatia In these regions, one of the most frequently affected cultivars was 'Chardonnay'. No phytoplasmas were detected in Dalmatia and Istria which was in accordance with the absence of grapevine yellows symptoms in these regions. In some areas of Istria, however, 'Chardonnay' is becoming more attractive to growers. Since this variety is known for its high incidence of phytoplasmoses, this region should continue to be monitored for possible phytoplasma outbreaks.

KEY WORDS

grapevine yellows, phytoplasmas, *Vitis vinifera* L.

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OVERVIEW

What are phytoplasmas?

Phytoplasmas (formerly called mycoplasma-like organisms or MLOs) are known to be the causal agents of several hundreds plant diseases (McCoy et al., 1989), including yellows diseases of the grapevine (*Vitis vinifera* L.). Many yellows diseases of plants were considered to be caused by viruses until 1967, when Doi and co-workers using transmission electron microscopy provided the first evidence that yellows diseases were caused by MLOs. This term was used until 1993, when the term "phytoplasmas" was proposed and accepted (Tully, 1993).

Phytoplasmas belong to the class *Mollicutes*. They are prokaryotes without the cell wall and, as a consequence, they can change shape (pleomorphic organisms). They are genetically simpler than most plant pathogenic bacteria and, so far, they have not been cultivated *in vitro*.

Phytoplasma detection and characterization

Traditionally, phytoplasmas were differentiated and characterized on the basis of their biological properties including host range, disease symptoms on natural hosts and insect vector specificity. Recently, the development of molecular biology-based tools, namely nucleic acid hybridization and polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis, provided rapid, simple and reliable means for diagnosis of these pathogens (Davis et al., 1993). The approach using RFLP analysis of PCR-amplified 16S rDNA fragments is the preferred method for the routine differentiation and characterization of phytoplasmas (Namba et al., 1993; Schneider et al., 1993). This approach is the foundation of a new phytoplasma classification according to which phytoplasmas are currently classified into 14 groups and 38 subgroups (Lee et al., 1998). However, there are gene sequences or DNA regions other then 16S rDNA that could be used as alternatives for classification (Boudon-Padieu et al., 1997; Schneider et al., 1997). Hybridization and serology techniques can also be successfully employed (Kuszala et al., 1993; Webb et al., 1999), while electron microscopy enables insight in phytoplasma distribution in plant tissues (Marcone and Ragozzino, 1996).

Grapevine yellows

Grapevine yellows diseases (GY) have been reported in many of the world's viticultural regions (Padovan et al., 1996). Studies have shown that phytoplasmas associated with these diseases belong to diverse groups including: aster yellows (16SrI group), X-disease (16SrIII group), elm yellows (16SrV group) and stolbur (16SrXII group) (Prince et al., 1993; Davis et al., 1997). Mixed infections of single grapevine plant with phytoplasmas from distinct groups and subgroups have also been reported (Alma et al., 1996; Daire et al., 1997). These findings further complicate the etiological picture of the GY diseases.

The symptoms on grapevines usually appear at the beginning of summer as leaf yellowing and downward rolling of the leaf blades. As a result, leaves assume typical triangular form. Blades turn yellow, but the veins may remain green. One of the most devastating symptoms is improper lignification of the young shoots that freeze in the following winter. The symptoms may also appear on the fruits where parts of the bunches dry out. As a consequence, the yield significantly drops in the first year after the onset of symptoms and the diseased plant usually dies within 2-3 years. Up till now, no successful therapy for diseased plants has been established. Economic losses caused by the phytoplasmas can be significant.

RESEARCH IN CROATIAN VINEYARDS

Since 1994, symptoms typical for the GY diseases have been observed on cultivars 'Pinot gris' and 'Chardonnay' in the Zagreb area (north-western Croatia), but no molecular evidence existed at that time to prove the relationship between typical GY symptoms and a phytoplasma agent. In the late '90s a collaborative research project involving researchers from the Department of Enology and Viticulture, Faculty of Agriculture and the Department of Biology, Faculty of Science at the Zagreb University was started. The aim of this project was to develop efficient means for the grapevine yellows disease agent detection and characterization, to use these techniques to survey for disease, and eventually, to develop strategies for controlling this disease not only in Croatian vineyards but also in the grapevine propagation material. This project continues at this writing.

The first molecular evidence for the presence of Bois Noir (stolbur) phytoplasmas infecting Croatian grapevines was presented by Šarić et al. (1997). Stolbur-related phytoplasma PCR-detection tests were also positive for 5 out of 6 'Pinot gris' grapevine plants gathered in Božjakovina (Zagreb region) in the following year (Škorić et al., 1998). Two weed species collected in that vineyard, *Taraxacum officinale* Web. and *Polygonum lapathifolium* L., were also found to host the same type of pathogen. The most sensitive detection system proved to be the one that employed general primers R16F1/R0 followed by R16F2/R2 (Lee et al., 1994; 1995) and specific primers R16(I)F1/R1 (Lee et al., 1994) and/or M1/M2 (Gibb et al., 1995). The most convenient tissue for the phytoplasma detection turned out to be the phloem tissue from leaf midribs gathered in late summer. The system for phytoplasma detection set up in this research (Škorić et al., 1998) has been used for further investigations.

The report about the incidence of the grapevine yellows in terms of geographical distribution and cultivar susceptibility was published by Šeruga et al. (2000). Results confirmed the presence of the Bois

Noir phytoplasmas in the vineyards of eastern and northwestern Croatia. The most frequently infected cultivar was 'Chardonnay'; other cultivars were found infected including 'Pinot Noir', 'Riesling' and 'Sauvignon'. In Istria and Dalmatia, where indigenous cultivars are predominately grown ('Teran', 'Malvazija Istria', 'Debit', 'Plavina', 'Plavac Mali'), no phytoplasmas have been detected so far, based on both visual symptoms and molecular analyses. In some areas of Istria 'Chardonnay' is becoming more attractive to growers. Since it is known for its high incidence of phytoplasmoses, it is necessary to keep monitoring this region for possible phytoplasma outbreaks.

CONCLUSIONS AND RESEARCH PLANS

The absence of phytoplasmas in Istria and Dalmatia as compared to continental Croatia could be the result of interaction between different factors including climate, soil characteristics, vector species and their feeding preferences. Geographical isolation of the Croatian islands and possible higher resistance level of autochthonous cultivars should also be taken into consideration.

Phytoplasmas in the vineyards of continental Croatia could have been introduced by import of foreign cultivars or they could have naturally spread from the neighboring countries (Italy, Slovenia, Hungary) where phytoplasmas had been previously detected in the grapevine. Therefore, in the future, all imported grapevine propagation material should be regularly checked.

In nature, phytoplasma-associated diseases are transmitted primarily by vectors belonging to the families *Cicadelloidea* (leafhoppers) and *Fulgoroidea* (planthoppers) (Lee et al., 1998). It is already known that in Europe, the most common vectors capable of spreading Flavescence dorée and GY are *Scaphoideus titanus* and *Hyalestes obsoletus*, respectively. A search for other possible insect vectors, as well as a screening of weed species, is needed in order to control already existing phytoplasmoses in Croatian vineyards.

The host range of subgroup 16SrXII-A (Bois Noir or stolbur) strains is known to include plants such as nectarine, pear (Lee et al., 1995) and vineyard weeds (Maixner et al., 1995). In Croatia, stolbur-like symptoms had been reported in tomato, pepper, eggplant, potato, *Cirsium* sp. and *Convolvulus arvensis* (Panjan, 1957) and there was an electron microscopy evidence for the phytoplasma presence in Croatian diseased potatoes (Panjan et al., 1970) as well as in apples and pears (Šarić and Cvjetković, 1985). Therefore, the molecular methods for phytoplasma investigations used in Croatian viticulture should be applied to other areas of plant production to provide information about the occurrence of this pathogen in other Croatian crops.

LITERATURE CITED

Alma A., Davis R. E., Vibio M., Danielli A., Bosco A., Arzone A., Bertaccini A. (1996). Mixed infection of grapevines in northern Italy by phytoplasmas including 16S rRNA RFLP subgroup 16SI-B strains previously unreported in this host. Plant Dis 80: 418-421

Boudon-Padieu E., Daire X., Clair D., Laviña A., Batlle A., Reinert W., Maixner M. (1997). Differentiation of grapevine phytoplasmas in the elm yellows and the stolbur group with the use of RFLP of non-ribosomal DNA. Proc 12th Meeting ICVG, Lisbon, Portugal, pp 55-56

Daire X., Clair D., Larrue J., Boudon-Padieu E. (1997). Survey for grapevine yellows phytoplasmas in diverse European countries and Israel. Vitis 36: 53-54

Davis R. E., Dally E. L, Tanne E., Rumbos I. C. (1997). Phytoplasmas associated with grapevine yellows in Israel and Greece belong to the stolbur phytoplasma subgroup, 16SrXII-A. J Plant Pathol 79: 181-187

Davis R. E., Dally E. L., Bertaccini A., Credi R., Osler R., Savino V., Carraro L., Di Terlizzi B., Lee I.-M., Barba M. (1993). Restriction fragment length polymorphism analyses and dot blot hybridization distinguish mycoplasmalike organisms associated with Flavescence dorée and Southern European grapevine yellows disease in Italy. Phytopathology 83: 772-776

Doi Y., Teranaka M., Yora K., Asuyama H. (1967). Mycoplasma-or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches'-broom, aster yellows or paulownia witches'-broom. Ann Phytopathol Soc Jap 33: 259-266

Gibb K. S., Padovan A. C., Mogen B. A. (1995). Studies on sweet potato little-leaf phytoplasmas detected in sweet potato and other plant species growing in Northern Australia. Phytopathology 85: 169-174

Gundersen D. E., Lee I.-M. (1996). Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. Phytopath Medit 35: 144-151

Kuszala C., Caudwell A., Cazzeles O., Credi R., Granata G., Kriel G., Magarey P., Pearson R. C., Refatti E., Tanne E. (1993). Grapevine yellows in different areas of the world: investigation by ELISA using Flavescence dorée specific antibodies. Proc 11th Meeting ICVG, Montreux , Swizerland, pp 99-100

Lee I.-M., Bertaccini A., Vibio M., Gundersen D. E. (1995). Detection of multiple phytoplasmas in perennial fruit trees with decline symptoms in Italy. Phytopathology 85: 728-735

Lee I.-M., Gundersen D. E., Bertaccini A. (1998). Phytoplasma: ecology and genomic diversity. Phytopathology 88: 1359-1366

Lee I.-M., Gundersen D. E., Hammond R. W., Davis R. E. (1994). Use of mycoplasmalike organism (MLO) group-specific oligonucleotid primers for nested-PCR assays to detect mixed-MLO infections in a single host plant. Phytopathology 84: 559-566

Maixner M., Ahrens U., Seemüller E. (1995). Detection of the German grapevine yellows (Vergilbungskrankheit) MLO in grapevine, alternative hosts and a vector by a specific PCR procedure. Eur J Plant Pathol 101: 241-250

Marcone C., Ragozzino A. (1996). Comparative ultrastructural studies on genetically different phytoplasmas using scanning electron microscopy. Petria 6: 125-136

McCoy R.E., Caudwell A., Chang C. J., Chen T. A., Chiykowsky L. N., Cousin M. T., Dale J. L., de Leeuw G. T. N., Golino D. A., Hackett K. J., Kirkpatrick B. C., Marwitz R., Petzold H., Sinha R. C., Sugiura M., Whitcomb R. F., Yang I. L., Zhu B. M., Seemüller E. (1989). Plant diseases associated with mycoplasma-like organisms. In: The Mycoplasmas (R. F. Whitcomb, J. G. Tully, eds.). Acad Press, New York, 545-640

Namba S., Kato S., Iwanami S., Oyaizu H., Shiozawa H., Tsuchizaki T. (1993). Detection and differentiation of plant-pathogenic mycoplasmalike organisms using polymerase chain reaction. Phytopathology 83: 786-791

Padovan A. C., Gibb K. S., Daire X., Boudon-Padieu E. (1996). A comparison of the phytoplasma associated with Australian grapevine yellows to other phytoplasmas in grapevine. Vitis 35: 189-194

Panjan M. (1957). Stolbur virus. Glasnik zaštite bilja 5: 51-54

Panjan M., Šarić A., Wrischer M. (1970). Mycoplasmanliche Gebilde in Tomatenpflanzen nach Infektion mit Kartoffelgelbsucht. Phytopathologische Zeitschrift 69: 31-35

Prince J. P., Davis R. E., Wolf T. K., Lee I.-M., Mogen B. D., Bertaccini A., Credi R., Barba M. (1993). Molecular detection of diverse mycoplasmalike organisms (MLOs) associated with grapevine yellows and their classification with aster yellows, X-disease, and elm yellows MLOs. Phytopathology 83: 1130-1137

Schneider B., Ahrens U., Kirkpatrick. B. C., Seemüller E. (1993). Classification of plant-pathogenic mycoplasma-like organisms using restriction-site analysis of PCR-amplified 16S rDNA. J Gen Microb 139: 519-527

Schneider B., Gibb K. S., Seemüller E. (1997). Sequence and RFLP analysis of the elongation factor Tu gene used in differentiation and classification of phytoplasmas. Microbiology 143: 3381-3389

Šarić A., Cvjetković B. (1985). Nalaz mikoplazmama sličnih organizama u jabuci sa simptomima proliferacije i kruški sa simptomima propadanja. Poljoprivredna znanstvena smotra 68: 61-67

Šarić A., Škorić D., Bertaccini A., Vibio M., Murari E. (1997). Molecular detection of phytoplasmas infecting grapevines in Slovenia and Croatia. Proc 12th Meeting ICVG, Lisbon, Portugal, pp 77-78

Šeruga M., Ćurković Perica M., Škorić D., Kozina B., Mirošević N., Šarić A., Bertaccini A., Krajačić M. (2000). Geographic distribution of Bois Noir phytoplasmas infecting grapevines in Croatia. J Phytopathol 148: 239-242

Škorić D., Šarić A., Vibio M., Murari E., Krajačić M., Bertaccini A. (1998). Molecular identification and seasonal monitoring of phytoplasmas infecting Croatian grapevines. Vitis 37: 171-175

Tully J.G. (1993). International Committee on Systematic Bacteriology, Subcommittee on the Taxonomy of Mollicutes. Minutes of the interim meetings, 1st-2nd Aug 1992, Ames, Iowa. Int J System Bacteriol 43: 394-397

Webb D. R., Bonfiglioli R. G., Carraro L., Osler R., Symons R. H. (1999). Oligonucleotides as hybridization probes to localize phytoplasmas in host plants and insect vectors. Phytopathology 89: 894-901

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