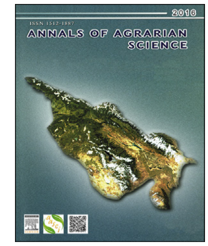
Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/annals-of-agrarian-science



Grapevine phytoplasma disease in Georgia

N.M. Chkhaidze [a,*], Mz.I. Lobjanidze [a], I.I. Dzmanashvili [a], Sh.Z. Barjadze [a,b], D.N. Maghradze [a]

[a] Agricultural University of Georgia, 240, David Aghmashenebeli Ave, Tbilisi 0159, Georgia
[b] Institute of Zoology of Ilia State University, Giorgi Tsereteli 3, Tbilisi 0162, Georgia

ARTICLE INFO

Article history:
Received 26 January 2016
Accepted 8 April 2016
Available online 26 June 2016

Keywords:
Grapevine
Phytoplasma
Insect vectors
Resistance

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*

* Corresponding author.
E-mail address: no.chkhaidze@agruni.edu.ge (N.M. Chkhaidze).
Peer review under responsibility of Journal Annals of Agrarian Science.
http://dx.doi.org/10.1016/j.aasci.2016.04.001

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 district (Western Georgia). The local cultivars are well adapted to the region.

Visual diagnostics of the disease

The symptoms of the disease are variable according to cultivar, region and intensity. The most obvious symptom is yellowing of leaves (on white grapevine cultivars, Shardonnay, Ganjuri) or reddening (on red grapevine cultivars Saperavi, Shavkapito) (Fig. 1), deformation of leaves, necrosis, inhibition of growth and drying of diseased plants in spring and summer. It should be mentioned that red-grapevine cultivars Aladasturi, Saperavi, Shavkapito, and Tavkveri may have not only red coloring, but yellow as well. Aladasturi and Chardonnay are characterized by similar symptoms of leaf deformation. The leaf size is reduced in all symptomatic cultivars.

Cytochemical detection of the phytoplasmas

By using Dienes' stain healthy and phytoplasma infected cultivars of grapevine and other host plant species of the potential phytoplasma vector cicadas were identified. The diseased phloem is always being stained dark blue (Fig. 2). In the epidemic areas of the disease the presence of healthy grapevine leaf with plants not carrying the infection is very rare (Table 1). In the examined material only Noah samples collected in 2010 in Guria, resulted symptomless (Fig. 2). The plant had no character of disease – neither externally not cyto-chemically. In 2015 in the same area in healthy looking leaves, from Noah the Dienes' reagent has shown positive reaction. In general, intensifying of visual disease symptoms is accompanied by intensive staining of the phloem in all the studied varieties (Fig. 2 and Table 1).

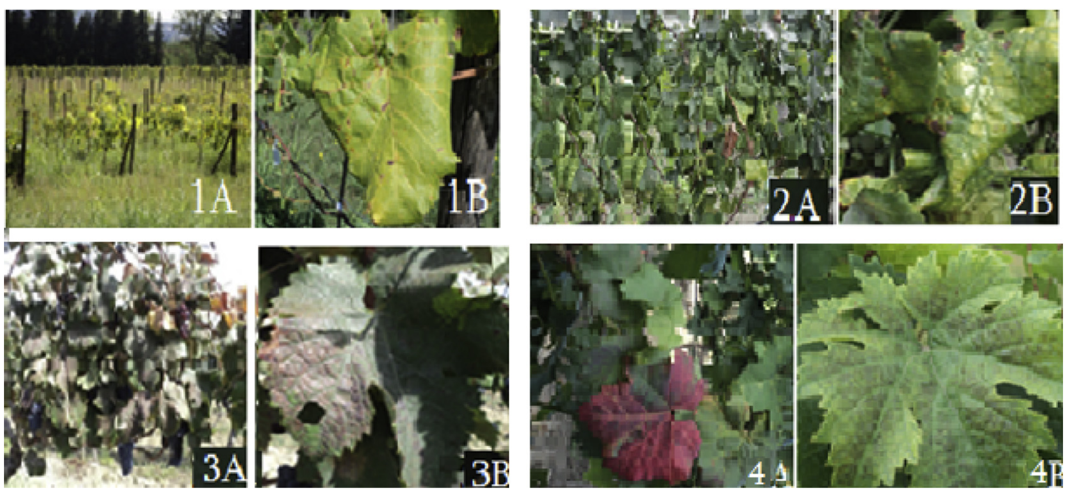


Fig. 1 – 1. Ganjuri, white. Mtskheta, Kartli; 2. Chardonnay, white. Mtskheta, Kartli; 3. Shavkapito, red. Mtskheta, Kartli; 4. Saperavi, red. Sighnaghi, Kakheti; A. General view of the plant. B. Diseased leaves.

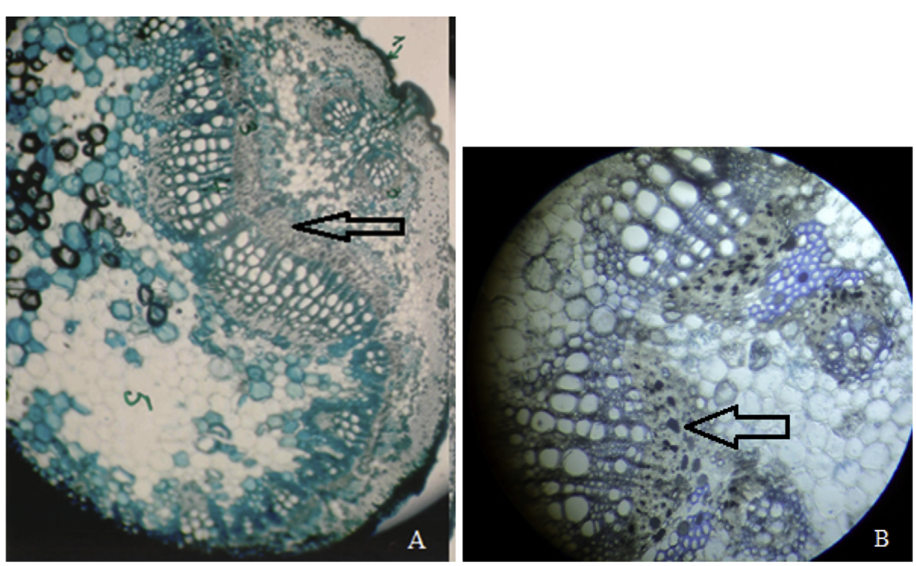


Fig. 2 – Hand cut sections of visual healthy cultivar Noah (A) and phytoplasma-infected cultivar Saperavi (B) leaf petioles stained with Dienes' stain. Arrows show healthy (A) and diseased (B) phloems. x40.

Table 1 – Structure of leaf petiole in middle part of visual healthy and diseased grapevine cultivars.

Cultivar	Degree of the disease	Conductive veins, number			Vessel, number				Vessel, diameters				Xylem			
		Main		Additional	Main		Additional		Main		Additional		Main		Additional	
		Number	%	Number	Number	%	Number	%	mk	%	mk	%	mk^2	%	mk^2	%
1. Rkatsiteli	Visual healthy	19.3	100.0	2–3	223.0	100.0	18.8	100.0	3.3	100.0	1.9	100.0	735.9	100.0	35.7	100.0
	Very diseased	17.3	89.7	2	178.7	80.1	11.7	62.2	2.6	78.8	1.9	100.0	464.6	63.1	22.2	62.2
2. Saperavi	Visual healthy	29.5	100.0	2	247.0	100.0	11.5	100.0	2.9	100.0	2.1	100.0	716.3	100.0	24.2	100.0
	Very diseased	13.0	44.1	2	130.0	52.6	7.0	60.9	2.7	93.1	2.3	109.5	351.0	49.0	16.1	66.5
3. Aladasturi	Visual healthy	26.3	100.0	2–3	236.6	100.0	28.5	100.0	3.3	100.0	3.2	100.0	780.8	100.0	91.2	100.0
	Very diseased	24.8	94.3	2	225.0	95.1	21.5	75.4	2.7	81.8	2.3	71.9	607.5	77.8	49.5	54.3
4. Kachichi	Visual healthy	18.0	100.0	2	246.0	100.0	18.0	100.0	4.2	100.0	2.4	100.0	1033.2	100.0	43.2	100.0
	Very diseased	17.0	94.4	2	201.0	81.7	12.0	66.7	2.7	64.3	1.6	66.7	542.7	52.5	19.2	44.4
5. Noah	Visual healthy	32.0	–	2	317.0	–	24.0	–	4.6	–	3.3	–	1046.1	–	79.2	–
	Diseased	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Detection by electron microscopy

Electron microscopic study carried out for the first time on diseased leaf of cultivar Saperavi. Only yellow spots were visible on the leaf, caused by sucking insects; it had not external symptoms of phytoplasma disease. Dienes' reaction shown the phytoplasma presence in the middle part of the leaf petiole and the electron microscopy allow to visualize their forms and sizes (Fig. 3). It has been determined that their form is slightly round, sizes vary within 0.2–1.0 μm, membrane is well visible. Phytoplasmas have been mentioned in the sieve cells of phloem. Phytoplasma cells are light colored (Fig. 3).

Infection-transmitting cicadas

In the infected areas of Eastern Georgia, 10 species of cicadas, which fed on grapevine as well as on other species, were collected and most of them resulted carrying phytoplasmas:

Agalmatium grylloides (Fabricius, 1794) – distributed all over Georgia, especially in Eastern Georgia. Its ability to transmit phytoplasmosis is not studied.

Cicadella viridis (Linnaeus, 1758) – distributed all over Georgia is hygrophilous. Its bio-ecology and ability to transmit phytoplasma are not studied.

Dictyophara europaea (Linnaeus, 1767) – polyphagous found on grapevine in Eastern Georgia (Sighnaghi district) in 2014–2015. The insect is considered in Europe as one of the vector of the Flavescence dorée phytoplasma.

Empoasca vitis (Gothe, 1875) – distributed all over Georgia is hygrophilous. It feeds on herbaceous plants and shrubs. Its ability to transmit phytoplasma is not studied.

Erythroneura imeretina Dekanoidze, 1962 – found with high population density in Mtskheta district (Mukhrani) in 2013 on the cultivar Saperavi, phytoplasma infected. Its ability to transmit phytoplasma is not studied.

Hyalesthes mlokosieviczi Signoret, 1879 – morphologically very similar to *Hyalesthes obsoletus*. It is distributed in Kartli and Kakheti regions. It was found in the 50-ies of the XX century in Eastern Georgia. It is possibly one of the vectors of phytoplasmas.

H. obsoletus Signoret, 1865 – found on grapevine (*V. vinifera*), sunflower (*Helianthus annuus*), liquorice (*Glycyrrhiza glabra*) and bindweed (*Convolvulus arvensis*) in Sighnaghi district in 2014–2015. Its specimens were found in June, July and August. It is a vector of Bois noir disease in Europe.

Lepyronia coleoptrata (Linnaeus, 1758) – fed on grapevine (*V. vinifera*), small nettle (*Urtica urens*), blackberry (*Rubus*), liquorice (*G. glabra*), sunflower (*H. annuus*), agrimony (*Agrimonia eupatoria*) and goosefoot (*Chenopodium album*).

Pentastiridius leporinus (Linnaeus, 1761) – found in Sighnaghi district on diseased grapevine (*V. vinifera*), small nettle (*U. urens*), blackberry (*Rubus*), black locust (*Robinia pseudoacacia*), liquorice (*G. glabra*), sunflower (*H. annuus*), agrimony (*A. eupatoria*) and goosefoot (*C. album*).

Philaenus spumarius (Linnaeus, 1758) – distributed all over Georgia, it is hygrophilous. It feeds on herbaceous plants

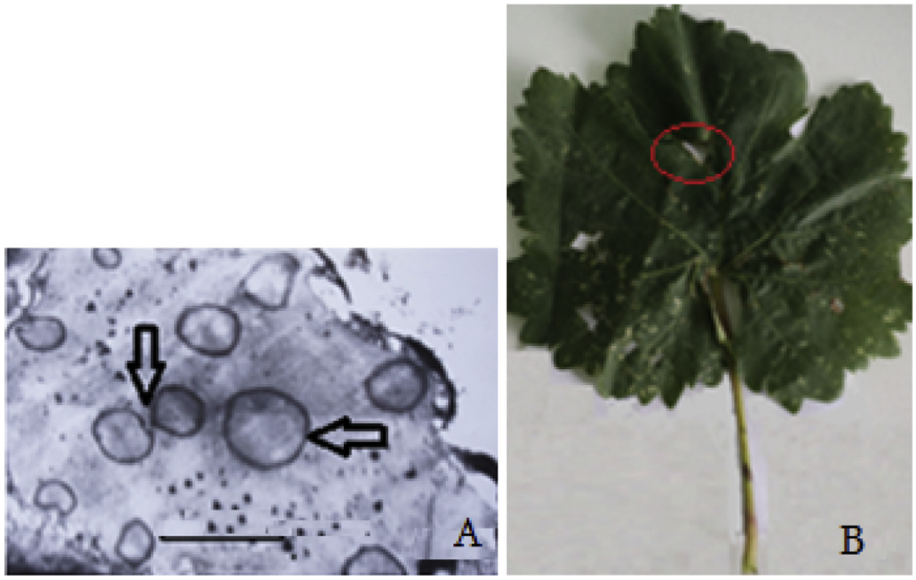


Fig. 3 – Phytoplasmas (arrows) in the phloem cells (A) of the visual healthy leaf (B) of cultivar Saperavi. A: X39,000.

and shrubs. Its ability to transmit phytoplasma is not studied.

In the diseases areas in Lanchkhuti district (Western Georgia) in 2015 the two species of cicadas listed below, which feed on diseased grapevine and other plant species were collected.

Metcalfa pruinosa – wide polyphagous insect damaging many subtropical woody plants and shrubs. Colonies were found on the following species: grapevine (*V. vinifera*, *V. labrusca*), medlar (*Mespilus germanica*), walnut-tree (*Juglans*), blackberry (*Rubus*), mulberry (*Morus*), small nettle (*U. urens*), male-fern (*Dryopteris*), black locust (*R. pseudoacacia*), tea (*Camellia sinensis*), Chinese wood-oil tree (*Aleurite scordata*), braken (*Pteridium tauricum*), agrimony (*A. eupatoria*), common ragweed (*Ambrosia artemisiifolia*), hazelnut (*Corylus colchica*), orange (*Citrus sinensis* var. washington navel), colchis ivy (*Hedera colchica*), trifoliate orange (*Poncirus trifoliata*). The Dienes' reaction has shown that except for *Morus* and *M. germanica*, all the above mentioned plant species were carrying phytoplasmas.

Ricania japonica Melich – is widely distributed in Western Georgia, it is a polyphagous species. It was collected together with *M. pruinosa* on the above mentioned plant species. They both may be carriers of phytoplasmas.

Pathoanatomical changes in grapevine infected by phytoplasmas

It has been defined that phytoplasmas induce structural disorders in the middle part of petiole of leaf, which extent depends on cultivar and disease severity (Table 1). The number of main conducting bundles in the middle parts of a petiole of visually healthy leaves varied within 18–32. The number of additional bundles was mainly 2, rarely 3 or 4. Diameter of vessels and water conducting area is higher in the cultivars growing in Lanchkhuti district –Aladasturi, Kachichi and Noah, than in Rkatsiteli and Saperavi, distributed in Sighnaghi district. Drastic changes are observed in the structure of middle part of petiole of symptomatic grapevine leaf. In particular, the numbers of main conductive bundles are decreased in Rkatsiteli – by 10.4%, in Saperavi – by 55.9%, and in Aladasturi – by 5.7%, the number of additional bundles decreased by unit only in Rkatsiteli, Aladasturi (Table 1) and Goruli mtsvane. In all the studied cultivars the number of vessels decreased in main and additional bundles. The number of vessels to the additional bundles decreased in all varieties, and the diameter of vessels decreased only in Kachichi and Aladasturi, in the cultivar Rkatsiteli it remained unchanged, and in Saperavi – increased by 9.5%. The changes in number and sizes of vessels were reflected in the total area of vessels, i.e. water conductive area of the petiole (Fig. 4). The latter is reduced in all diseased cultivars, especially in the cultivars of dry climate – Rkatsiteli and Saperavi growing in the conditions of dry climate (Kakheti) have less total area of water transmitting system of middle part of leaf petiole (area of main vascular system + area of companion vascular system) (772 μm^2 and 740 μm^2, respectively), than in Aladasturi (872 μm^2), Kachichi (1072 μm^2) and Noah (1125 μm^2), growing in the conditions of high moisture (Guria region) (Fig. 4).

Pathophysiological changes in grapevine phytoplasma infected

At the end of September in the petiole of leaf of diseased grapevine, in comparison with the visually healthy one, big quantity of starch content is observed. The starch has been accumulated mainly in the parenchyma cells adjacent to conductive system of petiole.

The content of plastid pigments is disordered in the leaves of phytoplasma infected grapevines. Rkatsiteli had small, yellowish colored leaves and Saperavi – internally twisted, dark red colored, also small leaves. By using of Dienes' staining phytoplasmas in a small quantity was revealed in visually healthy leaves of both cultivars, so they may be considered as latently infected. The area of visually diseased leaves was very much decreased, especially in the cultivar Saperavi. In particular, in the diseased cultivar Rkatsiteli the area of leaf reduced approximately by 70%, while in Saperavi – by 39% (Fig. 5). In the visually diseased leaves of the cultivar Saperavi chlorophylls "a" and "b" are reduced by equal quantities – approximately by 44%, in comparison with visually healthy ones. In the visually diseased leaves of the cultivar Rkatsiteli chlorophyll "a" is reduced by 8% (Fig. 6) and chlorophyll "b" by 23% (Fig. 7).

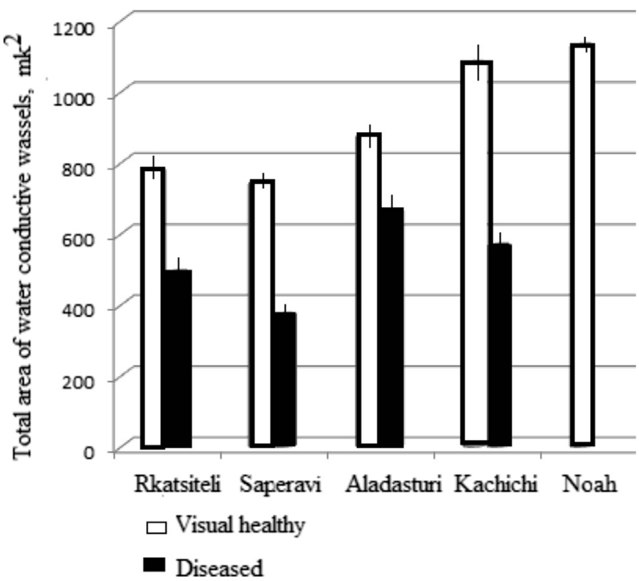


Fig. 4 – Total water conducting area in the middle part of visual healthy and phytoplasma diseased grapevine leaf petioles. Cultivar Noah did not have symptoms of disease.

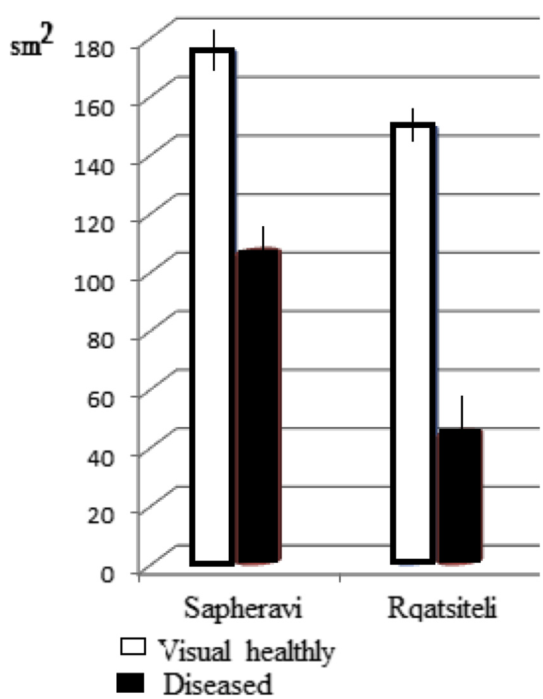


Fig. 5 – Areas in visual healthy and diseased leaves blades of grapevine infected by phytoplasmas.

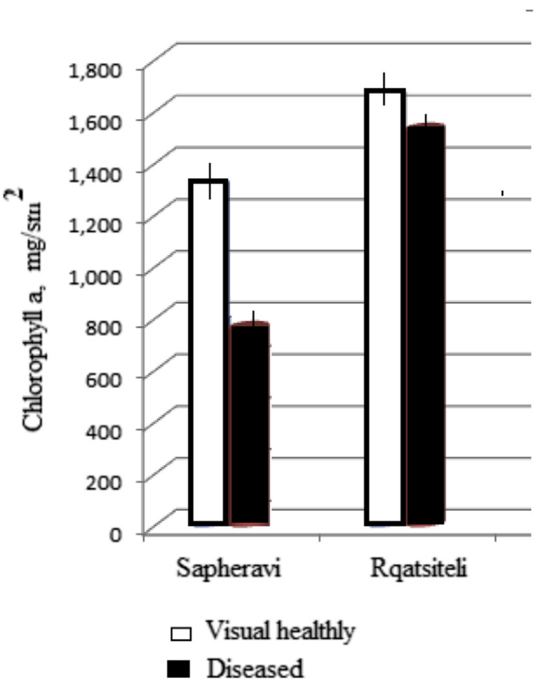


Fig. 6 – Content of chlorophyll "a" in visual healthy and diseased grapevine leaves.

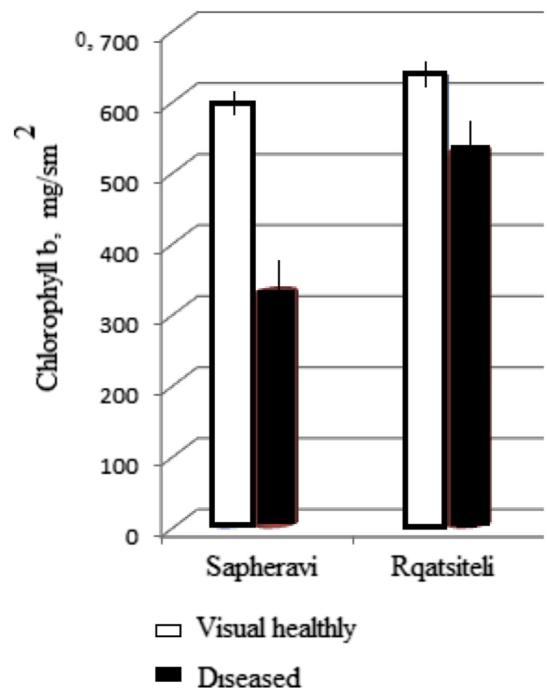


Fig. 7 – Content of chlorophyll "b" in visual healthy and diseased grapevine leaves.

Discussion

The laboratory and field studies carried out in 2005–2015 have shown that the area of distribution of grapevine phytoplasma diseases is more than it was known up to 2013. In particular, the districts Mtskheta and Lanchkhuti have been added to Telavi, Kvareli, Oni and Sighnaghi districts. The symptoms vary according to the cultivars and intensity of disease. Typical, as well as, specific symptoms are mentioned, e.g. yellowing of leaves instead of reddening on red cultivars–Aladasturi, Isabella and Tavkveri, whereas in Shavkapito and Saperavi the external manifestation of the disease begins directly with reddening of leaves. Yellowing of leaves on the cultivar Saperavi instead of reddening was mentioned by D. Giorgadze [20]. The definite causes of such modification of external symptoms are not known for us. It may be caused by different immunity of cultivars or existence of different strains. It was reported that in Eastern Georgia (Mtskheta district) on grapevine and bindweed two phytoplasma strains are associated with grapevine yellows '*Candidatus* Phytoplasma solani' (subgroup 16SrXII-A) and '*Ca*. P.a convolvuli' (subgroup 16SrXII-H) [22,23] respectively. Phytoplasmas are characterized by high degree of genetic differentiation [40] and GY are associated with genetically different phytoplasmas, the most important in Europe are Flavescence dorée (FD) and Bois noir (BN). There are two groups of FD – 16SrV-C and 16SrV-D, which differ from each other by geographic distribution [5,29,40–44]. The versatility of external symptoms of the disease suggests that in the endemic Georgian grapevine cultivars, except for the reported '*Ca*. P. solani' and '*Ca*. P. convolvuli' (subgroup 16SrXII-H), other groups of phytoplasmas should exist. The fact that the phytoplasma diseases in the main industrial cultivars in Georgia has not the character of epiphytoty [19,23], as for example, in France, Spain and Northern Italy [41,45–47], is especially noteworthy. It points out that the Georgian cultivars in general would have higher resistance against phytoplasmas. It may be caused by coevolution of endemic cultivars of grapevine and defined strains of phytoplasmas in a restricted geographic area. As it is known, the cultivars resistant to a disease are usually met in the natural epidemic source of origin of the cultivated plants, where evolution of parasite and its host-organism proceeds simultaneously [47]. The South Caucasus, in particular Georgia, is the oldest center of origin and domestication of grapevine [48].

The 13 species of cicadas registered in Georgia that are feeding on grapevine [20,49–52] deserve special investigation. This should be focused on the insect families Aphrophoridae, Cicadellidae, Cixiidae, Delphacidae, Derbidae, Dictyopharidae, Flatidae, Issidae, Psyllidae, and Ricaniidae, detected in the epidemic areas of grapevine phytoplasma diseases. The species composition of phytoplasmas depends on the vector insect that is the host organism for a phytoplasma [53,54]. An insect may transmit certain group of phytoplasmas. It has been already established that the BN disease is present and spread [19–23] in Eastern Georgia. *H. obsoletus*, found in large quantities in one epidemic area in Eastern Georgia – Sighnaghi district, must be considered the main insect vector candidate of BN. This insect is widespread in other regions as well [50–55], therefore *H. mlokosieviczi* is very interesting as also the representative of the genus *Hyalesthes* and *D. europaea*, known as grapevine phytoplasma vectors in Europe [56–59]. The existence of different groups of phytoplasmas adapted to various vector insects in Georgia is assumed. This idea fully agrees to the theory of coevolution of host and parasite. There is symbiotic interaction between phytoplasma and insect vectors [54–56]. *H. obsoletus* and *D. europaea* were not detected in Guria region, however out of the 14 feeding plant species of *M. pruinosa* and *R. japonica* 12 gave positive reaction with Dienes' staining, i.e. phloem contained phytoplasmas so both species of cicadas should be considered as potential vectors of phytoplasma diseases.

An effective measure of phytoplasma disease control is the selection of resistant genotypes and their propagation. Phytoplasmas are localized in the conductive system of a plant; they alter the process of formation of conductive bundles and leaf morphology on the early stage of differentiation [60,61]. In the visually diseased deformed and etiolated leaf the ratio of spongy-like and palisade-like tissues is lost [62], chlorophyll destroyed and chloroplasts are fragmented [33,62,63]; in the conductive system the number of vessel-fiber bundles and water-transmitting area is reduced [64,65]. The anatomical structure of the middle part of leaf petiole is a cultivar-specific character [65,66]. It was successfully used for selection of mulberry resistant to phytoplasma disease. This study shown that phytoplasma presence induces reduction of water-transmitting area in the middle part of grapevine leaf petiole. Such condition is accompanied by water deficit and arising of physiological dryness in mesophyll. Phytoplasmas cause disorders characteristic for water deficit in other species, e.g., closing of leaf stomata [33,65], inducing plasmolysis of chloroplasts, decay of chlorophylls, reduction of activity of ribulose-1,5-bisphosphate carboxylase, destruction of proteins in the membranes of thylakoids and water splitting system, accompanied by inactivation of photosystem II. The photosystem I is damaged only in severe diseases and only partially [63,64,67–71].

Decay of chlorophyll is a typical symptom of phytoplasma disease in many species, including grapevine [69,70,72]. Reduction of quantity of chlorophyll "a" per unit leaf area calculated per one leaf causes decreasing of the intensity of photosynthesis, generation of energy and mass, slowing of growth and reducing of immunity. Plant becomes small, thin and colorless, easily dies in winter. Photosynthesis in a leaf is weakened and respiration is intensified [62,70]. Sugar transportation from symptomatic leaves is hindered. Starch is accumulated in the mesophyll [33,61] and in the petiole of diseased leaf. Accumulation of starch is a signal of breaking down of sink–source system in the diseased grapevine [73].

Intensity of the disease depends on plant resistance. According to the percentage of damage in the leaves anatomy in the conditions of Guria the cultivar Noah (visually undamaged) is highly resistant, Aladasturi is relatively resistant (damage 25%) and Kachichi is moderately resistant (damage 50%). In the conditions of dry climate (Kakheti region) Rkatsiteli (damage 40%) and Saperavi (damage 50%) are moderately resistant. The higher ability of retention of plastid pigments by Rkatsiteli confirms the higher resistance of this cultivar in comparison with Saperavi.

Conclusion

The existence of the GY disease in Georgia is known from 2002. Nowadays this disease has been detected in four regions of Georgia. The symptoms of phytoplasma disease have been observed on 10 cultivars of grapevine, 6 of them – Rkatsiteli, Saperavi, Shavkapito, Tavkveri, Aladasturi and Goruli mtsvane – are Georgian endemics, four cultivars – Isabella, Ganjuri, Chardonnay and Noah – are introduced. The presence of diverse symptomatology suggests that different strains of phytoplasmas should exist in different regions of Georgia. Almost all studied asymptomatic cultivars are showing phytoplasma presence. Despite of this, epiphytoty is not present in Georgian industrial cultivars, that is the evidence of resistance of Georgian cultivars. The idea is also confirmed by the results these anatomical studies, according to which the damage of structure in the studied cultivars is less than 50%. We have recorded 12 species of cicadas on the investigated areas: *A. grylloides*, *C. viridis*, *D. europaea*, *E. vitis*, *E. imeretina*, *H. obsoletus*, *H. mlokosieviczi*, *L. coleoptrata*, *M. pruinosa*, *P. leporinus*, *P. spumarius*, and *R. japonica*. A number of these species of cicadas with high probability may be vectors of grapevine phytoplasma infection in Georgia.

The studied cultivars according to anatomical and physiological characteristics showed the following descending series of resistance: Noah, Aladasturi, Rkatsiteli, Kachichi, Saperavi. The analysis of results of study of the grapevine phytoplasma disease in Georgia during the last 10 years shows that it is necessary to continue comprehensive study of phytoplasma disease in all regions, in order to establish disease distribution and effective methods of control.

Acknowledgments

We would like to express our gratitude to Mr. Sergei Osipian (head of the laboratory of electron microscopy of the Agricultural University of Georgia) for the assistance.

REFERENCES

[1] Phytoplasmas and phytoplasma disease management: how to reduce their economic impact, in: Assunta Bertaccini (Ed.), Food and Agriculture COST Action FA0807 Integrated Management of Phytoplasma Epidemics in Different Crop Systems, Alma Mater Studiorum – University of Bologna, Italy, 2014, p. 288.

[2] A. Bertaccini, Current Status and Perspective of Phytoplasma Disease Research and Management. COST Action FA0807. Integrated Management of Phytoplasma Epidemics in Different Crop Systems, Publishing House of University of Bologna, Bologna, Italy, 2014.

[3] A. Bertaccini, B. Duduk, S. Paltrinieri, N. Contaldo, Phytoplasmas and phytoplasma diseases: a severe threat to agriculture, Am. J. Plant Sci. 5 (2014) 1763–1788.

[4] F. Quaglino, Y. Zhao, P. Casati, D. Bulgari, P.A. Bianco, W. Wei, R.E. Davis, '*Candidatus* Phytoplasma solani', a novel taxon associated with stolbur- and bois noir-related diseases of plants, Int. J. Syst. Evol. Microbiol. 63 (2013) 2879–2894.

[5] H.D. Mohr, Farbatlas Krankheiten, Schadlinge und Nutzlinge an der Weinrebe, Verlag, Stuttgart, 2005 (in German).

[6] 1st European Bois noir Work shop, Book of Abstracts, Weinsberg, Germany. 11.11–13.11, 2008, 33 pp..

[7] G. Belli, P.A. Bianco, M. Conti, Invited review grapevine yellows in Italy: past, present and future, J. Plant Pathol. 92 (2010) 301–324.

[8] I.A. Nakaidze, Soil Condition and Chlorosis of Grapevine in Georgia, Agricultural Institute of Georgia, Tbilisi, 1965 (in Russian).

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

[10] M. Kakulia, I. Chotorlishvili, H. Zedginidze, Z. Putkaradze, Mulberry Dwarf Disease, Methods of Diagnostics in Field, Agricultural Institute of Georgia, Tbilisi, 1979 (in Russian).

[11] M. Kakulia, Diseases of Mulberry, Agricultural Institute of Georgia, Tbilisi, 1983 (in Georgian).

[12] T.I. Zakarashvili, T.I. Skripal, N.I. Pavchenko, N.I. Mashkovskiy, Physic-chemical characters DNA of mycoplasmas-causative agents of mulberry dwarf disease, Microbiol. J. 45 (1983) 76–79 (in Russian).

[13] T.I. Zakarashvili, V.I. Prima, N.I. Pavchenko, L.P. Skripal, Genome size of mycoplasmas – causative agents of mulberry dwarf disease, Microbiol. J. 45 (1983) 49–52 (in Russian).

[14] A. Silvere, D. Giorgadze, Mycoplasma and virion like structures in salivary gland cells of *Hishimonus sellatus* Uhler., diseased with mulberry dwarf disease, Izvestia AN Estonia SSR, Biol. 23 (1978) 9–15 (in Russian).

[15] M. Kakulia, Recommendation–Materials on Resistance of Mulberry Varieties, Polyploids and Local Forms to Dwarf Disease, Agricultural Institute of Georgia, Tbilisi, 1982 (in Russian).

[16] D. Shalamberidze, N. Chkhaidze, Anatomical Method of Resistance Mulberry to Dwarf Disease, Serificate U1995000444, Tbilisi, Georgia, 1999, p. 14.

[17] N. Chkhaidze, Anatomical method for selection dwarf disease resistant mulberry (*Morus*), in: Proceedings of the Reports Presented at the International Jubilee Scientific Conference Problems of Maintenance and Utilization of Mulberry Silkworm Genetic Resources, September 25–29, Vratza, 2007, p. 101.

[18] N. Srinivasan, Simple diagnostic technique for plant disease of mycoplasmal etiology, Curr. Sci. 51 (1982) 883–885.

[19] N. Chkhaidze, I. Dzmanashvili, Danger of grapevine phytoplasma diseases in Georgia, Probl. Agric. Sci. 33 (2005) 71–73 (in Georgian).

[20] D. Giorgadze, New disease of grapevine in Georgia, Zas. Karantin Ras. 4 (2005) 45 (in Russian).

[21] D. Giorgadze, N. Zurabishvili, M. Oshkhereli, G. Aleksidze, Preliminary data for establish the etiology of grapevine disease turn red of leaf in Georgia, Bull. Acad. Agric. Sci. Geo 23 (2009) 116–118 (in Georgian).

[22] F. Quaglino, D. Maghradze, N. Chkhaidze, P. Casati, O. Failla, P.A. Bianco, First report of '*Candidatus* Phytoplasma solani' and '*Candidatus* Phytoplasma convolvuli' associated respectively with grapevine bois noir and bindweed yellows in Georgia, Plant Dis. 98 (2014), 1,151.2–1,151.2.

[23] F. Quaglino, N. Chkhaidze, D. Magradze, P. Casati, A. Ravazio, Surveys on Grapevine Yellows Incidence on Georgian Grapevine Varieties, COST, Conference abstracts, Oct. 2014, p. 43.

[24] A. Bertachinni, B. Duduk, Phytoplasma and phytoplasma disease: a review of recent research, Phytopathol. Mediterr. 48 (2009) 355–378.

[25] P.G. Weintraub, L.A. Beanland, Insect vectors of phytoplasmas, Annu. Rev. Entomol. 51 (2006) 91–111.

[26] I. Cordova, P. Jones, N.A. Harrison, C. Oropeza, *In situ* detection of phytoplasma DNA in embryos from coconut palms with lethal yellowing disease, Mol. Plant Pathol. 4 (2003) 99–108.

[27] A.J. Khan, S. Botti, A.M. Paltrinieri, A. Bertachinni, Phytoplasmas in alfalfa seedlings: infected or contaminated seeds?, in: 14th International Organization of

Mycoplasmology Conference, July 07–12, 2002, p. 6. Vienna, Austria.
[28] M. Algaghi, S. Massart, S. Steyer, M. Lateur, M. Haissam Jijakli, Study of diverse grafting techniques for their capability in rapid and efficient transmission of apple proliferation disease to different host plants, Bull. Insectology 60 (2) (2007) 381–382.
[29] S. Botti, A. Bertaccini, Phytoplasma infection trough seed transmission: further observations, in: 16th Congress IOM, Cambridge, 9–14 July, 2006, 113 pp.
[30] I.M. Lee, D.E. Gundersen-Rindal, R.E. Davis, I.M. Bartoszyk, Revised classification scheme of phytoplasmas based on RFLP analysis of 16S rRNA and ribosomal protein gene sequences, Int. J. Syst. Bacteriol. 48 (1998) 1153–1169.
[31] S.A. Hogenhout, K. Oshima, E.D. Ammar, S. Kakizawa, H.N. Kingdomand, S. Namba, Phytoplasmas: bacteria that manipulate plants and insects, Mol. Plant Pathol. 9 (2008) 403–423.
[32] I.M. Lee, R.E. Davis, T.A. Chen, L.N. Chiykowski, J. Fletcher, C. Hiruki, D.A. Schaff, A genotype-base system for identification and classification of mycoplasma like organisms (MLOs) in the aster yellows MLO strain cluster, Phytopathology 82 (1992) 977–986.
[33] N. Chkhaidze, Physiological mechanisms of resistance in mulberry (*Morus*) diseased with phytoplasma disease, in: International Scientific Conference “Genetical Resources of Cultivated Plants and Their Application in Agriculture”. Conference Papers. Gori, 2008, pp. 268–271 (in Georgian).
[34] S. Santi, F. De Marco, R. Polizzotto, S. Grisan, R. Musetti, Recovery from stolbur disease in grapevine involves changes in sugar transport and metabolism, Front. Plant Sci. 4 (2013) 1–12.
[35] N. Chkhaidze, Selection of dwarf disease resistant mulberry (*Morus*) forms by means of anatomical method, in: International Workshop on Silk Handcrafts Cottage Industries and Silk Enterprises Development in Europe, Central Asia and Near East, Bursa, Turkey, 6–10 March, 2006, pp. 26–27.
[36] N. Ketskhoveli, M. Ramishvili, D. Tabidze, Ampelography of Georgia, Publisher of the Georgian Academy of Sciences, Tbilisi, 1960 (in Georgian).
[37] Q. Tskhakaia, E. Mirianashvili, Plant Anatomy, Practical Course, Tbilisi State University, Tbilisi, 1957 (in Georgian).
[38] N.N. Tretijakov, Practicum in Plant Physiology, Agropromizdat, Moscow, 1990 (in Russian).
[39] N. Elerdashvili, Collection and Fixation of Insects (Methods), Agricultural Institute of Georgia, Tbilisi, 1975 (in Georgian).
[40] S.N. Borkhsenius, O.A. Chernova, V.M. Chernov, M.S. Vonskiy, Mycoplasmas: Molecular and Cellular Biology, Interaction with Immune System of Mammals, Pathogenicity, Diagnostics, Nauka, Saint-Petersburg, 2002 (in Russian).
[41] M. Martini, S. Botti, C. Marcone, C. Marzachì, P. Casati, P.A. Bianco, R. Benedetti, A. Bertaccini, Genetic variability among Flavescence dorée phytoplasmas from different origins in Italy and France, Mol. Cell. Probes 16 (2002) 197–208.
[42] B. Duduk, S. Botti, M. Ivanović, B. Krstić, N. Dukić, A. Bertaccini, Identification of phytoplasmas associated with grapevine yellows in Serbia, J. Phytopathol. 152 (2004) 575–579.
[43] A. Alma, R.E. Davis, M. Vibio, A. Danielli, D. Bosco, A. Arzone, A. Bertaccini, Mixed infection of grapevines in Northern Italy by phytoplasmas including 16S rRNA RFLP subgroup 16SrI-B strains previously unreported in this host, Plant Dis. 80 (1996) 418–421.
[44] A. Gajardo, N. Fiore, S. Prodan, S. Paltrinieri, S. Botti, A.M. Pino, A. Zamorano, J. Montealegre, A. Bertaccini, Phytoplasmas associated with grapevine yellows disease in Chile, Plant Dis. 93 (2009) 789–796.
[45] M. Engelbrecht, J. Joubert, T.J. Burger, First report of aster yellows phytoplasma in grapevines in South Africa, Plant Dis. 94 (2010) 373.
[46] E. Angelini, D. Clair, M. Borgo, A. Bertaccini, E. Boudon-Padieu, Flavescence dorée in France and Italy —occurrence of closely related phytoplasma isolates and their near relationships to palatinate grapevine yellows and an alder yellows phytoplasma, Vitis 40 (2001) 79–86.
[47] E. Torres, S. Botti, J. Rahola, M.P. Martin, A. Bertaccini, Grapevine yellows diseases in Spain: eight years survey of disease spread and molecular characterization of phytoplasmas involved, An. Jard. Bot. Madr. 62 (2005) 127–133.
[48] N.I. Vavilov, in: P.M. Jukovskij, F.X. Bakhteev (Eds.), Selected Works, vol. IV, Nauka, Moscow-Leningrad, 1964, pp. 314–435.
[49] S. Imazio, D. Maghradze, G. De Lorenzis, R. Bacilieri, V. Laucou, P. This, A. Scienza, O. Failla, From the cradle of grapevine domestication: molecular overview and description of Georgian grapevine (*Vitis vinifera* L.) germplasm, Tree Genet. Genomes 9 (2013) 641–665.
[50] Ir. Batiashvili, G. Dekanoidze, Entomology, Ganatleba, Tbilisi, 1974 (in Georgian).
[51] Mz.I. Lobjanidze, N.J. Robakidze, Carriers of disease of plants in Georgia, actual problems of parasitology in Georgia, in: Sh. Potskhveria (Ed.), Association of Parasitologists of Georgia. Proceedings Scientific Works, 2009, pp. 175–180. Sveti.
[52] N. Chkaidze, Mz Lobjanidze, Grapevine phytoplasma disease and its potential carriers in Georgia, in: Science Works of the International Conference, Kherson, Ukraine, 2012, pp. 149–152 (in Russian).
[53] H. Kawakita, T. Saiki, W. Wei, W. Mitsuhashi, K. Watanabe, M. Sato, Identification of mulberry dwarf phytoplasmas in the genital organs and eggs of leafhopper *Hishimonoides sellatiformis*, Phytopathology 90 (8) (2000 Aug) 909–914.
[54] N.M. Christensen, K.B. Axelsen, M. Nicolaisen, A. Schultz, Phytoplasmas and their interactions with their hosts, Trends Plant Sci. 10 (2005) 526–535.
[55] E. Gonella, I. Negri, M. Marzorati, M. Mandrioli, L. Sacchi, M. Pajoro, E. Crotti, A. Rizzi, E. Clementi, R. Tedeschi, C. Bandi, A. Alma, D. Daffonchio, Bacterial endosymbiont localization in *Hyalesthes obsoletus*, the insect vector of Bois Noir in *Vitis vinifera*, Appl. Environ. Microbiol. 77 (2011) 1423–1435.
[56] F. Lessio, A. Alma, Host plants and seasonal presence of *Dictyophara europaea* in the vineyard agro-ecosystem, Bull. Insectology 61 (2008) 199–200.
[57] P.G. Ploalie, C. Chireceanu, Experimental proofs regarding the association of cell wall deficient bacteria (mycoplasma like organisms, phytoplasmas) with grapevine yellows disease in Romania, Romanian Biotechnol. Lett. 17 (3) (2012) 7261–7269.
[58] C. Linder, M. Cavadini, *Dictyophara europaea* an alternative host of Flavescence dorée in Switzerland, IOBC WPRS Bull. 105 (2014) 191–194.
[59] L. Filippin, J. Jović, T. Cvrkovic, V. Forte, D. Clair, I. Toševski, E. Boudon-Padieu, M. Borgo, E. Angelini, Molecular characteristics of phytoplasmas associated with Flavescence dorée in clematis and grapevine and preliminary results on the role of *Dictyophara europaea* as a vector, Plant Pathol. 58 (2009) 826–837.
[60] N. Chkhaidze, Mitosis in mulberry (*Morus*) diseased with dwarf disease, Probl. Agric. Sci. 36 (2006) 54–55.
[61] N. Chkhaidze, N. Sichinava, Differentiation of conductive system in mulberry shoot diseased with dwarf disease, Probl. Agric. Sci. 37 (2006) 134–136.
[62] N. Kandelaki, Ratio of Palisade and Spongy Parenchima in Mesophyll of Mulberry Leaf Diseased with Dwarf Disease,

Questions of Sericulture in Georgia, Scientific Works, Tbilisi, 1986, pp. 116–121 (in Georgian).
[63] T.I. Zakarashvili, Electron-microscopic researches of mycoplasmas-causative agents of mulberry dwarf disease, Microbiol. J. 45 (1983) 71–76 (in Russian).
[64] M. Bertamin, N. Nedunchenzian, F. Tomasi, M.S. Grando, Phytoplasma [stolbur-subgroup (bois noir-BN)] infection inhibits photosynthetic pigments, ribulose-1,4-bisphosphate carboxylase snd photosynthetic activities in field grown grapevine (*Vitis vinifera* L. *cv*. *Chardonnay*) leaves, Physiol. Mol. Plant Pathol 61 (2002) 357–366.
[65] D. Shalamberidze, Selection of Mulberry Forms Resistance to Dwarf Disease by Means of Anatomical Structure (Dissertation), Tbilisi, 1998 (in Georgian).
[66] N. Chkhaidze, Leaf petiole structure of grapevine (*Vitis vinifera* L.) diseased with phytoplasma, Probl. Agric. Sci. 40 (2007) 67–70.
[67] T.V. Vekua, Leaf petiole architecture of grapevine varieties distributed in West Georgia, Sci. Works Georgian State Agric. Inst. 1 (1940) 41–45.
[68] N. Chkhaidze, Mycoplasmal infection and water economy in mulberry (*Morus*), Probl. Agric. Sci. (2001) 190–193 (in Russian).
[69] M. Bertamini, M.S. Grando, N. Nedunchezhian, Effects of phytoplasma infection on pigments, chlorophyll-protein complex and photosynthetic activities in field grown apple leaves, Biol. Plant. 47 (2003) 237–242.
[70] M. Bertamini, N. Nedunchezhian, Effects of phytoplasma [stolbur-subgroup (bois noir-BN)] on photosynthetic pigments, saccharides, ribulose 1,5-bisphosphate carboxylase, nitrate and nitrite reductases, and photosynthetic activities in field-grown grapevine (*Vitis vinifera* L. cv. Chardonnay) leaves, Photosynthetica 39 (2001) 119–122.
[71] Z. Khidesheli, Biochemical, physiological and ultrastructural changes in tissues of tomato diseased with mycoplasma, Sci. Works Acad. Agric. Sci. Georgia (1996) 191–194.
[72] N. Chkhaidze, CO_2 changes and chlorophyll content in different resistant mulberry (*Morus*) leaves diseased with phytoplasma, in: 5th BACSA International Conference “Sericulture for Multiproducts – New Prospects for Development.” Seriprodev, Bucharest, Romania, April 11th–15th, 2011, pp. 70–73.
[73] Yu.V. Gamalei, The nature of the trophic tract in vascular plants, Cytology 51 (2009) 375–387.