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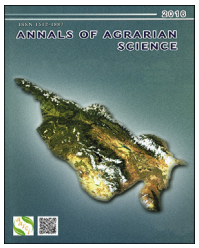

Grapevine phytoplasma disease in Georgia

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ABSTRACT

Results of grapevine yellows disease (GY) studies in 2005–2015 are reported. Based on symptoms and Dienes' staining method the disease was detected in Kartli, Kakheti and Guria regions on *Vitis vinifera* (L.) Rkatsiteli, Saperavi, Shavkapito, Tavkveri, Aladasturi, Kachichi, Ganjuri, Chardonnay, *Vitis labrusca* (L.) cultivar Isabella, species hybrid cultivar Noah. The phytoplasmas were also visualized by using electron microscopy. The disease seriously modifies the structure of leaf the grapevine phloem, the chlorophyll content and the functioning of sink–source system. According to anatomical characters the studied cultivars showed a decreasing resistance from Noah, to Aladasturi, Rkatsiteli, Kachichi and Saperavi. In the areas of disease epidemic 12 leafhopper species were identified: *Agalmatium grylloides* (Fabricius, 1794), *Cicadella viridis* (Linnaeus, 1758), *Dictyophara europaea* (Linnaeus, 1767), *Empoasca vitis* (Gothe, 1875), *Erythroneura imeretina* Dekanoidze, 1962, *Hyalesthes mlokosieviczi* Signoret, 1879, *Hyalesthes obsoletus* Signoret, 1865, *Lepironia coleoptrata* (Linnaeus, 1758), *Pentastiridius leporinus* (Linnaeus, 1761), *Philaenus spumarius* (Linnaeus, 1758), *Metcalfa pruinosa* (Say, 1830), *Ricania japonica* (Melichar, 1898), which may be possible vectors of grapevine phytoplasmas in Georgia.

Introduction

Phytoplasmas are very small bacteria without cell wall, which cause severe and untreatable diseases of wild and cultivated plants and also of grapevine [1–4]. Grapevine phytoplasma diseases with the general name "grapevine yellows" (GY) are widely spread in the regions of intensive vine-growing all over the world and some of them are subject to quarantine [2,5–7].

Yellowing of grapevine leaves or chlorosis is known in Georgia since older times. According to the causes it was divided into infectious (caused by viruses, fungi, bacteria, pests) and non-infectious (disorder of soil conditions for grapevine nutrition) [8]. In the last century, nobody suggested phytoplasma infection as one of possible causes of yellowing of grapevine in Georgia, despite a disease – curly leaf of mulberry or mulberry dwarf disease has been detected in Georgia in 1964, which mycoplasmic nature has been established in 1967 [9]. Georgia was the center of study of mycoplasmosis in the former Soviet Union: the diagnostic method for mulberry dwarf disease has been elaborated for field conditions in 1964–1999 [10], the indicator variety of mulberry [11], mycoplasma strains [12,13], the vector insect *Hishimonus*

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sellatus (Uhler, 1896) have been reported [14] based on selection mulberry cultivars resistant to phytoplasma have been obtained [15], and a unique anatomical method for forecasting the possible resistance of mulberry to the disease was settled [16,17].

Leaves of GY symptomatic grapevine were provided by farmers from Sighnaghi district in 2002. As a result of the visual and cytochemical analyses (Dienes' method) [18] of samples of the cultivars Rkatsiteli and Saperavi phytoplasma were detected [19]. The existence of a disease associated with possible phytoplasma presence in the cultivar Aleksandrouli in Racha region [20] and Saperavi in Kakheti region [21] was also reported. In 2014, by PCR method it has been established that the phytoplasma disease in grapevine is associated with "stolbur" group in Eastern Georgia [22,23].

Phytoplasmas are characterized by complicated life cycle and the diseases are distributed by sap feeding insects belonging to the families Cicadellidae, Cixiidae, Psyllidae, Delphacidae and Derbidae [24], by parasitic plants (*Cuscuta* sp.) [25], by seed [26,27], by grafting [5,28]. The systematics of phytoplasmas [29,30], structure of genome [31], interaction with the host organisms [1,2], plant resistance [32,33] are intensively studied. Decreasing of losses due to phytoplasma diseases is possible by selection and practical use of resistant cultivars [2,3,11,34,35]. Despite intensive investigations, the available knowledge about phytoplasma biology and mechanisms of plant resistance is insufficient, rendering not possible to manage them with eradication control methods. Moreover, the areas of distribution of phytoplasma diseases and the species affected are quickly widening.

Even more questions are still present about the grapevine phytoplasma disease in Georgia. It is not established – whether there are different groups of phytoplasmas and different insect vectors in the various vine-growing regions and what is the resistance of the Georgian cultivars against the GY. The symptoms of the disease, pathological changes (anatomical, physiological, biochemical, genetic) according to the cultivars, the markers necessary for selection of resistant individuals are also not studied. Study of these issues in the homeland of cultivated grapevine – Georgia, where more than 500 endemic cultivars of *Vitis vinifera* (L.) are described ampelographically in various agri-environmental conditions [36], is especially important from the theoretical and practical points of view.

Results of the investigation carried out on phytoplasma disease of grapevine in Georgia during last 10 years are presented.

Objectives and methods

The study has been conducted on the Georgian cultivars of the grapevine *Vitis vinifera* (L.): Rkatsiteli, Saperavi, Shavkapito, Tavkveri, Aladasturi, Kachichi, and on the Azerbaijan cultivar Ganjuri, on the French cultivar Chardonnay, *Vitis labrusca* (L.) on the cultivar Isabella (synonym Odessa), on the interspecific hybrid cultivar Noah. Visual assessment of the disease was made in farmers' vineyards (Sighnaghi and Lanchkhuti districts), in the ampelographic collection of the Agricultural University of Georgia (Mtskheta district), in the national center of production of saplings of grapevine and fruit trees (Mtskheta district). Sighnaghi district is located in the eastern part of Tsiv-Gombori Range at 500–800 m a.s.l. Dry continental climate dominates there. Winter is cold, summer hot and drought-afflicted. The annual precipitation is 400–500 mm. The snow cover is rarely formed. The soils there are chernozem and cinnamonic; many aboriginal varieties grow there. The best quality wines are produced there. The main industrial cultivars for wine production are Rkatsiteli, Saperavi and Goruli mtsvane. Mtskheta district is located at 400–600 m a.s.l., is characterized by dry subtropical climate with relatively cold winter, annual precipitation is ca. 500–600 mm. Alluvial-calcareous and meadow cinnamonic soils are mainly present there. The industrial cultivars are: Goruli mtsvane, Chinuri, Tavkveri. High quality wines are produced. Lanchkhuti district is located in Western Georgia, at height 50–100 m a.s.l. The humid subtropical climate dominates there. Due to vicinity to Black Sea it is characterized by warm winter and moist climate. Summer is hot. The annual precipitation is ca. 2000 mm. Mainly yellow and red soils are there. The widespread cultivars are Odessa, Noah, Aladasturi. There are many other local cultivars. Original wines are produced there.

Grapevine is cultivated by trellis method in Eastern Georgia and on pergola in Western Georgia. The plants were assessed according to external symptoms: healthy (without visual symptoms), weakly diseased (up to 25% of the plants are symptomatic), moderately (up to 50% of the plants are symptomatic) and severely diseased (more than 50% symptomatic) [10]. Visually healthy and symptomatic material has been fixed in ethanol and formalin. Temporary slides were made from the fixed material for establishment of disease and patho-anatomical disorders. Preparation of anatomical sections, staining and microscopic study of structure was made by standard methods [37]. For determination of content of starch, the J + JK solution was used [37]. The quantitative content of plastid pigments in leaves was measured by spectrophotometric method [38]. Diagnostic of phytoplasmas in petiole of visually healthy and diseased leaves has been carried out by Dienes' cytochemical method [18]. The material for the detection of quantity of plastid pigments was sampled in the vineyards of the village Kvemo Bodbe, Sighnaghi district. Cicadas were collected by insect net and placed in glass tubes for species determination [39]. For study plant cells and tissues and insects the transmission microscope TESLA BS-500; the light microscope МБИ-3, stereomicroscope МБС-9 were used. The microphotographs have been taken by digital camera Sony (12.3 megapixels). The experiments were repeated three times. The study has been carried out in 2005–2015.

Results and analysis

Spread of disease

In 2005–2015, epidemics of GY disease were detected in regions differing in agri-environmental conditions: Kakheti region – Sighnaghi district, Kartli region – Tbilisi and Mtskheta districts (Eastern Georgia) and Guria region – Lanchkhuti

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