

Article

Performance of Georgian Grapevine Varieties in a Vineyard Infected by Flavescence Dorée Phytoplasma in Piedmont, Northwestern Italy

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Abstract

In Europe, Flavescence dorée (FD), the only epidemic disease within the phytoplasma-associated grapevine yellows complex (GY), reduces productivity and has a negative impact on berry composition and wine quality. Recent studies have shown that Georgian *Vitis vinifera* L. varieties have low susceptibility to Bois noir (BN), another GY disease. This study investigated the performance of some Georgian grapevine varieties in a highly FD-affected area in Piedmont (northwestern Italy), exploring their susceptibility to FD and testing their oenological potential through berry and wine quality analyses. Activities, conducted in a case-study vineyard containing central–western European, Georgian, and PIWI (fungus-resistant grape varieties) varieties, included field surveys and molecular analyses. Mortality and infection percentage index were significantly higher in Georgian and central–western European varieties, respectively. All Georgian varieties exhibited none or mild symptoms without a reduction in the number of symptomless berries. Only the FD phytoplasma (FDp) genotype M54 was identified in infected grapevines, suggesting that differences in symptom severity were related to a variety-specific response to infection. Despite infection, Georgian varieties maintained stable berry and wine quality parameters, showing no significant changes in acidity, sugar content, and flavor profile. Thus, Georgian varieties had great oenological potential and responded well to both FDp infection and local agroecosystem conditions.

Keywords: grapevine yellows; oenological potential; *map* gene

Academic Editors: Grazyna Podolska and Anna Szafranska

Received: 16 July 2025

Revised: 15 September 2025

Accepted: 19 September 2025

Published: 21 September 2025

Citation: Portaccio, L.; Paissoni, M.A.; Giacosa, S.; Passera, A.; Barbieri, C.; Maghradze, D.; Rolle, L.; Gerbi, V.; Failla, O.; Bianco, P.A.; et al. Performance of Georgian Grapevine Varieties in a Vineyard Infected by Flavescence Dorée Phytoplasma in Piedmont, Northwestern Italy. *Agriculture* **2025**, *15*, 1988. <https://doi.org/10.3390/agriculture15181988>

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1. Introduction

Phytoplasmas are cell wall-less bacteria of the Mollicutes class. They are phloem-restricted obligate intracellular parasites of a wide variety of plants, in which they cause numerous diseases [1]. They are transmitted from plant to plant by phloem-feeding insect

vectors [2]. Based on molecular and biological features, phytoplasmas are classified into species within the provisional genus ‘*Candidatus Phytoplasma*’; moreover, taxonomic groupings have been defined by similarity coefficients calculated on collective enzymatic digestion profiles of 16S rRNA gene amplicons [3,4].

In Europe, Flavescence dorée (FD) is the only epidemic and the most economically damaging disease within the phytoplasma-associated grapevine yellows complex (GY) [5]. FD is caused by a phytoplasma known as grapevine Flavescence dorée phytoplasma (FDp), belonging to the taxonomic subgroups 16SrV-C and -D [6]. FD affects various parts of the vine, including leaves, branches, and bunches, manifesting symptoms such as the following: (i) chromatic changes in the leaf lamina (including the veins) and rolling/curling of the leaf margins, (ii) lack of lignification and the appearance of blackish pustules on shoots, and (iii) dehydration, desiccation (in June/July), and withering (in August/September) of the bunches [7]. Moreover, FD causes significant growth delays of affected plants and, during veraison, induces marked reductions in budburst rate, fertility index, leaf area, and chlorophyll content. Furthermore, infected plants exhibited a severe drop in productivity, mainly due to the decreased number of bunches. Microscope analysis of leaf tissues from diseased plants revealed ultrastructural changes, abnormal callose buildup in sieve plates, and increased lipid/plastoglobuli content in chloroplasts within phloem parenchyma cells [8]. Moreover, ultrastructural changes also have been observed in xylem tissues of FD-affected vines [9]. Such changes can partly explain the disease’s negative effects on plant growth and yield.

Berry quality parameters do not seem to be altered by FD [8], but only a few studies have thoroughly investigated this aspect. Interestingly, several studies have focused on berry and wine quality from vines affected by Bois noir (BN), another disease of the GY complex widespread in Europe and in other continents that is associated with ‘*Candidatus Phytoplasma solani*’ [10]. Analyses of wines produced from grapes of BN-infected vines showed decreased alcohol, epicatechin, and iron contents, along with increased organic acids, titratable acidity, catechin, and calcium contents. Sensory evaluation confirmed these wines had unfavorable characteristics, such as higher acidity, bitterness, and often pinkish discoloration. The negative impact on berry composition and wine quality was particularly pronounced in vintages with favorable weather conditions for grapevine production. In contrast, the negative effects of BN infection were less noticeable or even masked in vintages with unfavorable weather (wet and cool) [11].

FDp is transmitted from vine to vine by the vector *Scaphoideus titanus*, which completes its life cycle solely on grapevines [12]. Given its highly epidemic nature, FDp is classified as a quarantine pathogen. Its containment is regulated by a mandatory control decree that includes insecticide treatments targeting *S. titanus* populations and the uprooting of symptomatic vines [7]. Recent studies have provided significant insights into FD epidemiology: (i) extensive genetic diversity among FDp and related strains has been revealed through nucleotide sequence analyses of variable genes (e.g., *map* and *vmp*) [13–18], (ii) epidemic FDp strains are primarily transmitted by *S. titanus*, but some can be spread from other plant hosts (such as *Alnus glutinosa* and *Clematis vitalba*) to grapevines by alternative vectors like *Dictyophara europaea*, *Orientus ishidae*, and *Allygus mixtus* [17,19], (iii) FDp-related strains can move from alder to grapevine via the vector *Oncopsis alni*, although these non-epidemic strains associated with Palatinate grapevine yellows (PGY) are not transmitted by *S. titanus* [17], (iv) potential vectors (*Phlogotettix cyclops*, *Hishimonus hamatus*) have been identified as capable of acquiring FDp under controlled conditions [17,20,21], and (v) there is a growing list of FDp-host plants (e.g., *Ailanthus altissima*, *Corylus avellana*, *Salix* spp.) [14,18,22]. These findings demonstrate that FD epidemiology extends beyond the simple grapevine-*S. titanus* pathosystem to include additional vectors and reservoir plants. Although these alternative vectors may not transmit FDp from vine

to vine, they ensure the pathogen's continuous presence within vineyards, facilitating its potential rapid spread by *S. titanus*. The withdrawal of broad-spectrum insecticides used in viticulture against leafhoppers might also contribute to the frequent FD recrudescence observed in recent years. This dynamic evolution of the FD epidemiology scenario underscores its increasing complexity [14,17,18,22]. Thus, studying host plants, potential vectors, and FDp strains in the vineyard agro-ecosystem is essential for understanding FD epidemiology and devising effective intervention strategies.

An ambitious strategy for controlling GY phytoplasma diseases involves selecting plant varieties that are resistant, tolerant, or not susceptible, whether cultivated or wild, to serve as sources of resistance genes for breeding programs. Previous studies have identified plant species or varieties with low susceptibility to phytoplasma infections [23]. However, so far, none of the examined *Vitis* spp. and *Vitis vinifera* L. varieties have shown immunity or strong resistance to the phytoplasma associated with grapevine yellows (GY) [24].

Over the past decade, Georgian native germplasm, including over 500 cultivars exhibiting a unique genetic pool [25,26], has been the focus of extensive research [27]. Furthermore, recent studies have indicated that grapevine varieties selected in domestication centers of grapevine such as Georgia show tolerance or resistance to plant pathogens like *Plasmopara viticola* (Berk. & M. A. Curtis) Berl. & De Toni, which causes downy mildew [28,29] and *Erysiphe necator* Sch., which causes powdery mildew [30]. Surveys conducted in the vineyards of the Kakheti and Shida (Inner) Kartli regions in eastern Georgia revealed a widespread presence of Bois noir (BN), while Flavescence dorée (FD) was not reported [31]. Most native Georgian grapevine cultivars displayed only mild symptoms of BN and maintained full berry production, whereas internationally known cultivars showed severe symptoms [31]. As widely documented for phytoplasma-associated diseases, symptom severity in infected plants can be influenced by both the pathogen's virulence and the plant host's susceptibility [32,33]. Molecular characterization and phylogenetic analyses revealed that BN phytoplasma strains in Georgia form a bindweed-related population genetically distinct from those found in central–western Europe. Notably, the presence of the same phytoplasma strain in grapevine cultivars with varying symptom severity suggested that Georgian local cultivars have low susceptibility to BN [31].

Based on such evidence of low susceptibility to BN of Georgian grapevine cultivars in the South Caucasus region, the present study aimed to investigate the adaptability of some Georgian *V. vinifera* varieties to grow in a case-study vineyard located in a highly FD-affected area in Piedmont (northwestern Italy), exploring their susceptibility to FD and testing their berry and wine quality.

2. Materials and Methods

The study was based on (a) monitoring and mapping grapevine yellows symptoms exhibited by three groups of grapevine varieties [central–western European, Georgian, and PIWI (fungus-resistant grape varieties obtained through crossbreeding between European *Vitis vinifera* vines and disease-resistant American or Asian *Vitis* species)], (b) identifying the grapevine-infecting phytoplasma(s) by molecular analyses, (c) evaluating berry and wine quality from Georgian varieties.

2.1. Characteristics of the Case-Study Vineyard

The investigation was conducted in a vineyard located in Mombaruzzo (AT) (44°45'40" N, 8°24'20" E; Piedmont Region, northwestern Italy), within an area known to be highly affected by FD [16,22]. In the vineyard, rows were north–south oriented and grapevines were trained using the one-side Guyot system (distance between rows 2.8 m; plant distance along the row 1.0 m). The vineyard was bordered by buildings (houses) to

the north, by other vineyards to the east and to the west, and by a forest (including plants species previously reported as FDp hosts, such as *Ailanthus altissima*, *Corylus avellana*, *Juglans regia*, *Prunus avium*, *Robinia pseudoacacia*, and *Sambucus nigra* [14,22]) to the south. In the vineyard, *S. titanus* populations were controlled through mandatory insecticide treatments carried out at least twice a year according to the indications by Regional Phytosanitary Service. The vineyard, planted in 2013, was divided into three distinct sectors: the first one including 10 locally cultivated western European grapevine varieties [8 Piedmont native (Barbera, Brachetto, Cortese, Dolcetto, Freisa, Grignolino, Moscato bianco, Nebbiolo) and 2 French (Chardonnay, Pinot noir) cultivars]; the second one including 12 Georgian native grapevine varieties (Aladasturi, Alexandrouli, Chinuri, Goruli Mtsvane, Okhtoura, Ojaleshi, Paneshi, Rkatsiteli, Sapena, Saperavi Atenis, Shavkapito, Tchvitiluri); the third one including 16 grapevine varieties furnished by Vivai Cooperativi Rauscedo (VCR), located in a village Rauscedo, UD, Italy [12 PIWI varieties: 18-080 (Tocai x Bianca), 80-024 (Tocai x Bianca), 80-100 (Tocai x Bianca), Early Sauvignon (Sauvignon Blanc x Kozma 20-3), Fleurtai (Tocai x Kozma 20-3), Julius (Regent x Kozma 20-3), Petit Cabernet (Cabernet Eidos: Cabernet Sauvignon x Bianca), Petit Sauvignon (Sauvignon Nepis: Sauvignon x Bianca), Petra (Kunbarat x Pinot Noir), Royal Cabernet (Cabernet Volos: Cabernet Sauvignon x Kozma 20-3), Soreli (Tocai x Kozma 20-3), Viorica (Seibel 13-666 x Aleatico); two European varieties: Palava (Czech cultivar, central Europe) and Tocai friulano (northeastern Italy cultivar, western Europe); two Georgian native varieties: Mtsvane Kakhuri and Saperavi)]. PIWI varieties, utilized in this study, were previously selected by VCR (PIWI-VCR) through breeding programs for carrying resistance genes (coming from wild American or Asian grapevine species) to *Plasmopara viticola* and/or *Erysiphe necator* (aetiological agents of downy and powdery mildew, respectively). The two Georgian varieties (Mtsvane Kakhuri and Saperavi) and the two European varieties (Palava and Tocai friulano) by VCR were grouped with the other Georgian varieties and the other locally cultivated western European varieties, respectively, in results interpretation and statistical analyses. The group including central–western European varieties will be referred as ‘European’ varieties from this point. In each vineyard sector, grapevine varieties were planted based on a randomized block scheme in which three blocks per variety (10 plants per block) were used, except for Barbera (nine blocks) and Moscato bianco (six blocks) (Figure 1).

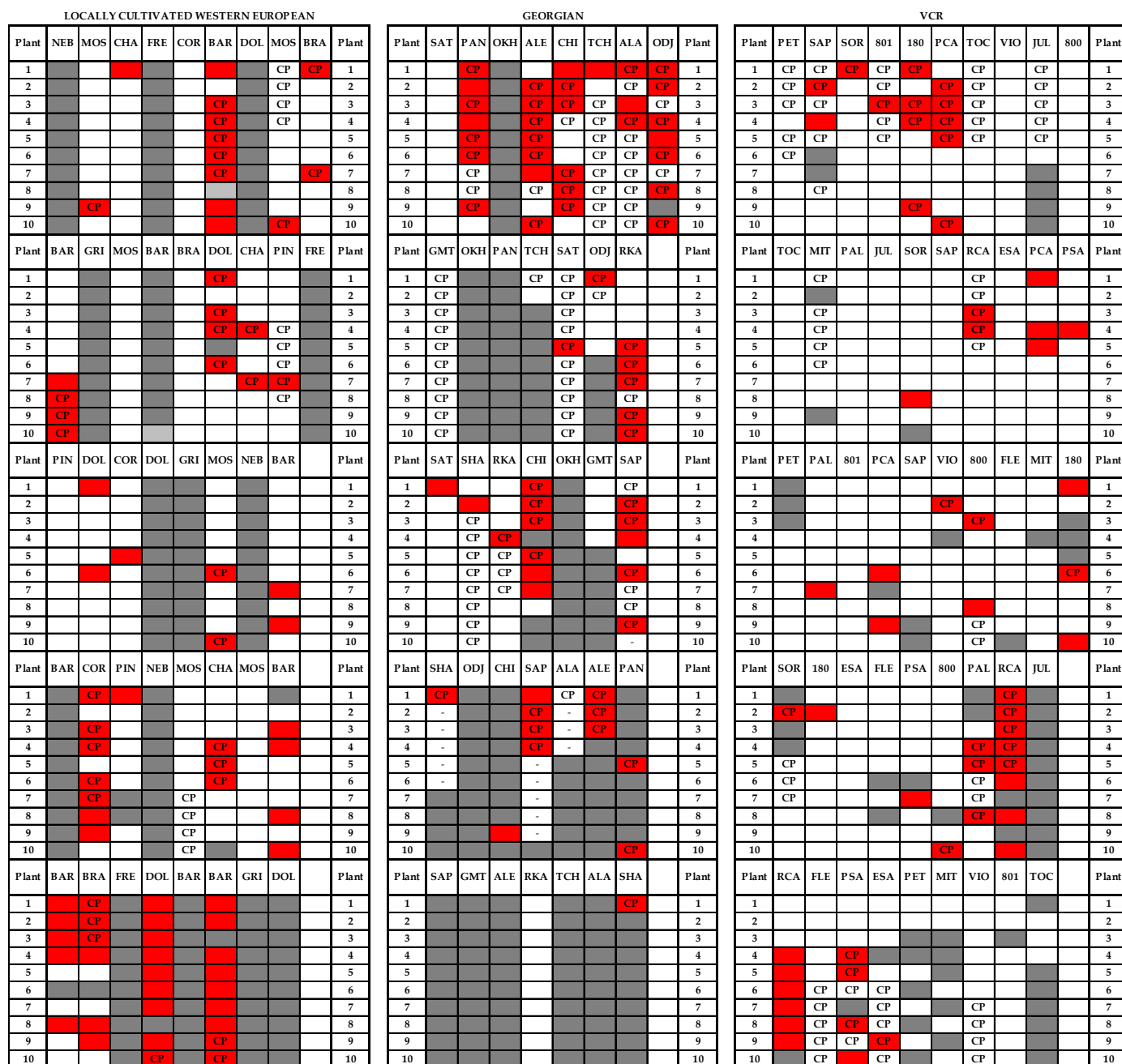


Figure 1. Schematic representation of the case-study vineyard in Mombaruzzo. In each sector [locally cultivated western European, Georgian, VCR (12 PIWI-VCR, two Georgian, and two European varieties)], the positions of variety-related blocks are defined by variety name abbreviations as follows: (i) native (BAR, Barbera; BRA, Brachetto; CHA, Chardonnay; COR, Cortese; DOL, Dolcetto; FRE, Freisa; GRI, Grignolino; MOS, Moscato bianco; NEB, Nebbiolo; PIN, Pinot noir); (ii) Georgian (ALA, Aladasturi; ALE, Alexandrouli; CHI, Chinuri; GMT, Goruli Mtsvane; OKH, Okhtoura; ODJ, Odjaleshi; PAN, Paneshi; RKA, Rkatsiteli; SAP, Sapena; SAT, Saperavi Atenis; SHA, Shavkapito; TCH, Tchvitiluri); (iii) VCR (PIWI-VCR: 180, 18-080; 800, 80-024; 801, 80-100; ESA, Early Sauvignon; FLE, Fleurta; JUL, Julius; PCA, Petit Cabernet; PSA, Petit SauvignoN; PET, Petra; RCA, Royal Cabernet; SOR, Soreli; VIO, Viorica. Georgian: MTS, Kakhuri_VCR; SAP, Saperavi_VCR. European: PAL, Palava_VCR; TOC, Tocai friuliano_VCR). In the vineyard scheme, each box represents a plant and its color is related to the plant healthy condition: white box, symptomless plant; grey box, dead/absent plant; red box, symptomatic plant. In the vineyard map, the position (box) of collected grapevine is indicated by the acronym CP (collected plant).

2.2. Symptom Observation and Plant Sampling

Surveys on GY symptoms were carried out in September 2017. The inspection was carried out by two people inspecting both sides of the grapevine plants in order to evaluate the presence of dead plants and accurately check the presence and the severity of GY symptoms and, at the same time, exclude other causes of similar symptoms (e.g., leaf color alteration and curling caused by incisions on the upper part of the vine canes made by *Stictocephala bisonia* females and larval bites). Plant mortality was calculated as the percentage of dead plants out of the planted ones. GY incidence was calculated as the percentage of symptomatic vines out of the observed ones for each variety. The severity of symptoms was classified and sorted according to a GY symptom scale from 0 to 2, modified from the ones previously proposed [34,35], as follows: (i) symptom severity class 0 = plants with no symptoms; (ii) symptom severity class 1 = plants with leaf symptoms; (iii) symptom severity class 2 = plants with leaf symptoms and berry shrivel. Starting from severity classes, infection percentage index (I%I) was calculated using the formula by Townsend and Heuberger [36] as follows: $I\%I = \sum i1(n_i \times v_i)/N \times V$, where v_i is the symptom severity class, n_i is the number in one class, N is the total number, V is the highest class, and i is the number of classes. One-way ANOVA followed by post hoc Tukey test ($p < 0.05$) was used to compare plant mortality and I%I between varieties and variety groups (European, Georgian, PIWI-VCR). Statistical analyses were performed using the software SPSS version 27 (IBM, Armonk, NY, USA).

For each examined variety, petioles from five leaves per plant were collected from about five (European and PIWI-VCR) and ten (Georgian) vines showing GY symptoms. If no symptomatic vines were present for a certain variety, leaf petioles were collected from symptomless plants. In detail, leaf petioles were collected from 245 grapevines (60 plants of European varieties, 120 plants of Georgian varieties, 65 plants of PIWI-VCR varieties). Collected samples, kept at 4 °C, were transferred to laboratory (DiSAA, University of Milan), washed, weighed (1 g per sample), placed in extraction plastic bags (BIOREBA AG, Reinach, Switzerland), and stored at −30 °C until molecular analyses.

2.3. Identification and Genotyping of GY-Associated Phytoplasmas

Total DNA was extracted from examined plants as previously described [5], with some modifications. Briefly, prewarmed cetyltrimethylammonium bromide (CTAB)-based buffer (2.5% [wt/vol] CTAB, 100 mM Tris [pH 8.0], 1.4 M NaCl, 50 mM EDTA [pH 8]) was added to leaf petioles (1 g) within the extraction plastic bags, homogenized by mechanical pestle (SEDIAG SAS, Bretenière, France), and held at 60 °C for 20 min. After incubation, DNA was extracted by adding chloroform/isoamyl alcohol (24:1, vol/vol) solution and precipitated by incubation with isopropanol at −20 °C for 20 min. A nucleic acid pellet was washed with 70 and 80% ethanol, air dried, suspended in 150 µL of deionized autoclaved water, measured for quality and concentration by Nanodrop system, and maintained at −30 °C until use.

Total DNAs (50 to 100 ng), extracted from the samples collected from the 245 examined vines, were utilized as templates in nested PCR reactions conducted for detecting (i) phytoplasmas belonging to taxonomic group 16SrV (including FDp) through the amplification of *map* gene performed using the primer pair FD9F5/MapR1 in direct PCR, followed by nested PCRs with the primer pair FD9F6/MapR2 [13], and (ii) '*Ca. P. solani*' (associated with BN) through the amplification of *stamp* gene, performed using the primer pair StampF/StampR0 in direct PCR, followed by nested PCRs with the primer pair StampF1/StampR1 [37]. Primer sequences and reaction conditions were as previously described [13,37].

Total nucleic acids from periwinkle plants infected by phytoplasma strains STOL ('*Ca. P. solani*', subgroup 16SrXII-A, Acc. No. AF248959, [9]), AY1 ('*Ca. P. asteris*',

subgroup 16SrI-B, Acc. No. M30790, [38]), and EY1 ('*Ca. P. ulmi*', subgroup 16SrV-A, Acc. No. AY197655, [39]) were used as reference controls. The reaction mixture devoid of nucleic acids was used as the negative control. PCR products were verified by electrophoresis on 1% agarose gel in TBE buffer and visualized under a UV transilluminator.

FD9F6/MapR2 amplicons were sequenced in both strands (2X coverage per base position) by a commercial sequencing service (Eurofins Genomics, Germany). Obtained nucleotide sequences were assembled by the Contig Assembling Program and trimmed to the annealing sites of the FD9F6 and MapR2 primers in the software BioEdit version 7.2.6 [40]. *Map* gene nucleotide sequences, obtained in this study from 16SrV phytoplasma strains identified in grapevines, were aligned using the ClustalW Multiple Alignment program in the software BioEdit and analyzed by Sequence Identity Matrix to calculate their genetic diversity. Finally, *map* sequence variants were aligned with representative sequences of 157 *map* genotypes previously defined within taxonomic subgroup 16SrV [13–19,22,41,42], and their evolutionary relatedness and genealogy were defined through genotype networks generated using the software PopART version 1.7 (<http://popart.otago.ac.nz>, accessed on 22 October 2024) by performing a median-joining (MJ) calculation, maintaining the parameter $e = 0$. The generated overall network covered the *map* genotypes described in this and previous studies and included their main biological features.

2.4. Grape Sampling and Berry Analyses

The Georgian white (Goruli Mtsvane, Mtsvane Kakhuri, Rkatsiteli, Sapena, and Tchvitiluri) and red (Aladasturi, Odjaleshi, Paneshi, Saperavi, and Shavakapito) varieties were subjected to grapes analyses, as well to wine production and analyses to evaluate their oenological potential and features. Concerning grapes, the method used was to sample five clusters for each replicate. The number of invaiate berries and the number of total berries were counted for each cluster. The veraison berries were evaluated from the point of view of color and mechanical texture. Next, the percentage of veraison of each cluster was measured. At the end of veraison, representative samples of the clusters were obtained for each replicate after refractometric analysis. The samples were brought to the laboratory, pressed, and their juice was used to analyze the total soluble solids (by refractometry, expressed as Brix), pH under stirring [43], and total acidity by acid–base titration [44], the latter expressed in g/L as tartaric acid.

A batch of ten berries was also obtained, weighed to obtain the average berry weight, and placed in a freezer to evaluate the polyphenolic profile of the skin and seeds at the laboratories of the University of Milan at the Department of Agricultural and Environmental Sciences. The method for the evaluation of total anthocyanins consists of separating the berry from the skin and seeds, which were weighed for the determination of these average values [45]. The skins were immersed in tubes with 20 mL of ethanol hydrochloric (70% ethanol, 30% water with the addition of 1% concentrated (37%) hydrochloric acid) and made to rest 16–19 h in the dark to avoid oxidation. The same procedure applies to seeds, counting them before being infused. After the elapsed time, the test tubes were opened and the solid part removed to obtain an extract to be analyzed through spectrophotometry. By means of the analysis of the skins, the total anthocyanin content was assessed with a 540 nm reading after proper dilution (150 μ L extract in 10 mL total volume of hydrochloric ethanol solution). The measured absorbance value gives the total anthocyanin index (TA) expressed in mg/L as malvidin-3-O-glucoside, with the following relationship: $TA \text{ (mg/L)} = E_{540 \text{ nm}} \times 16,17 \times d$. The result was then converted to mg/kg berries.

To evaluate the total polyphenol index, the method by [45] was used. Skin and seed polyphenol extracts were diluted in 0.5 mL of 30 μ L with 470 μ L of ethanol hydrochloric

and 100 µL with 400 µL of ethanol hydrochloric, respectively, and the addition of 3.5 mL of water and 0.5 mL of Folin–Ciocalteu reagent. The solution was subsequently added with 2 mL of 10% Na₂CO₃ and a further 3.5 mL of water to obtain a final volume of 10 mL. After 30 min, the spectrophotometric reading was carried out at 700 nm. The final polyphenol index of the skin or seeds was obtained in mg/L as (+)-catechin with the following relationship: PB (mg/L) = 186.5 × E700 nm × d, and then converted into mg/kg berries.

2.5. Winemaking Protocol of Georgian Varieties

At technological maturity, the grapes (10–25 kg) were harvested and processed at the University of Turin experimental cellar ‘Bonafous’ in Chieri (Italy) using standardized procedures in glass containers. Two different protocols were applied for white or red varieties.

White varieties (Goruli Mtsavane, Mtsvane Kakhuri, Rakatsiteli, Sapena, Tchvitiluri) were harvested and the grapes kept in a thermo-controlled room at 0 °C overnight, prior to be destemmed and crushed (TEMA destemmer–crusher, Enoveneta, Piazzola Sul Brenta, Italy), added of 35 mg/L of SO₂ and then inoculated with 20 g/hL of *Saccharomyces cerevisiae* active dry yeast (Fermol Chardonnay, AEB Group, Brescia, Italy) and 20 g/hL of GoFerm and 15 g/hL of Fermaid E (Lallemand Inc., Montreal, QC, Canada) yeast autolysate and yeast nutrition supplementer. Skin contact was performed for 48 h at a 20–25 °C temperature, and then the juice–wine was devatted, continuing the fermentation process at the same temperature range. An addition of nitrogen was performed at ¼ of the alcoholic fermentation with 5 g/hL of Aleavit One (Alea Evolution, Molinella, Italy). At the end of alcoholic fermentation, the wine was racked, added of 70 mg/L of SO₂, and treated with 3 g/hL of bentonite. The clear wine was subsequently bottled.

For red varieties (Aladasturi, Odaleshi, Paneshi, Saperavi, Shavakapito), the grapes were picked at technological ripeness and stored at 15 °C overnight in a thermo-controlled room prior to being destemmed and crushed. Sulphur dioxide and yeast inoculation were performed in the same way as the white wine protocol, but by using a different active dry yeast (*S. cerevisiae* F15, Laffort, Bordeaux, France). The maceration–fermentation was performed at a 20–25 °C temperature for six days, with cap management consisting in punching downs (twice a day for the first three days) and then pumping overs (twice a day for the remaining three days). Finally, the wine was devatted, and malolactic fermentation was performed through *Oenococcus oeni* addition (Malotabs, dosage equivalent to >10¹¹ colony forming units/hL; Lallemand Inc., Montreal, QC, Canada). When the malic acid was completely consumed, the wines were racked and added to 50 mg/L of SO₂, and subsequently bottled. Both white and red wines were neither subjected to tartaric stabilization nor filtration treatments.

2.6. Wine Analysis

The bottled wines were analyzed for basic parameters and polyphenolic characterization. pH and total acidity were assessed using the same methods of juice analysis [43,44]. Organic acids, residual sugars, ethanol, and glycerol were determined by an Agilent HPLC 1260 coupled with a UV–Refractive Index detector (Agilent Technologies, Santa Clara, CA, USA), equipped with a 300 × 7.8 mm cation exchange column (Aminex HPX-87H) and a Cation H⁺ Microguard cartridge (Bio-Rad Laboratories, Hercules, CA, USA) using the chromatographic conditions reported by [46].

For red wines, the polyphenolic characterization was performed with a UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The analysis involved total anthocyanin (TA) and total flavonoid (TF) indexes obtained by wine dilution in hydrochloric ethanol solution and expressed as mg/L of malvidin-3-O-glucoside chloride and mg/L of (+)-catechin, respectively [47]. Total polyphenol index (TPI) was obtained by the Folin–

Ciocalteu method and expressed as mg/L of (+)-catechin [47]. Color characteristics were calculated from the spectra acquired in the Vis (360–800 nm) as color intensity ($I = A_{420} + A_{520} + A_{620}$), hue ($T = A_{420}/A_{520}$) [48], and CIELab coordinates [49]. Lightness (L^*), red/green color coordinate (a^*), and yellow/blue color coordinate (b^*) are reported. Monomeric anthocyanin profile was investigated using an Agilent 1260 HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a RP-18 column (5 μ m, 25 $\times$ 0.4 cm; Merck, Darmstadt, Germany), and a diode array detector (DAD), with the chromatographic conditions reported in [50]. Prior to injection, the wine samples were purified using solid phase extraction (1-g C18 Sep-Pak cartridges, Waters Corporation, Milford, MA, USA), the anthocyanins recovered with methanol, evaporated in a rotary evaporator, reconstituted in methanol/ H_3PO_4 10^{-3} mol/L (60:40 v/v), and filtered with PTFE 0.45 μ m filter membranes. Data were elaborated using Agilent ChemStation software version 2.2 (Agilent Technologies, Santa Clara, CA, USA), and expressed as percentage of individual anthocyanins calculated as peak area at 520 nm wavelength.

2.7. Sensory Analysis of Wines

The experimental wines were subjected to an informed sensory evaluation by a panel of sixteen tasters conducted at the University of Milan. The assessors were asked to evaluate the wines with two different tasting sheets, one sheet for red wines and one for white wines. For both types of wines, an unstructured scale of 10 cm has been used for the evaluation of color intensity and color hue, in-mouth properties (namely acidity, bitterness, astringency, body, and balance). Regarding aroma characteristics, the assessors were asked to mark the presence/absence of five attributes (red fruit, black fruit, floreal, vegetal, spice). In the white wine evaluation forms, red and black fruit descriptors were replaced by fruity and citrus (e.g., lemon and orange). Scale-rated attributes are reported as the average of the sixteen evaluators, while aroma descriptors are reported by citation frequency.

3. Results

3.1. Incidence of Grapevine Yellows

Surveys conducted in the case-study vineyard showed that 32% (398 out of 1260) of the examined plants were dead: 43% (179 out of 420) for Georgian varieties, 35% (169 out of 480) for European varieties, and 14% (50 out of 360) for PIWI-VCR varieties (Table 1). Statistical analyses, carried out considering the variety groups (European, Georgian, PIWI), showed that mortality of Georgian varieties was significantly higher in comparison with European and PIWI-VCR varieties, which had no significant difference (Figure 2A). However, statistical analyses, carried out considering the single varieties, did not evidence any significant difference (Figure 2B). The following varieties were not evaluated due to their absence (mortality 100%): Nebbiolo, Freisa, and Grignolino (European), and Okhtoura (Georgian).

Based on the observation of available plants, it was found that 22% (189 out of 862) of the present grapevines showed typical GY symptoms: 27% (64 out of 241) for Georgian varieties, 25% (79 out of 311) for European varieties, and 15% (46 out of 310) for PIWI-VCR varieties (Table 1). Within European varieties, the most relevant symptoms in the red berry varieties were curling of the leaves downwards into a triangular shape, non-woody shoots, and shriveled bunches. In contrast to the others, cv. Barbera and Dolcetto had a more intense red coloration of the leaves, while Cortese and Brachetto plants showed symptoms only in one shoot. For the white berry varieties, Moscato bianco showed non-lignified shoots, leaf curling, and perinerv yellowing. As all the symptomatic plants showed total absence or a very limited presence of berries, symptoms observed in all the

locally cultivated European varieties were classified in severity class 2 (Figure 3A–C). Interestingly, Tocai friulano did not exhibit any symptoms. Within PIWI-VCR varieties, 80-024, Fleurtaï, Julius, and Petra did not show any GY symptoms. Symptoms affected the whole plant only in 80-100 and Petit Sauvignon. The most affected varieties were 18-080, Petit Cabernet, and Royal Cabernet. On Early Sauvignon and Petit Cabernet, symptoms were present on the whole canopy but the bunches were not desiccated (severity class 1). All the other PIWI-VCR varieties showed total absence or very limited presence of berries; symptoms observed in such PIWI-VCR varieties were classified in severity class 2 (Figure 3D–F). Within Georgian varieties, Goruli Mtsvane and Mtsvane Kakhuri did not show any GY symptoms. The variety Chinuri showed leaf curling and color changes, un lignified shoots and nodes on the whole canopy. Only a few plants of the varieties Alexandrouli, Paneshi, and Odjaleshi could be evaluated as cause of death. In the last two, the bunches had no symptoms that could be traced back to GY. For the varieties Aladasturi, Saperavi, Saperavi Atenis, Shavkapito, and Tchvitiluri, most of the vines were asymptomatic and only a few showed mild symptoms. As all the symptomatic plants maintained an unaltered presence of berries, symptoms observed in all the Georgian varieties were classified in severity class 1 (Figure 3G–I).

Table 1. Surveys on GY symptoms in European, Georgian, and PIWI-VCR varieties in Mombaruzzo vineyard. n.a., not available.

Group	Variety	Number of Grapevines				Mortality (%)	GY Incidence (%)	Symptom Severity Class
		Planted	Dead	Observed	Symptomatic			
European	Barbera	90	34	56	32	38	57	2
	Brachetto	30	1	29	8	3	28	2
	Chardonnay	30	1	29	6	3	21	2
	Cortese	30	0	30	8	0	27	2
	Dolcetto	60	32	28	15	53	54	2
	Freisa	30	30	0	n.a.	100	n.a.	n.a.
	Grignolino	30	30	0	n.a.	100	n.a.	n.a.
	Moscato bianco	60	0	60	4	0	7	2
	Nebbiolo	30	30	0	n.a.	100	n.a.	n.a.
	Palava_VCR	30	2	28	4	7	14	2
	Pinot noir	30	2	28	2	7	7	2
	Tocai friulano_VCR	30	7	23	0	23	0	0
Total		480	169	311	79	35	25	
Georgian	Aladasturi	30	16	14	3	53	21	1
	Alexandrouli	30	17	13	10	57	77	1
	Chinuri	30	12	18	11	40	61	1
	Goruli Mtsvane	30	16	14	0	53	0	0
	Mtsvane Kakhuri_VCR	30	8	22	0	27	0	0
	Odjaleshi	30	16	14	8	53	57	1
	Okhtoura	30	30	0	n.a.	100	n.a.	n.a.
	Paneshi	30	18	12	9	60	75	1
	Rkatsiteli	30	0	30	6	0	20	1
	Sapena	30	11	19	9	37	47	1
	Saperavi_VCR	30	4	26	2	13	8	1
	Saperavi Atenis	30	0	30	2	0	7	1
	Shavkapito	30	13	17	3	43	18	1
	Tchvitiluri	30	18	12	1	60	8	1
Total		420	179	241	64	43	27	
PIWI-VCR	18-080	30	3	27	8	10	30	2
	80-024	30	10	20	0	33	0	0
	80-100	30	1	29	1	3	3	2
	Early Sauvignon	30	1	29	1	3	3	1
	Fleurtaï	30	3	27	0	10	0	0

Julius	30	14	16	0	47	0	0
Petit Cabernet	30	0	30	10	0	33	2
Petit Sauvignon	30	2	28	6	7	21	2
Petra	30	8	22	0	27	0	0
Royal Cabernet	30	3	27	16	10	59	1
Soreli	30	4	26	3	13	12	2
Viorica	30	1	29	1	3	3	2
Total	360	50	310	46	14	15	

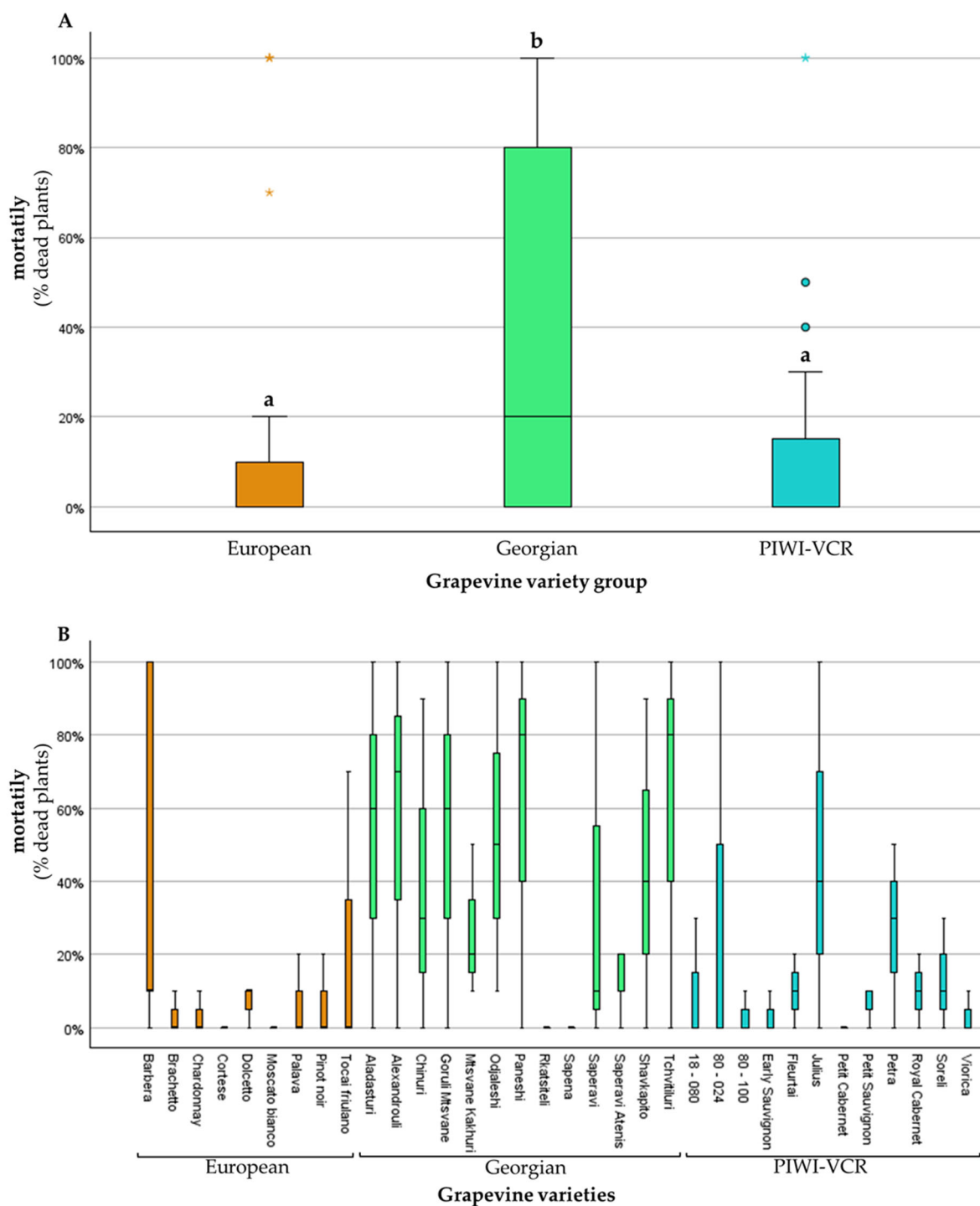


Figure 2. Statistical analyses of grapevine plants mortality rate considering variety groups (European, Georgian, PIWI-VCR) (A) and single varieties (B). Different letters on the graphic bars indicate significant differences obtained by ANOVA test results ($p < 0.05$).

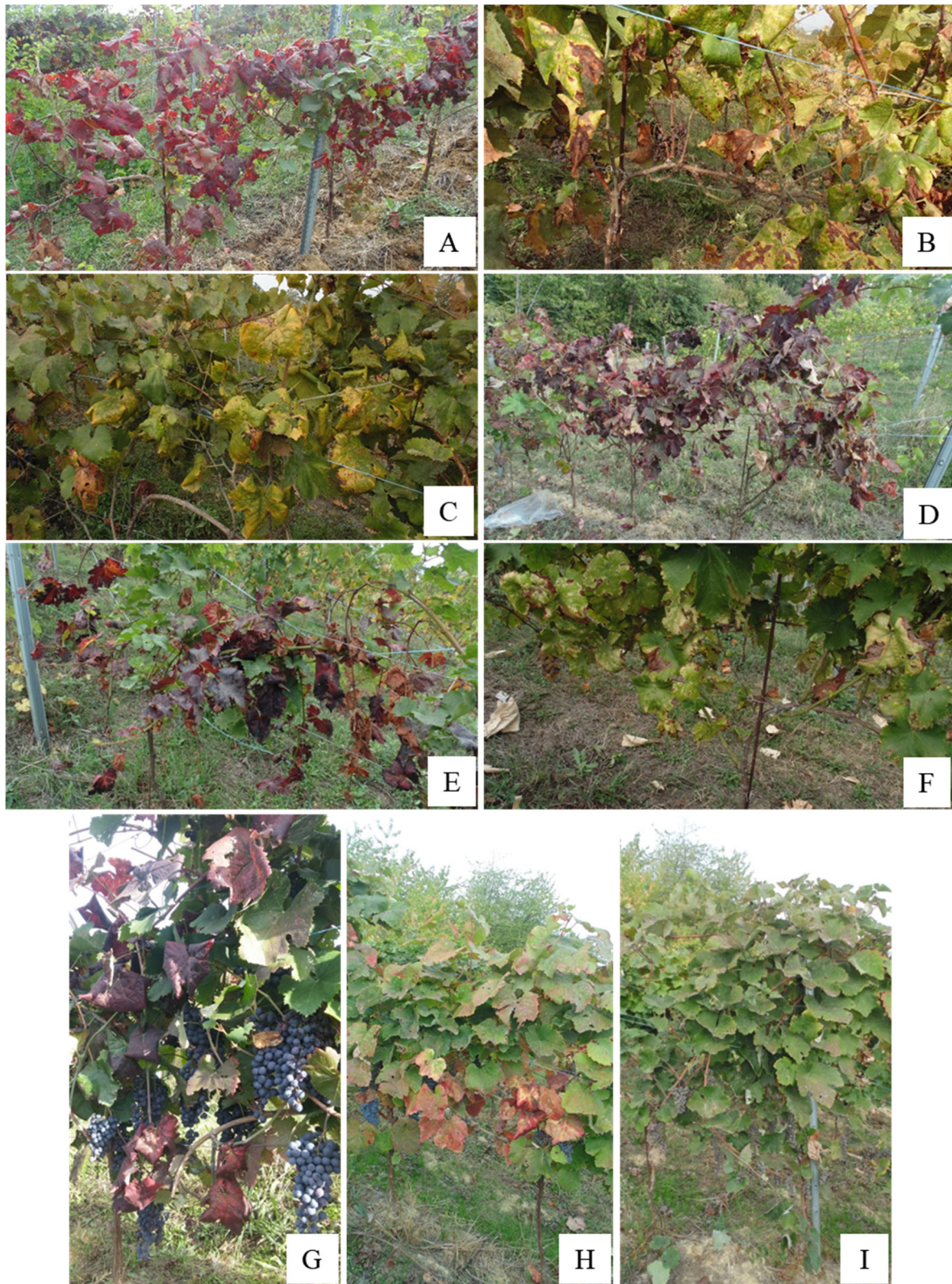


Figure 3. Grapevine yellows symptoms exhibited by European ((A) Barbera; (B) Chardonnay; (C) Moscato bianco), PIWI-VCR ((D) 18-080; (E) Royal Cabernet; (F) Petit Sauvignon), and Georgian ((G) Odjaleshi; (H) Paneshi; (I) Shavkapito) varieties.

Considering the incidence percentage index (I/I), statistical analyses, carried out considering the variety groups (European, Georgian, PIWI-VCR), showed that I/I of European varieties was significantly higher in comparison with PIWI-VCR varieties, while Georgian varieties showed an intermediate behavior (Figure 4A). Interestingly, I/I of

Georgian and PIWI-VCR varieties were not significantly different but determined by distinct component: for Georgian varieties the main component was represented by percentage of symptomatic plants, while for PIWI-VCR varieties by symptom severity class 2. Statistical analyses, carried out considering the single varieties, evidenced a significant difference between I%I of Barbera and Dolcetto (locally cultivated European) and I%I of Tocai friulano (European), Goruli Mtsvane, Mtsvane Kakhuri (Georgian), 80-024, Fleurtai, Julius, and Petra (PIWI-VCR) (Figure 4B).

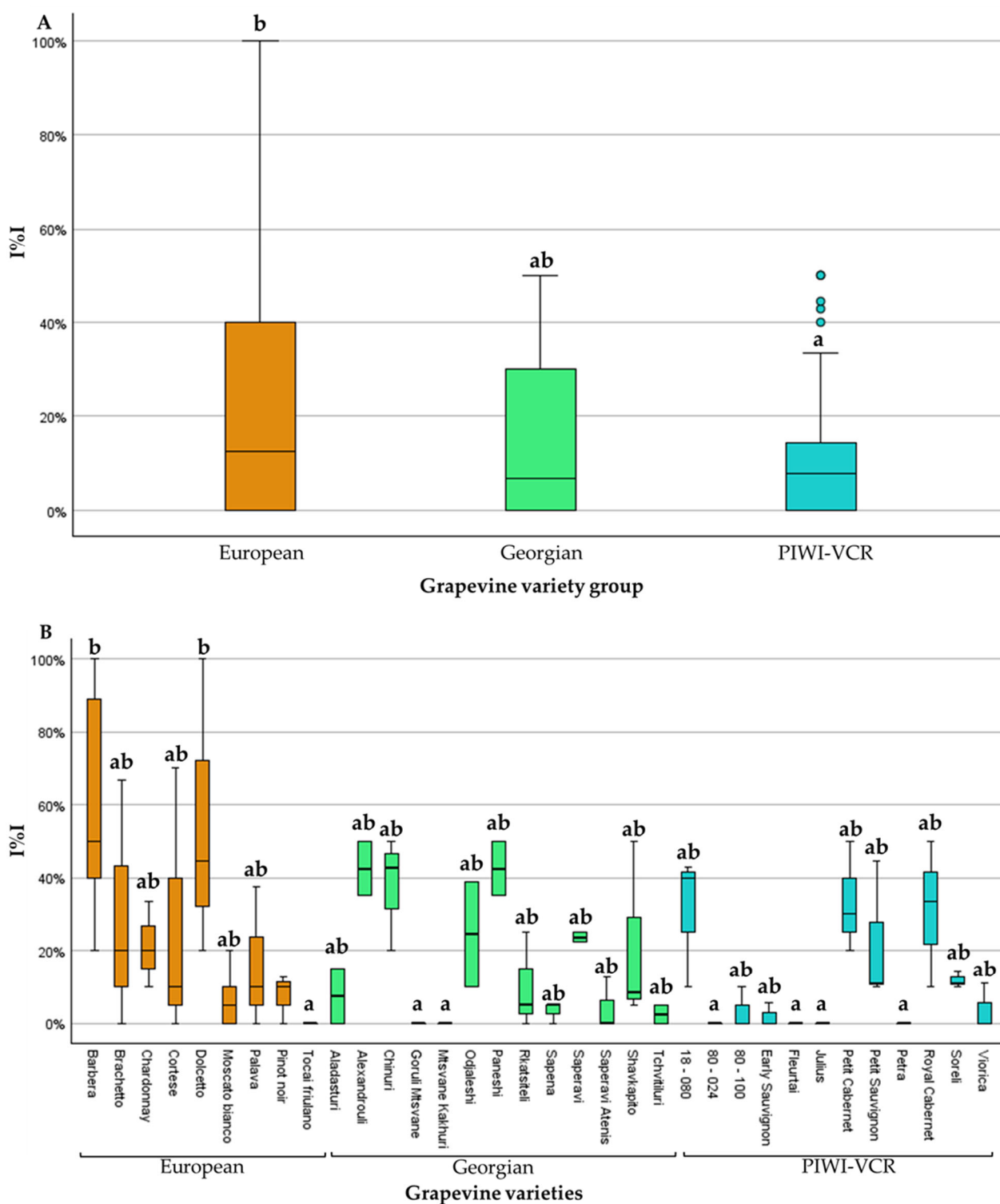


Figure 4. Statistical analyses of infection percentage index (I%I) considering variety groups (European, Georgian, PIWI-VCR) (A) and single varieties (B). Different letters on the graphic bars indicate significant differences obtained by ANOVA test results ($p < 0.05$).

3.2. Phytoplasmas Identified in Symptomatic Vines

For the amplification of *map* (16SrV phytoplasmas) and *stamp* ('*Ca. P. solani*') genes, total DNAs extracted from the collected leaf samples were used as templates in nested PCR reactions. In total, 60 local vines (37 symptomatic, 23 symptomless), 120 Georgian vines (50 symptomatic, 70 symptomless), and 65 PIWI-VCR vines (27 symptomatic, 38 symptomless) were examined. *Map* gene amplification identified 16SrV phytoplasmas in 36% (90 out of 245) of the examined grapevines: 67% of symptomatic (76 out of 114) and 11% of symptomless (14 out of 131) grapevines (Table 2). In detail, 16SrV phytoplasmas were detected: (i) in 52% (31 out of 60) of the European variety grapevines [81% of symptomatic (30 out of 37) and 4% of symptomless (1 out of 23) grapevines], with all the Barbera, Cortese, Dolcetto, and Moscato bianco symptomatic plants found infected; (ii) in 37% (44 out of 120) of the Georgian variety grapevines [64% of symptomatic (32 out of 50) and 17% in symptomless (17 out of 70) grapevines], with almost all the Alexandrouli, Rkatsiteli, and Saperavi Atenis symptomatic plants found infected; (iii) in 23% (15 out of 65) of the PIWI-CVR variety grapevines [52% of symptomatic (14 out of 27) and 3% in symptomless (1 out of 38) grapevines], with all the 18-080, Soreli, and VioriCa symptomatic plants found infected (Table 2). No PCR products were obtained from the '*Ca. P. solani*'-specific amplification of *stamp* gene. Reliability of the results was supported by the specific amplification in positive controls (STOL and AY1 for *stamp* gene; EY1 for *map* gene) and by the absence of DNA amplification in the reactions of healthy plant control and the negative control (PCR mixture devoid of DNA). The varieties Pinot noir (European), Saperavi (Georgian), 80-024, 80-100, Early Sauvignon, Fleurtaï, and Julius (PIWI-VCR) were found not infected by GY-associated phytoplasmas.

Table 2. Identification of 16SrV phytoplasmas in European, Georgian, and PIWI-VCR varieties.

Group	Variety	Status	N. of Plants 16SrVp-Infected/Sampled	16SrVp Map Genotype
European	Barbera	S	10/10	M54 (FDp)
	Brachetto	S	3/5	M54 (FDp)
	Chardonnay	S	3/5	M54 (FDp)
	Cortese	S	5/5	M54 (FDp)
	Dolcetto	S	5/5	M54 (FDp)
	Moscato bianco	S	3/3	M54 (FDp)
		A	0/12	
	Palava_VCR	S	1/3	M54 (FDp)
		A	0/2	
	Pinot noir	S	0/1	
		A	0/4	
	Tocai friulano_VCR	A	1/5	M54 (FDp)
	Total	S	30/37	
Georgian		A	1/23	
	Aladasturi	S	1/2	M54 (FDp)
		A	1/8	M54 (FDp)
	Alexandrouli	S	8/9	M54 (FDp)
		A	0/1	
	Chinuri	S	4/9	M54 (FDp)
		A	0/1	
	Goruli Mtsvane	A	4/10	M54 (FDp)
	Mtsvane Kakhuri_VCR	A	0/5	
	Odjaleshi	S	4/7	M54 (FDp)

		A	0/3	
	Paneshi	S	3/7	M54 (FDp)
		A	0/3	
	Rkatsiteli	S	6/6	M54 (FDp)
		A	0/4	
	Saperavi_VCR	S	0/1	
		A	0/4	
	Saperavi Atenis	S	1/1	M54 (FDp)
		A	1/9	M54 (FDp)
	Sapeva	S	4/6	M54 (FDp)
		A	0/4	
	Shavkapito	S	1/2	M54 (FDp)
		A	4/8	M54 (FDp)
	Tchvitoluri	A	2/10	M54 (FDp)
Total		S	32/50	
		A	12/70	
PIWI-VCR	18-080	S	5/5	M54 (FDp)
	80-024	S	0/2	
		A	0/3	
	80-100	S	0/1	
		A	0/4	
	Early Sauvignon	S	0/1	
		A	0/4	
	Fleurtaï	A	0/5	
	Julius	A	0/5	
	Petit Cabernet	S	2/5	M54 (FDp)
	Petit Sauvignon	S	1/3	M54 (FDp)
		A	0/2	
	Petra	A	1/5	M54 (FDp)
	Royal Cabernet	S	3/7	M54 (FDp)
		A	0/3	
	Soreli	S	2/2	M54 (FDp)
		A	0/3	
	Viorica	S	1/1	M54 (FDp)
		A	0/4	
Total		S	14/27	
		A	1/38	

All the 90 gene *map* amplification products, obtained from grapevines (31 for European varieties, 44 for Georgian varieties, 15 for PIWI-VCR varieties), were sequenced. Bioinformatics analyses revealed that 16SrV phytoplasma strains, identified in 90 infected grapevines, shared an identical *map* gene nucleotide sequence, undistinguishable from the sequence of the FDp strain M54 (Acc. No. AM384886) (Table 2). Network analyses based on *map* gene sequence analysis highlighted that M54 FDp strains, identified in the present study, belong to the cluster Map-FD2, including other two FDp strains (M148 and M155) recently reported in Serbia (Figure 5).

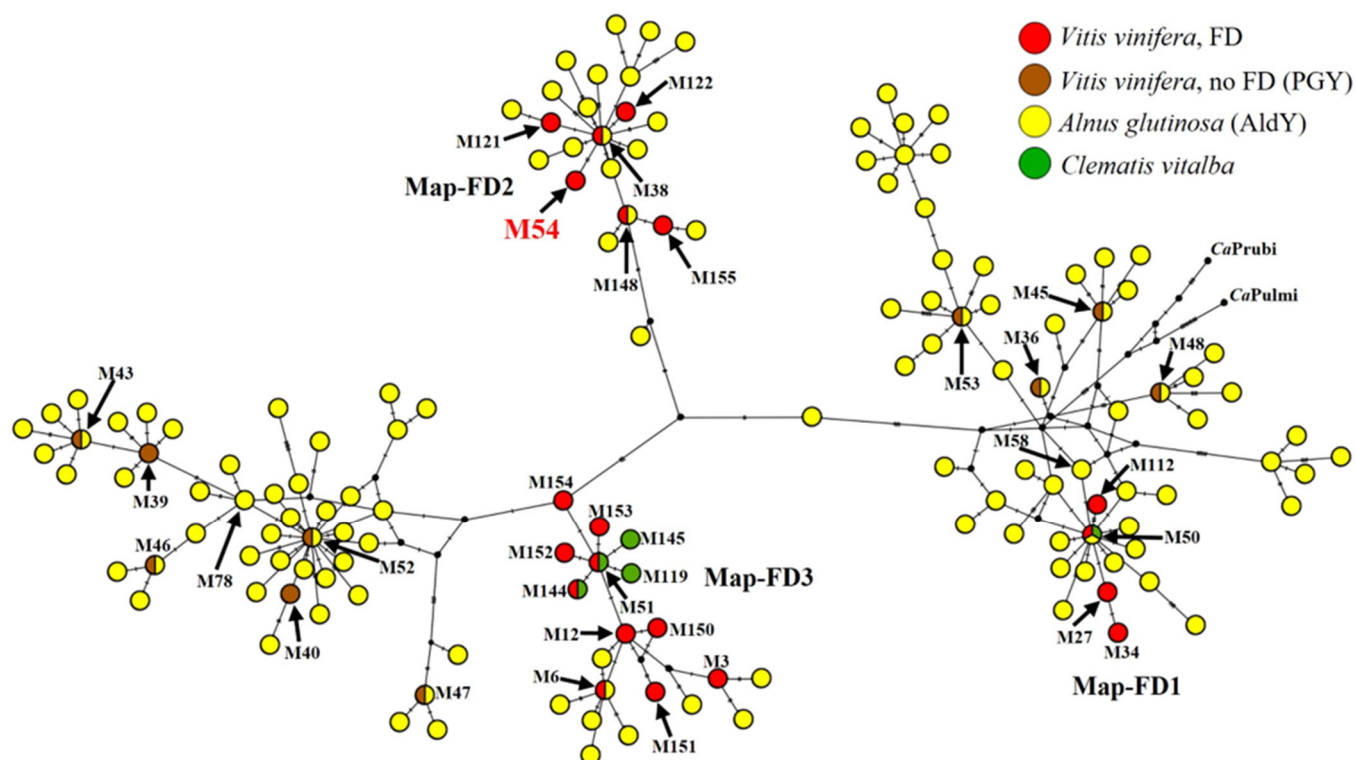


Figure 5. Median-joining networks inferred from map genotypes of FDp and related strains. Genotypes are represented by circles and paired genotypes are connected by a line. Each SNP mutation is represented by a hatch mark, while black dot vertices represent median vectors. The network of this manuscript includes 157 *map* genotypes present in the literature [13–19,22,41,42]. Genotype(s) identified in the present study is(are) written in red and bold letters.

3.3. Georgian Varieties Oenological Potential: Grapes and Wines Analysis

The analysis of technological maturity (Table 3) showed that, for white grapes, the Sapena variety had the highest values of Brix (21.9), sugars (223 g/L), bunch weight (271.67 g), and berry weight (3.19 g), while the highest value of total acidity (10.7 g/L as tartaric acid) was noted for the Tchivtiluri variety. The variety Rkatsiteli has a pH (3.22) slightly higher than other white varieties, indicative of a lower acidity trait, for example, Goruli Mtsvane. The total acidity value (7.2 g/L as tartaric acid) is quite high, contributing to a lively freshness of the wine, relevant for the balance of mouthfeel/flavors. The weight of the berry (2.56 g) and the weight of the bunch (185.44 g) correspond to an average size in the sample set analyzed. Harvesting took place relatively late compared to other varieties, which could indicate a greater time needed for complete maturation and the optimal accumulation of sugars and aromas. As for the variety Goruli Mtsvane, it showed a concentration of soluble solids (22.3 Brix) similar to that of Rkatsiteli although sugar content was deviating by 24 g/L. The pH (3.13) is lower than that of Rkatsiteli, while the total acidity (6.5 g/L tartaric acid) was slightly lower than Rkatsiteli, but still high enough to guarantee a good freshness. The bunches of Goruli Mtsvane are slightly heavier (211.83 g) than the Rkatsiteli, while the berries are slightly smaller (2.38 g).

From the analysis of red wines, the highest values of Brix (25.6) and sugars (227 g/L) were found for the Aladasturi variety. The highest acidity value was present in the Odjaleshi variety. Regarding grape characteristics, the Aladasturi variety had high values of bunch weight, number of seeds, seed weight (g), and seed weight as berry percentage. The cultivar Shavkapito presented higher value regarding berry weight. Phenolic determination showed that the highest values of TA (1973.85 mg/kg malvidin-3-O-glucoside chloride), seed TPI (360.08 mg/kg (+)-catechin), and skin TPI (3399.22 mg/kg (+)-catechin)

emerged in the variety Odjaleshi. The harvest of the Saperavi grapes showed that the amount of anthocyanins in the 2018 grapes was 1347.72 mg/kg, whereas the Shavkapito grapes harvested in 2018 had a quantity of anthocyanins of 980 mg/kg.

Table 3. Parameters of the technological maturity of Georgian white and red wine grapes.

Parameter (unit)	White Winegrapes					Red Winegrapes				
	Goruli Mtsvane	Mtsvane Kakhuri	Rkatsit eli	Sapena	Tchiviti luri	Aladas turi	Odjale shi	Paneshi	Sapera vi	Shavka pito
<i>Grape juice basic parameters</i>										
Total soluble solids (Brix)	22.2	nd	22.3	21.9	21.5	25.6	25.2	24.2	24.3	23.6
Sugars (g/L)	222	nd	198	223	182	227	222	207	211	200
pH	3.13	nd	3.22	3.16	3.10	3.25	3.05	3.42	3.16	3.36
Total acidity (g/L tartaric acid)	6.5	nd	7.2	8.5	10.7	7.2	8.8	5.0	6.1	6.7
<i>Grape characteristics</i>										
Bunch weight (g)	211.83	nd	185.44	271.67	134.50	257.00	127.17	161.39	126.25	237.59
Berry weight (g)	2.38	nd	2.56	3.19	1.83	2.61	1.67	2.16	1.70	2.79
Average number of seeds/berries	nd	nd	nd	nd	nd	3.2	2.4	1.8	1.9	3.0
Seeds weight (g)	nd	nd	nd	nd	nd	2.08	1.07	0.79	0.84	1.45
Skins weight (% w/w)	nd	nd	nd	nd	nd	26.82	40.37	27.39	31.91	19.10
Seeds weight (% w/w)	nd	nd	nd	nd	nd	7.96	6.39	3.71	4.90	5.90
<i>Phenolics determination</i>										
TA (mg/kg malvidin-3-O-glucoside chloride)	nd	nd	nd	nd	nd	1082 b	1974 c	452 a	1348 b	980 b
TPI seeds (mg/kg (+)-catechin)	nd	nd	nd	nd	nd	295 bc	360 c	152 ab	238 b	51 a
TPI skins (mg/kg (+)-catechin)	nd	nd	nd	nd	nd	1475 a	3399 c	2278 b	2495 b	2292 b
TPI from seeds (% w/w)	nd	nd	nd	nd	nd	16.6%	9.6%	6.3%	8.7%	2.2%
Harvest date	21 Sept, 2018	21 Sept, 2018	27 Sept, 2018	21 Sept, 2018	27 Sept, 2018	23 Oct, 2018	23 Oct, 2018	27 Sept, 2018	08 Oct, 2018	27 Sept, 2018
Harvest weight (kg)	23	24	12	16	10	21	12	12	25	25

For each phenolic parameter, different letters among cultivars indicate significant differences ($p < 0.05$). nd: not determined.

As demonstrated in Table 4, the analysis of the white wines showed that the ethanol content ranged between 12.2 and 14.1% *v/v*, with Sapena showing the highest content. Nevertheless, these wines showed a generally sustained total acidity (5.8–7.9 g/L as tartaric acid) but variable pH (3.16–3.75). Tchivtiluri showed the highest tartaric acid content (2.35 g/L), and together with Sapena a high malic acid content (2.26 and 3.11 g/L, for Tchivtiluri and Sapena, respectively). Therefore, Sapena demonstrated the ability to accumulate sugars, while maintaining a good amount of organic acids. This was demonstrated during a general survey of the population of Georgian vines from an eno-carpological point of view, where it was found that they have smaller berries than non-Georgian vines and a thicker skin. This characteristic means that while they achieve high sugar levels, they retain a good content of organic acids and less tannins, especially in seeds [51]. Another possible source of variability in wines can be attributable to the vineyard that is located on a slope and made it difficult to carry out monitoring and intervention operations, resulting in increased differences during the ripening. Regarding the glycerol content, the Sapena variety has the highest concentration. The color evaluation with the CIELab method showed a golden yellow color with no visible oxidation traits, although the values of absorbance at 420 nm were moderately high, in the range of 0.161–0.231.

Regarding red wines, the alcoholic content ranged from 11.2 to 13.7% *v/v*, with the Aladasturi variety showing the lowest ethanol content and total acidity (6.0 g/L of tartaric acid). Lactic acid and glycerol values were highest in the Shavkapito and Paneshi varieties, respectively. In this study, the Paneshi variety was the poorest in anthocyanins and the second richest in total polyphenols. Like Shavkapito, it is characterized by a low concentration of anthocyanins but a high concentration of total polyphenols and total flavonoids.

The wine produced from the Paneshi variety had the lowest color intensity and orange hue, and in line, the lightness (L^*) was the highest together with the highest yellow component according to CIELab analysis. Regarding anthocyanins, the wine of the Paneshi variety was the lowest in anthocyanins (120 mg/L) according to its color intensity, and its monomeric color was composed mainly by malvidin-3-O-glucoside (49.14%) and by cinnamoyl derivatives (12.26%). These anthocyanins are known for the high stability and the more deep-red absorption wavelength. In contrast, the wine produced from the Odjaleshi variety is the most colorful (color intensity 8.07 A.U., total anthocyanins 428 mg/L) followed by Aladasturi (color intensity 7.41 A.U., total anthocyanins 325 mg/L). In relation to what has been stated previously [52], there is a direct proportional correlation between the content of monomeric anthocyanins and antioxidant activity where, as the antioxidant activity increases, the quantity of monomeric anthocyanins also increases.

Table 4. Wines produced from Georgian varieties: basic parameters, phenolics, and color characteristics.

Parameter (unit)	White Wines					Red Wines				
	Goruli Mtsvane	Mtsvane Kakhuri	Rkatsiteli	Sapena	Tchvitoluri	Aladasturi	Odjaleshi	Paneshi	Saperavi	Shavkapito
Basic parameters										
Ethanol (% <i>v/v</i>)	13.0	13.8	12.2	14.1	12.3	11.2	12.8	13.7	13.0	12.7
Residual sugars (g/L)	2.33	2.25	2.61	3.11	3.19	0.22	nd	0.23	0.11	0.16
pH	3.71	3.75	3.43	3.65	3.16	3.48	3.38	3.55	3.53	3.63
Total acidity (g/L as tartaric acid)	6.2	5.8	6.8	7.0	7.9	6.0	7.1	7.1	7.1	7.3
Acetic acid (g/L)	0.26	0.31	0.16	0.30	0.25	0.43	0.36	0.64	0.44	0.58
Tartaric acid (g/L)	1.83	1.71	1.77	1.51	2.35	2.13	1.79	1.15	1.26	1.05
Malic acid (g/L)	2.16	1.81	1.82	3.11	2.26	nd	nd	nd	nd	nd
Citric acid (g/L)	0.47	0.14	0.36	0.42	0.23	nd	0.07	nd	0.06	nd
Lactic acid (g/L)	0.29	0.29	0.28	0.29	0.30	1.77	1.59	1.36	1.83	1.96
Glycerol (g/L)	10.17	10.16	9.20	10.49	9.31	8.76	9.95	11.65	10.80	10.78
Phenolics and color characteristics										
Total phenolics (mg/L (+)-catechin)	-	-	-	-	-	1050	1487	1872	1431	2249
Total flavonoids (mg/L (+)-catechin)	-	-	-	-	-	1170	1903	2044	1458	2554
Total anthocyanins (mg/L malvidin-3-O-glucoside chloride)	-	-	-	-	-	325	428	120	283	274
L^*	96.1	96.9	95.5	96.8	96.5	23.1	21.5	31.8	26.5	22.5
a^*	0.34	0.01	0.25	0.63	0.03	54.96	53.73	55.47	55.14	52.98
b^*	9.60	12.97	12.83	11.15	15.53	35.18	35.33	38.19	35.86	32.86
$A_{420\text{ nm}}$ (A.U., O.P. 10 mm)	0.161	0.182	0.207	0.163	0.231	-	-	-	-	-
Color intensity (A.U., O.P. 10 mm)	-	-	-	-	-	7.410	8.070	4.800	5.690	6.310
Hue	-	-	-	-	-	0.541	0.647	0.886	0.789	0.760
Visible color transposition										
Anthocyanin profile										
Delphinidin-3-O-glucoside (%)	-	-	-	-	-	3.52	5.50	6.51	6.77	12.87
Cyanidin-3-O-glucoside (%)	-	-	-	-	-	0.30	3.63	2.21	4.29	1.76
Petunidin-3-O-glucoside (%)	-	-	-	-	-	5.97	8.52	10.44	9.84	13.94
Peonidin-3-O-glucoside (%)	-	-	-	-	-	6.57	17.61	12.22	21.97	7.16
Malvidin-3-O-glucoside (%)	-	-	-	-	-	68.11	41.01	49.15	44.69	42.89
Σ acetylated anthocyanins (%)	-	-	-	-	-	10.57	14.15	7.22	3.89	3.88
Σ cinnamoylated anthocyanins (%)	-	-	-	-	-	4.97	9.58	12.26	8.55	17.50

nd: not determined.

The wine produced by the Aladasturi variety showed a low contribution of acetyl derivatives, and a high impact of malvidinin-3-O-glucoside (68.11%). The Shavkapito variety presented higher TPI and TF values (2249 mg/L and 2554 mg/L, respectively).

From the sensorial analysis carried out on wines obtained from Georgian varieties, it emerged that in white wines (Figure 6A), all tasters perceived a fruity aroma, with a frequency of citation varying between 50.0% and 81.3%. Goruli Mtsvane and Tchivtitluri wines have distinguished themselves for their citrus (for both 62.5%) and floral aromas (50.0% and 43.8%, respectively). The vegetal aroma was most evident in Mtsvane Kakhuri, Sapena, and Rkatsiteli wines (62.5%, 50.0%, and 43.8% respectively). The spicy aroma was particularly perceived in Mtsvane Kahuri wine (43.8%). The Tchivtitluri wine showed the highest values color intensity, acidity, and astringency, while the differences in bitterness were limited (Figure 6B). Goruli Mtsvane scored the highest score for balance.

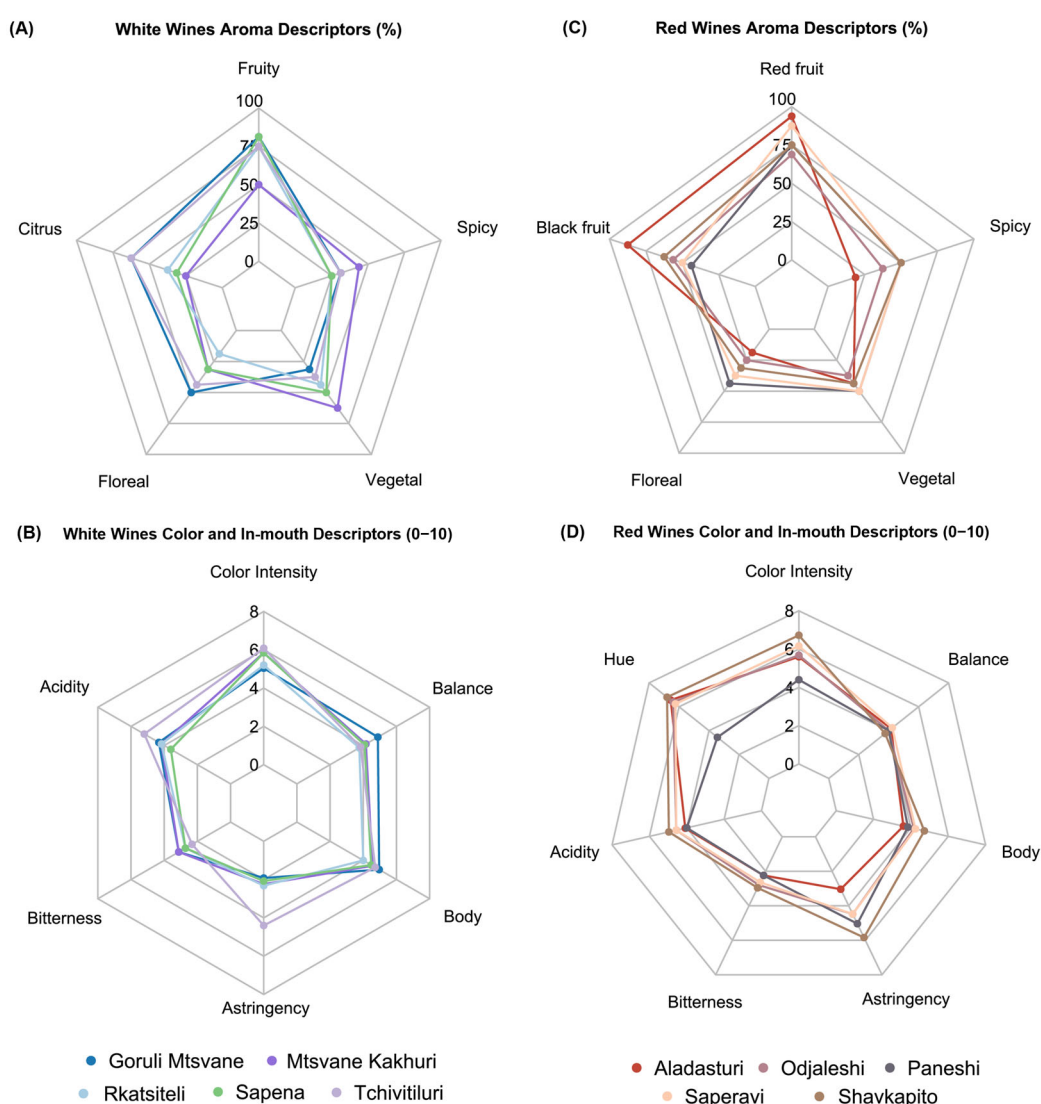


Figure 6. Sensory characteristics of white and red wines of Georgian varieties. Radar plot reporting aromatic descriptors as percentage frequency of citation (%) for white (A) and red (C) varieties, and color and in-mouth descriptors on a scale from 1 to 10 for white (B) and red (D) varieties.

As for red wines (Figure 6C), the aroma of red fruit was prominent in all samples, with a frequency of citation between 68.8% and 93.8%. The aroma of black fruit was

particularly evident in Aladasturi wine (87.5%). Floral, vegetal, and spicy aromas were not relevant, except for Paneshi and Saperavi wine, which showed vegetal (both 50%) and, together with Shavkapito, spicy notes (all 50%). The Shavkapito variety has produced wines with high color intensity, acidity, bitterness, astringency, and body (Figure 6D). Saperavi varietal wines have been recognized for the best balance.

4. Discussion

Results obtained from field surveys and molecular analyses confirmed the high FD infection pressure in the investigated area [16,22] and highlighted that all the examined Georgian varieties (except Okhtoura) adapted to the pedoclimatic conditions of the case-study vineyard and, even if infected by FDp, showed mild symptoms maintaining the complete berry production. These findings confirmed previously reported evidence of Georgian varieties tolerating '*Ca. P. solani*' infection in the South Caucasus region [35,36]. A similar behavior (no significant differences in infection percentage index, I%I) was found for PIWI-VCR varieties, previously selected through breeding programs between *Vitis vinifera* and wild American or Asian grapevine species for carrying resistance genes for fungal diseases. However, all the Georgian varieties exhibited symptoms of severity class 1 (symptoms only on the leaves), while seven PIWI-VCR varieties showed symptoms of severity class 2 (symptoms on both leaves and berries). Interestingly, I%I of locally cultivated European varieties (all exhibiting symptoms of severity class 2) was significantly higher compared to the Georgian and PIWI-VCR ones. Unfortunately, several grapevine varieties were completely absent during the surveys probably due to abiotic stresses in parts of some vineyard sectors, and to the activity of wild boars feeding on grapevines at the south border of the vineyard, surrounded by a forest. As evidenced in previous study [53], Tocai friulano showed very low susceptibility to FD.

Molecular analyses identified FDp only in 67% of symptomatic grapevines, and '*Ca. P. solani*' associated with Bois noir (the other disease of GY complex present in Italy) was never detected. This can be explained by several hypotheses: (i) the phytoplasmas are unevenly distributed in phloem tissues of infected plants [54]; (ii) the phytoplasma concentration in plant tissues in the different sampling periods can be extremely low [55]; (iii) the difficulty of recognizing mild symptoms mainly on Georgian and PIWI-VCR varieties [31].

As largely reported for phytoplasma-associated diseases of stone fruit trees (i.e., apple proliferation and European stone fruit yellows), symptom intensity observed in infected plants can be influenced by both the virulence of the pathogen and the susceptibility level of the plant host [32,33]. In the case of phytoplasma diseases of grapevine, several studies reported that genetically distinct '*Ca. P. solani*' strains could show a variable range of virulence associated with symptom severity on infected grapevine [31,35]. Thus, it is reasonable to hypothesize that genetically distinct FDp strains (*map* genotypes), carrying distinct pools of genes encoding effector proteins [56], also could show a variable range of virulence. Intriguingly, in this study, all the grapevine plants infected by FDp carried the genotype M54 (taxonomic subgroup 16SrV-D), reinforcing the idea that the observed symptom severity is strictly connected with the grapevine variety response to infection. The FDp genotype M54 is the largely prevalent FDp genotype in north-western Italy and Canton Ticino (Switzerland) [14,16,41]. Based on the evidence from previous studies, the epidemiological pattern of FDp genotype M54 is strictly limited to the close system '*S. titanus*—grapevine', even if it was occasionally reported in additional host plants (*Corylus avellana* and herbaceous plants) and insects (*Orientus ishidae* and *Thamnotettix dilutior*) [14,22]. Recently, FDp genotype M54 was reported for the first time in *Ailanthus altissima*, *Juglans regia*, *Robinia pseudoacacia*, and *Sambucus nigra* in Piedmont [22], proving the increasing presence of FDp M54 additional hosts in the vineyard agro-ecosystem. Thus,

studying host plants, potential vectors, and FDp strains in the vineyard agro-ecosystem is essential for understanding FD epidemiology and devising effective intervention strategies.

In this complex epidemiological scenario, particularly in high-pressure disease areas like Piedmont, an ambitious strategy for FD control involves selecting *V. vinifera* varieties that are resistant, tolerant, or not susceptible. Previous studies demonstrated that, in grapevine varieties scarcely susceptible to Bois noir (showing symptoms only on canopy), the phytoplasma infection has a negative impact on berry composition and wine quality [10]. In this study, analyses have shown that Georgian grapevines, grown in the case-study vineyard in Mombaruzzo (AT), have not only a tolerance for FDp infections but also keep interesting oenological potential. In fact, despite the presence of phytoplasma, these first outcomes suggest that Georgian varieties maintain stable berry and wine quality parameters. Chemical analysis of the must and wine showed acidity, sugar content, and sensory profile in accordance with oenological objectives.

In more detail, white wines with sustained total acidity and variable pH content were produced from some of the tested Georgian varieties. The Sapena variety stood out for its ability to accumulate sugars while retaining a relevant content of organic acids, thus being well balanced in terms of freshness and body. Moreover, Sapena was found to have smaller berries and thicker skins compared to some non-Georgian vines [51].

As for the analysis of the red varieties, Aladasturi showed the highest sugar content among the red wines (227 g/L) and mild total acidity (6.0 g/L). The determination of anthocyanins and phenolic compounds showed that Odjaleshi wine had the highest content of total anthocyanins and polyphenols, was particularly colorful, and had good phenolic potential. Paneshi is the variety with the lowest anthocyanin content but a high content of total polyphenols and flavonoids, suggesting a high potential in terms of wine structure and body; however, this aspect has not emerged fully with the sensory analysis performed.

To make a comparison with previous study [57], an in-depth analysis was made of the differences in polyphenolic compounds between the international variety Cabernet Sauvignon and the Georgian variety Saperavi and it was found that there are no major differences in polyphenols in the two varieties. Only in the wine obtained from the Saperavi variety was there a higher content of polyphenols such as catechin (43.9 mg/L), caffeic acid (8.66 mg/L), p-coumarin acid (4.61 mg/L), chlorogenic acid (0.29 mg/L), and myricetin (2.69 mg/L) compared to Cabernet Sauvignon. In conclusion, it emerged that there is a more rigid coordination of metabolism in the synthesis of flavonoids of the vine Saperavi. In this last variety and Shavkapito varieties, the polyphenols in the skins and seeds are similar, but they differ in the average berry weight.

The observed FD tolerance in Georgian varieties could be attributed to host–pathogen interaction dynamics or physiological mechanisms. The practical application of these varieties in FD-affected regions could reduce the economic and epidemiological impact of the disease by limiting pathogen propagation and vector acquisition efficiency but further controlled inoculation studies and transcriptomic analyses are necessary to elucidate the tolerance mechanisms. Moreover, the Georgian *V. vinifera* germplasm provides novel allelic diversity for introgression into susceptible European varieties through conventional or marker-assisted breeding programs to facilitate the development of new varieties combining FD tolerance with desirable enological and agronomic traits. Furthermore, vineyard diversification through the introduction of Georgian varieties may be useful in adapting to climate change and maintaining viticultural productivity and economic viability across wine-growing regions.

5. Conclusions

We highlighted that Georgian *V. vinifera* varieties, examined in this study, show low susceptibility to FD and maintain high berry and wine quality even after infection. These varieties could represent a valuable resource for viticulture in Piedmont (and—probably—for other viticultural regions also including Georgia itself), offering a sustainable solution to reduce the FD economic impact and contribute to the diversification and resilience of local vineyards.

Author Contributions: Conceptualization, P.A.B., O.F., V.G., and D.M.; methodology, P.A.B., O.F., L.R., V.G., S.G., M.A.P., and F.Q.; software, L.P., A.P., C.B., S.G., M.A.P., and F.Q.; validation, L.P., A.P., S.G., M.A.P., L.R., V.G., O.F., D.M., and F.Q.; formal analysis, L.P., C.B., S.G., M.A.P., and F.Q.; investigation, P.A.B., O.F., V.G., D.M., and F.Q.; resources, P.A.B., O.F., L.R., V.G., and D.M.; data curation, L.P., A.P., C.B., S.G., M.A.P., and F.Q.; writing—original draft preparation, L.P.; writing—review and editing, L.P., M.A.P., S.G., A.P., C.B., D.M., L.R., V.G., O.F., P.A.B., and F.Q.; visualization, L.P., A.P., S.G., M.A.P., C.B., and F.Q.; supervision, F.Q.; project administration, P.A.B., O.F., D.M., and F.Q.; funding acquisition, P.A.B., O.F., D.M., and F.Q. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Cantina TRE SECOLI S.c.a., Via Stazione 15, Mombaruzzo (AT), Italy, grant GEVIR (GEnotipi di VItte Resistenti—Resistant GEnotypes of *Vitis vinifera*), and by LEPL National Wine Agency of Georgia, within the ‘Research Project on Study of Georgian Grape and Wine Culture’.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed at the corresponding author.

Acknowledgments: We would like to thank Flavio Pallanzone, Carlo D’Angelone, and Giorgio Moroni for their technical assistance and Paolo Triberti, owner of the case-study vineyard.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had a role in the design of the study (selection of the location and the grapevine varieties used for planting the examined vineyard). The funders had no role in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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