

Screening of Georgian grapevine germplasm for susceptibility to downy mildew (*Plasmopara viticola*)

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Summary

Downy mildew, caused by the obligate biotrophic parasite *Plasmopara viticola*, is one of the most serious grapevine diseases with worldwide distribution. Resistant grapevines can be used to reduce damages caused by the pathogen, recently different levels of susceptibility to *P. viticola* were detected in some Georgian autochthonous varieties. The aim of the present work was to classify additional Georgian autochthonous varieties into different groups according to their susceptibility to the downy mildew agent in the framework of COST Action FA1003 "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding". The leaf disk assay defined by the OIV 452-1 protocol was used for screening 61 native varieties of Georgia. Screened varieties showed different degree of resistance: very high – 7 accessions, high - 13, medium - 15, low – 23, and very low - 3 accessions. The results suggest that further resistant genotypes are likely to be found within more than 500 Georgian grapevine cultivars.

Key words: *Vitis vinifera*; resistance; OIV descriptor; South Caucasus.

Introduction

Plasmopara viticola (Berk. & M. A. Curtis) Berl. & De Toni is a biotrophic pathogen which causes downy mildew to members of the family *Vitaceae*, in particular to the cultivated Eurasian species *Vitis vinifera* L. (CADLE-DAVIDSON, 2008). The pathogen infects grapevines worldwide causing important economic losses. The current strategy to control the disease relies totally on the use of chemical fungicides. An alternative to the chemical control is the use of resistant varieties which is cost-effective and environmentally friendly. Up to now, resistance genes have been found mainly in American varieties, but their introgression in *V. vinifera* is not particularly successful, due to the poor quality of yield in resistant hybrids. Susceptibility to *P. viticola* has been observed in the majority of the most widely cultivated European grapevine varieties, but has not been tested in the many varieties present in the domestication area of *V. vinifera*, Caucasus, Asia Minor or Central Asia, which are likely characterized by a great genetic variability.

Leaf discs assays are widely used to study resistance towards *P. viticola* because they are space-saving and reproduce rather accurately field responses in plants (BROWN *et al.* 1999, SOTOLAR 2007). This method was used in current research for assessing the resistance level of Georgian *V. vinifera* varieties towards *P. viticola*. Therefore, the aim of the present study was to characterize the level of susceptibility to *P. viticola* of some Georgian grapevine varieties.

Material and Methods

Plant material: Sixty-one Georgian grapevine genotypes (55 autochthonous varieties and six wild accessions) and three resistant controls (rootstock 'Kober 5BB' and hybrids cultivars 'Isabella' and 'Moldova') were selected for this study. Georgian varieties and the control ones were sampled in the Skra germplasm repository in the Gori administrative district of East Georgia; the wild grapevine *Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi accessions were collected in natural forests in the same Gori region (Table and Fig. 1). Wood cuttings were collected in winter time, grown in controlled conditions, at 22-25 °C, in dark-light (14 h - 10 h) conditions and kept untreated against *P. viticola*. The 4th-5th completely unfolded leaves starting from the apex were collected for inoculation purposes. The experiments were repeated for two consecutive years.

Inoculum source: Naturally infected leaves were collected from several vineyards. The leaves were carefully washed with water and incubated overnight at 20 °C in order to induce sporulation. The sporangial suspension was prepared in tap water and adjusted to 25,000 spores·mL⁻¹ by using a hemocytometer.

Leaf disc assay. A total of four leaf discs per cultivar were cut out from the collected leaves with the cork borer, 1.5 cm in diameter. The leaf discs were placed on water-agar in Petri dishes with lower surface upwards. Inoculation was carried out by distributing 40 µL drops of sporangial suspension on the leaf discs (one drop per leaf disc). Each droplet was placed in the middle of the leaf discs. After an overnight incubation the droplets of the sporangial suspension were removed by blotting with a filter paper. The leaf discs were incubated at 25 °C in complete darkness. The results were checked after 5-8 d.

The degree of infection and correspondently – the resistance of the cultivars were estimated based on the intensity of sporangiophore formation according to the OIV

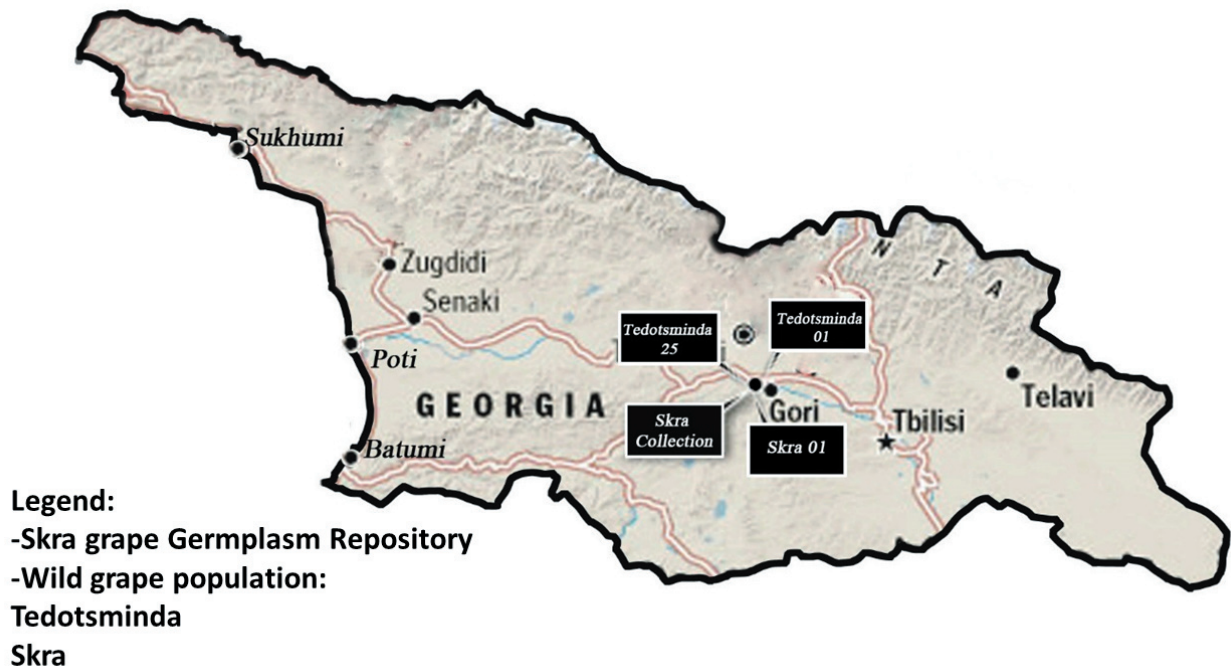


Fig. 1: Location of tested genotypes in Kartli Province, East Georgia.

452-1 descriptor (Orv, 2009) at: 9: no sporangiophores (very high resistance); 7: 1-5 sporangiophores (high resistance); 5: 6-20 sporangiophores (medium resistance); 3: > 20 sporangiophores (low resistance); 1: dense sporangiophore carpet (very low resistance).

Leaf disc assays were repeated two times during the first year. The varieties showing high susceptibility were excluded from the experiments carried out the second year during which the experiments were repeated six times.

Statistical analyses: Differences in resistance of the Georgian genotypes were assessed using Mann-Whitney test was used to compare resistance of all Georgian grapevine varieties vs. American very high resistant variety 'Isabella' (*V. labrusca*) and 'Kober 5BB' (*V. berlandieri* x *V. riparia*) rootstock, and resistant breeding cultivar 'Moldova' ('Guzal Kara' x 'Villard Blanc') from the Republic of Moldova (TARALASHVILI *et al.* 1989, TROSHIN 2006). Data were analyzed by using GraphPad Prism5 software (GraphPad Software, Inc., La Jolla, CA, USA). The *P*-values ≤ 0.05 were considered as significant.

Results and Discussion

Symptom expression: The earliest symptoms caused by *P. viticola* in some screened varieties, a localized necrosis at the abaxial surface, occurred within two days after the inoculation. Chlorotic spots appeared on leaf discs of susceptible genotypes four days after inoculation. Irregular shaped necrotic patches appeared and expanded progressively during later stages of incubation. Sporulation coverage increased and white sporangiophores were observed on the abaxial surface of the leaves. In the majority of the screened varieties necrosis was not detected on the abaxial surface of the leaves.

Necrosis was observed 5-6 d after inoculation on the following genotypes: 'Tedotsminda 10', 'Tedotsminda 02', 'Tedotsminda 05', 'Tedotsminda 15', 'Jvari', 'Saperavi Clone 359', 'Pitra', 'Tavtsitela', 'Tskobila', 'Zhghia', 'Ktsia', 'Kharistvala Meskhuri', 'Chinuri', 'Saperavi Bejashvilis', 'Rkatsiteli Clone', 'Beglaris Kurdzeni', 'Sapena', 'Chitiskvetchka Meskhuri', 'Bua Kurdzeni', 'Ikaltos Tsiteli', 'Rkatsiteli Vardisperi', 'Rkatsiteli', 'Supris Tetri', 'Saperavi Budeshuriseburi', 'Kisi', 'Kharistvala Shavi', 'Ubakluri' and 'Gomis Tetri'.

Responses to *P. viticola* infection in leaf disc assays. The inoculation experiments revealed a different susceptibility to *P. viticola* among the 61 Georgian autochthonous cultivars (Table, Fig. 2).

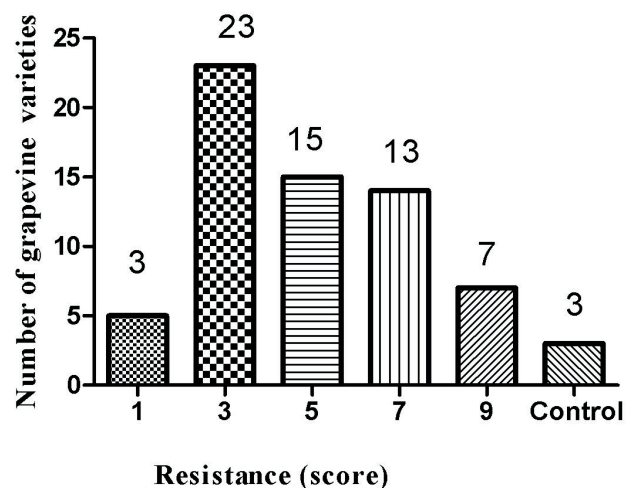


Fig. 2: Frequency distribution of resistance traits of native Georgian grapevine varieties to *Plasmopara viticola*: 1: very low, 3: low, 5: medium, 7: high, 9: very high.

Table
Tested native Georgian varieties and their resistance scores to *Plasmopara viticola* (2013-2014)

Variety	Berry color ¹⁾	Usage ²⁾	Region of origin	Distribution	Resistance ³⁾ to <i>P. viticola</i>		Final score
					Year		
					2013	2014	
1 Ghvinis Tetri	B	W	Kakheti	Germplasm	1	-	1b
2 Kurkena	B	W	Samegrelo	Germplasm	1	-	1d
3 Okroula	B	T-W	Kakheti	Germplasm	1	-	1d
4 Akhmetis Tsiteli	Rg	W	Kakheti	Germplasm	3	-	3d
5 Buera	B	T-W	Kakheti	Germplasm	3	-	3d
6 Chinuri	B	W	Kartli	Major	3	3	3d
7 Dzaghlirchamia	B	W	Kakheti	Germplasm	3	-	3d
8 Ghrubela Kakhuri	G	W	Kakheti	Germplasm	3	-	3d
9 GomisTetri	B	T-W	Imereti	Germplasm	5	3	3d
10 Ingilouri Kumsi	B	W	Kakheti	Germplasm	3	-	3d
11 Kharistvala Meskhuri	B	T	Meskheti	Germplasm	5	3	3d
12 Khikhvi	B	W	Kakheti	Minor	3	-	3d
13 Kisi	B	W	Kakheti	Minor	5	3	3d
14 Mirzaanuli Tetri	B	W	Kakheti	Germplasm	3	-	3d
15 Mtsvane Kakhuri, Clone 12	B	W	Kakheti	Major	3	-	3d
16 Mtsvane Kviteli	G	W	Kakheti	Germplasm	3	-	3d
17 Rkatsiteli	B	W	Kakheti	Major	3	-	3d
18 Rkatsiteli, Clone 48	B	W	Kakheti	Minor	3	3	3d
19 Sirgula	B	W	Lechkhumi	Germplasm	3	-	3d
20 Tavtsitela	B	W	Ratcha-Lechkhumi	Germplasm	5	3	3d
21 Tedotsminda 02	-	-	Kartli	Wild	-	3	3d
22 Tedotsminda 22	-	-	Kartli	Wild	-	3	3d
23 Tsnoris Tetri	B	W	Kakheti	Germplasm	3	-	3d
24 Ubakluri	B	W	Kakheti	Germplasm	5	3	3d
25 Zakatalis Tetri	B	W	Kakheti	Germplasm	3	-	3d
26 Zhghia	GR	W	Kakheti	Germplasm	-	3	3d
27 Saperavi Bejashvilis	N	W	Kartli	Germplasm	7	3	5d
28 Shavkapito	N	W	Kartli	Minor	7	3	5d
29 Beglaris Kurdzeni	B	W	Ratcha	Germplasm	5	5	5d
30 Bua Kurdzeni	B	W	Kakheti	Germplasm	-	5	5d
31 Kharistvala Shavi	N	T-W	Kakheti	Germplasm	7	5	5d
32 Kvira	N	W	Ratcha-Lechkhumi	Germplasm	7	5	5d
33 Chitiskvertska Meskuri	B	W	Meskheti	Germplasm	7	3	5d
34 Chitistvala Bodhuri	B	W	Kakheti	Germplasm	5	-	5d
35 Sapena	B	W	Kakheti	Germplasm	7	5	5d
36 Bazaleturi	G	W	Kartli	Germplasm	7	3	5d
37 Jvari	B	W	Kartli	Germplasm	7	3	5d
38 Saperavi Budeshuriseburi	N	W	Kakheti	Minor	-	5	5d
39 Supris Tetri	B	W-T	Kakheti	Germplasm	5	5	5d
40 Tedotsminda 05	-	-	Kartli	Wild	-	5	5d
41 Pitra	B	W	Ratcha-Lechkhumi	Germplasm	7	3	5d
42 Ikalts Tsiteli	N	W	Kakheti	Germplasm	5	7	7c
43 Krakhuna Clone	N	T	Imereti	Minor	7	-	7c
44 Ktsia	N	W	Kartli	Germplasm	9	7	7c
45 Mtsvane Kakhuri	B	W	Kakheti	Major	5	7	7c
46 Tsitska	B	W	Imereti	Major	7	9	7c
47 Tsitska, Clone	B	W	Imereti	Minor	7	9	7c
48 Rkatsiteli Vardisperi	Rg	W	Kakheti	Minor	5	7	7b
49 Saperavi, Clone 359	B	W	Kakheti	Minor	-	7	7b
50 Skra	-	-	Kartli	Wild	-	7	7b
51 Tedotsminda 10	-	-	Kartli	Wild	-	7	7b
52 Tedotsminda 15	-	-	Kartli	Wild	-	7	7c
53 Tskobila	N	W	Kakheti	Germplasm	-	7	7c
54 Goruli Mtsvane	B	W	Kartli	Major	3	9	7b
55 Chkhikoura	B	W	Imereti	Germplasm	9	9	9a
56 Dondghlabi Shavi	N	W	Imereti	Germplasm	-	9	9a
57 Dondghlabi Mtsvane	G	W	Imereti	Germplasm	7	9	9a
58 Kakhis Tetri	B	T-W	Kakheti	Germplasm	-	9	9a
59 Kesi	B	W	Kakheti	Germplasm	-	9	9a
60 Muradouli	B	W	Imereti	Germplasm	9	9	9a
61 Tsirkvalis Tetri	B	W	Imereti	Germplasm	9	9	9a
62 Kober 5BB (<i>V. berl.</i> x <i>V. rip.</i>) (Contr.)	N	R	Austria	Major	-	9	9a
63 Isabella (Contr.)	N	W	USA	Major	9	9	9a
64 Moldova (Contr.)	N	W	Moldova	Major	-	7	7b

¹⁾ B: green yellow, N: blue black, G: grey, Rg: dark red violet.

²⁾ W: wine, T: table, W-T: wine-table, T-W: table-wine, R: rootstock.

³⁾ 1: very low, 3: low, 5: medium, 7: high, 9: very high. Means followed by the same letter are not statistically different (Dunnet Posttest $p < 0.05$).

Two years screening revealed that the varieties such as: 'Chkhikhoura' (white, wine, Imereti), 'Tsirkvalis Tetri' (white, wine, Imereti), 'Dondghlabi Mtsvane' (white, wine, Imereti), 'Muradouli' (white, wine, Imereti), are characterized by a very high resistance to *P. viticola*. There are also several varieties like 'Dondghlabi Shavi' (red, wine, Imereti), 'Kesi' (white, wine, Kakheti) and 'Kakhis Tetri' (white, wine, Kakheti) in this germplasm demonstrated very high resistance after one-year evaluation and recognized prospective for next tests. All of them are no more cultivated and are available only in germplasm collections. It should be mentioned that among these seven resistant genotypes five are from the Imereti region of West Georgia with humid and mild climatic conditions and two of them from Kakheti region of East Georgia with less humid and more hot climatic conditions than in West Georgia.

Selected genotypes - especially the varieties 'Chkhikhoura' (Accession No GEO015-3-13B), 'Muradouli' (GEO015-2-10B), 'Tsirkvalis Tetri' (GEO015-3-13A) and 'Dondghlabi Mtsvane' (GEO015-3-9B) characterized by a high resistance level in both years need to be further investigated in order to be included in future grapevine breeding programs.

Further 13 varieties from East and West parts of Georgia revealed high resistance towards *P. viticola*, while, 15, 23 and 3 grapevine varieties originating from East and West Georgia showed medium, low and very low resistance, respectively. The varieties that showed medium, very low and low resistance exhibited significantly lower resistance in comparison with 'Isabella' ($P < 0,05$) (Table).

A few of the medium to very high resistant varieties are cultivated in small areas and the great majority of them are available exclusively in grapevine collections.

The results obtained in the present study suggest that the Georgian germplasm which includes more than 500 dif-

ferent grapevine varieties, is worth to be screened for its resistance level against the downy mildew agent. Moreover since the three genotypes belonging to *V. vinifera* L. ssp. *sylvestris* showed a high resistance level, it seems that also the wild accessions should be screened together with the cultivated ones.

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