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Evidence of resistance to the downy mildew agent *Plasmopara viticola* in the Georgian *Vitis vinifera* germplasm

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Summary

Grapevine downy mildew, caused by *Plasmopara viticola*, is one of the most important diseases at the international level. The mainly cultivated *Vitis vinifera* varieties are generally fully susceptible to *P. viticola*, but little information is available on the less common germplasm. The *V. vinifera* germplasm of Georgia (Caucasus) is characterized by a high genetic diversity and it is different from the main European cultivars. Aim of the study is finding possible sources of resistance in the Georgian autochthonous varieties available in a field collection in northern Italy. The resistance levels to *P. viticola* were estimated both by experimental inoculations and by disease assessment in field conditions in a multi-year activity. Of the 93 tested accessions, 'Mgaloblishvili N' showed a constant resistant behaviour, by reducing the disease severity and the pathogen sporulation. High levels of leaf hair density did not always associate with reduced disease gravity in experimental inoculations, confirming that this kind of preformed barrier is not completely efficient in preventing pathogen infections.

K e y w o r d s : grapevine; disease resistance; disease incidence.

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 species *Vitis vinifera* L. (Bellin *et al*. 2009). *P. viticola* was fortuitously introduced in France from North America during the nineteenth century and rapidly spread across Europe (Galet 1977). The pathogen infects all green parts of the plant causing, in favourable weather conditions, extensive losses in grape yield (Yu *et al.* 2012). The damages caused by the pathogen can lead to quantitative losses, by infecting inflorescences and bunches, and to qualitative losses, by causing an early defoliation of the plant.

Severe epidemics of downy mildew usually occur in temperate regions, characterized by grapevine vegetative seasons with frequent rainfall and moderate temperatures. On the contrary, high temperatures and reduced water availability during late spring and summer usually prevent the spread of the disease (Vercesi *et al*. 2010). The control of downy mildew on grapevine varieties requires regular application of fungicides. However, the intensive use of chemicals becomes more and more restrictive due to human health risk and negative environmental impact (Blasi *et al.* 2011).

Damages due to *P. viticola* could be reduced by using resistant grapevine varieties. The breeding programs are usually carried out by crossing *V. vinifera* with resistant species and in particular with American *Vitaceae* that co-evolved with the pathogen. The first generation hybrids, obtained from the end of the XIXth to the beginning of the XXth century, were unsuitable for the production of high quality wines, due to their unpleasant foxy aromas coming from the American species of the *Vitis* genus (Eibach and Töpfer 2015). Nowadays, thanks to numerous backcrossing cycles, the last generation breeding varieties possess resistant characteristics of the American species and qualitative characteristics similar to those of *V. vinifera* cultivars (Di Gaspero and Foria 2015). However, finding sources of resistance in *V. vinifera* could be a great innovation, because it could really simplify the breeding programs originating new varieties with the quality levels required for wine production. Interesting results concerning the European wild grape, a subspecies of the *V. vinifera* species, have been recently found (Schröder *et al.* 2015).

The *V. vinifera* species comprises cultivated (*V. vinifera* subsp. *vinifera*) and wild (*V. vinifera* subsp. *sylvestris* Gmelin) subspecies, originally dispersed from western Asia to Europe (Zohary and Horf 2000). At present, more than 12,000 accessions are recorded as individual cultivated varieties in Europe (Vitis International Variety Catalogue-VIVC; http://www.vivc.de/index.php). The origin of most of them is still questionable due to: the existence of several putative domestication centers, dispersed in all the distribution area of the wild progenitor; exchange of plant material among countries; and possible crossing among locally domesticated varieties and grapes imported from abroad. Georgia in the South Caucasian region, is considered one of the most important primary centers of domestication of cultivated grapevine, in the larger area comprising Oriental Anatolia, Syria and Northern Mesopotamia, where the first viticulture emerged, towards the middle of the VI millennium BC (Forni 2012). Actually paleobotanical and archaeological remains attesting the first development of the vinicultural

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production and grapevine domestications have been found in Southern Caucasus; moreover, the region is rich in grapevine diversity and wild grapevines are widely present in the area (Maghradze *et al.* 2012a).

The evaluation of Georgian grapevine germplasm is interesting for different reasons. This germplasm comprises a very wide range of cultivars (525 according to Ketskhoveli *et al*. 1960) with high genetic variability (De Lorenzis *et al*. 2015) and various ampelographic characters, agronomical traits and phenological diversity (Maghradze *et al.* 2012b). The viticultural and the enological features of this genetic material are also very different from Western European material (Imazio *et al*. 2013) and interesting because of their possible cultivation for innovative wine quality profiles, which could be different compared to wines from Western cultivars. There is also an interest in possible sources of useful genes for breeding programs for qualitative characters and/or for tolerance to biotic and abiotic stresses (Maghradze *et al*. 2012b, Quaglino *et al.* 2016). Indeed, some interesting Georgian varieties showed a high level of resistance to *P. viticola* in experimental inoculations (Bitsadze *et al*. 2015).

The main objective of the study was searching possible sources of resistance to *P. viticola* in Caucasian *V. vinifera* germplasm coming from Georgia and cultivated in a field collection in northern Italy by combining two approaches: experimental inoculations of leaf discs and evaluation of the disease incidence in vineyard.

Material and Methods

Plant material: The 94 Georgian grapevine varieties used in this study (Tab. 1) are grown in in a collection vineyard established in 2006 at the Regional Research Station of Riccagioia in Lombardy region of northern Italy. They are all *V. vinifera* varieties native to Georgia (South Caucasus region) apart from one: a *Vitis x labruscana* L.H.

Table 1

List of Georgian varieties in relation to their region of origin, berry colour and density of the hairs between the veins on the lower side of the leaf

ID	Name of variety	Region of origin	Berry colour*	Hair density**
L21A	Okroula	Kakheti	B	3
L21B	Tsnoris Tetra	Kakheti	B	3
L21C	Kurkena	Kakheti	B	3
L21D	Akhmetis Shavi	Kakheti	N	3
L21E	Saperavi Grdzelmtevana	Kakheti	N	3
L21F	Zakatalis Tsiteli	Kakheti	N	3
L22A	Mgaloblishvili	Imereti	N	7
L22B	Marguli Sapere	Imereti	N	5
L22C	Gabekhouri Tsiteli	Imereti	N	7
L22D	Endeladzis Shavi	Imereti	N	3
L22E	Mtsvane Onidan	Ratcha	B	5
L22F	Usakhelouri	Ratcha	N	3
L23A	Khushia Shavi	Imereti, Guria	N	7
L23B	Orona	Guria	N	5
L23C	Ikaltos Tsiteli	Kakheti	N	7
L23D	Okhtoura	Kakheti	N	7
L23E	Kistauris Saghvine	Kakhuri	N	5
L23F	Vertkvichalis Shavi	Imereti	N	5
L24A	Satsuravi	Adjara	N	7
L24B	Khrogi	Ratcha	N	3
L24C	Zakatalis Tetri	Kakheti	B	5
L24D	Mtsvivani Mskhvilmartsvala	Kakheti	B	3
L24E	Jghia	Kakheti	N	5
L24F	Chinuri	Kartli	B	3
M21A	Ghvinis Tsiteli	Kakheti	N	3
M21B	Kharistvala Shavi	Kakheti	N	3
M21C	Tkupkvirta	Kakheti	N	3
M21D	BudeshuriTsiteli	Kakheti	N	3
M21E	Buera	Kakheti	B	3
M21F	Goruli Mtsvane	Kartli	B	7
M22A	Zerdagi	Samegrelo	N	7
M22B	Paneshi	Samegrelo	N	5
M22C	Chkhucheshi	Samegrelo	B	3
M22D	Chkhaveri	Guria	N	7
M22E	Kamuri Shavi	Guria	N	5
M22F	Jani Bakhvis	Guria	N	5
M23A	Tkbili Kurdzeni	Kakheti	N	7
M23B	Kuprashviliseuli	Imereti	N	7
M23C	Dzelshavi Obchuri	Imereti	N	3

Tab. 1, continued

ID	Name of variety	Region of origin	Berry colour*	Hair density**
M23D	Mirzaanuli	Kakheti	B	3
M23E	Chkhikoura	Imereti	B	3
M23F	Kapistoni Tetri	Imereti	B	3
M24A	Asuretuli Shavi	Kartli	N	3
M24B	Tavkara	Kakheti	N	7
M24C	Argvetula	Imereti	N	7
M24D	*Vitis x labruscana*	Georgia	N	7
M24E	Ananura	Kartli	N	3
M24F	Tchvitiluri	Samegrelo	B	7
N21A	Gorula	Kartli	B	3
N21B	Tita Kartlis	Kartli	B	3
N21C	Adreuli Tkhelkana	Kartli	N	5
N21D	Shavkapito	Kartli	N	5
N21E	Ghrubela Kartlis	Kartli	B	3
N21F	Buza	Kartli	N	5
N22A	Otskhanuri Sapere	Imereti	N	7
N22B	Orbeluri Ojaleshi	Lechkhumi	N	5
N22C	Aleksandrouli	Ratcha	N	5
N22D	Rkatsiteli	Kakheti	B	3
N22E	Kumsmtevana	Kakheti	B	3
N22F	Sirgula	Kakheti	B	3
N23A	Tsolikouri Mtsvivani	Imereti	B	5
N23B	Bazaleturi	Imereti	B	5
N23C	Tsirkvalis Tetri	Imereti	B	5
N23D	Vertkvichalis Tetri	Imereti	B	5
N23E	Imeruli Shavi	Imereti	N	5
N23F	Adanasuri	Imereti	N	5
N24A	Ojaleshi	Samegrelo	N	5
N24B	Aladasturi	Guria	N	7
N24C	Tchumuta	Guria	N	7
N24D	Khushia Shavi	Imereti, Guria	N	7
N24E	Badagi	Guria	N	7
N24F	Acharuli Tetri	Adjara	B	-
O21A	Tamaris Vazi	Kartli	N	3
O21B	Saperavi Atenis	Kakheti	N	5
O21C	TkvlapaShavi	Imereti	N	5
O21D	Tavkveri	Kartli	N	3
O21E	Shavtsitska	Imereti	B	5
O21F	Dondghlabi	Imereti	B	5
O22A	Sapena	Kakheti	B	5
O22B	Ubakluri	Kakheti	N	5
O22C	Rkatsiteli Vardisperi	Kakheti	Rs	3
O22D	Tsqobila	Kakheti	N	5
O22E	Danakharuli	Kartli	B	3
O22F	Chitiskvertskha Meskhuri	Kartli	N	5
O23A	Maghlari Tvrina	Imereti	N	5
O23B	Rko Shavi	Imereti	N	5
O23C	Dziganidzis Shavi	Imereti	N	7
O23D	Didshavi	Imereti	N	7
O23E	Kvelouri	Imereti	B	7
O23F	Samarkhi	Guria	B	7
O24A	Avasirkhva	Abkhazeti	N	3
O24B	Kachichi	Samegrelo	N	5
O24C	Shonuri	Samegrelo	N	7
O24D	Aspindzura	Kartli	N	5
PN	Pinot noir	-	N	-

*N: noir (black); B: blanc (white); Rs: rose; ** 3: low; 5: medium; 7: high.

Bailey (accession M24D), belonging to the Georgian *Vitis* germplasm, was included in this survey as resistant control accession, whereas 'Pinot noir N ' was used as susceptible control. Plants were grafted on 1103 Paulsen (*V. berlandieri* x *V. rupestris*) rootstock, spaced at 2.5 m (inter-row) x 1 m (intra-row), trained to the Guyot system at a density of 4,000 plants·ha^{-1} with a two-bud spur and a 10- to 12-bud cane. The inter-row soil was kept weed free by two yearly glyphosate herbicide treatments. Each accession consists of five plants per variety. The site is located in the Oltrepò pavese viticultural area (long. 9°05', lat. 44°58', elevation 144 m a.s.l.) on a hilly terrace with a slight east exposition

with a typical clay soil (Udic Paleustalfs fine silly, mixed, superaclive, mesic following the USDA soil taxonomy by Soil Survey Staff 1999). The initial plant propagation material was taken from the grapevine collection of Georgian ancient cultivars established in an area locally named as 'Dighomi' located closed to the Georgia capital Tbilisi in 1967/1968 and belonging to the Agricultural University of Georgia.

The trichome density on the lower side of leaves was determined in the field following the OIV method code 84 (2001).

Fifteen accessions (L22A, L22B, L23A, L23F, M22C, M22E, M22F, M24C, M24D, N21F, N23E, N24D, N24E, N24F, O22B and O23D) of this collection derived from wooden cuttings were also grown in pots (20 cm diameter) in greenhouse at 24 °C with a 12:12 photoperiod. The plants were not treated with fungicides active against *P. viticola*.

Fungal material: The *P. viticola* inoculum used in the experimental procedure was collected from naturally infected leaves of a plot not treated with fungicides against the downy mildew agent in a vineyard located in northern Italy at Sirmione, in the province of Brescia, Lombardy region. The 'Cabernet Sauvignon N' plants, spaced at 2.4 x 1 m, are trained to the Guyot system in vineyard. Symptomatic leaves were excised, placed in zip bags and transported to the laboratory in a ice box. The leaves were rinsed with running tap water to remove sporangia and incubated overnight in growth chamber at 22 °C to induce fresh sporulation.

Experimental inoculation: Experimental inoculations with *P. viticola* inoculum were carried out on leaf samples collected from the field at the beginning of grapevine growing seasons 2011, 2012 and 2013. Three leaves (3^{rd}-5^{th} leaf starting from the shoot apex) were detached from each accessions. Three leaf discs (15 mm diameter) were cut from each leaf with a cork borer and placed lower surface upward on a moistened filter paper in a Petri dish (9 cm diameter). Three plates containing three leaf discs were obtained for each grapevine genotype. The leaf discs were sprayed with 1 mL *P. viticola* sporangia suspension ($5x10^4$ sporangia·mL^{-1}) and incubated in growth chamber at 22 °C for 7-10 d.

The disease severity was estimated by the Percentage Index of Infections (I%I) calculated from the formula of Townsend and Heuberger (1947) after scoring each leaf disc for the surface covered by sporulation at the stereo microscope (Leica Wild M10). The classes used to calculate the I%I ranged from 0 to 7, where: 0 = absence of sporulation; 1 = 0.1-2.5 % of the surface covered by sporulation; 2 = 2.5-5 %; 3 = 5-10 %; 4 = 10-25 %; 5 = 25-50 %; 6 = 50-75 %; and 7 = 75-100 % of the leaf area covered by sporulation (Toffolatti *et al*. 2012). The plants with I%I lower than 25 % were considered resistant.

Quantification of sporangia: In 2011, experimental inoculations were carried out as previously described by using leaves collected from the grapevine varieties cultivated in greenhouse in order to estimate the the number of sporangia produced by the pathogen 10 d after incubation at 22 °C. The fungal inoculum (C strain) used in this assay derives from a single germinating oospore (Toffolatti *et al*. 2012). Sporangia were detached from the sporangiophores by vortexing the leaf discs in a 2 mL tube containing 1 mL of 20 % glycerol:water (v:v). The sporangia suspension was kept at -20 °C until further analysis. The average number of sporangia per leaf disc was obtained as described by Toffolatti *et al.* (2012) and divided by the leaf disc diameter (cm^2). The I%I was calculated for each accession.

Field evaluation: The downy mildew incidence on the Georgian varieties cultivated in vineyard was assessed in July at BBCH 79 phenological phase (Lorenz *et al.* 1994) for four consecutive grapevine growing seasons (2012, 2013, 2014 and 2015) by calculating the percentage of infected leaves and bunches (I%D) over the total. The downy mildew incidence was estimated also in an untreated plot of *V. vinifera* 'Croatina N', fully susceptible to *P. viticola*, placed immediately nearby. The 'Croatina N' plot has the same characteristics already described for the Georgian varieties and consists of three rows 50 m long. The I%D in the 'Croatina N' plot was estimated by counting the diseased organs on 100 leaves and 100 clusters randomly chosen in four subplots of 12 vines each. Meteorological data, *i.e.* average temperature (°C) and rainfall amount (mm), were daily recorded in a weather station from April until the middle of July each year.

Statistical analysis: The existence of differences in the I%I values related to hairiness and in the sporangia/cm^2 produced by the pathogen on different accessions was estimated by Kruskal-Wallis H on the values transformed in ranks followed by pairwise comparison post-hoc test. Non parametric statistics (Spearman R and Kendall Tau coefficients) was used to test the relationship between the I%I and the number of sporangia/cm^2 and between the I%I values obtained during the experimental inoculations carried out on the cultivars cultivated in open field and in greenhouse. For this purpose, the values were transformed in ranks. The analyses were carried out by SPSS v. 23.

Results

Experimental inoculations on leaves collected from the field: The results obtained by experimentally inoculating the leaves collected from the field greatly varied during the three year period considered (Fig. 1). The *Vitis* x *labruscana* L.H. Bailey accession M24D, used as resistant control, showed the complete absence of the disease symptoms in all the assays. On the contrary, the susceptible reference variety 'Pinot noir N' showed I%I higher than 50 %. Apart from a few cases, the Georgian *V. vinifera* accessions showed a more variable behaviour when inoculated with *P. viticola*. In 2011, almost all the accessions showed high levels of susceptibility (I%I > 35 %), except for three accessions that were characterized by I%I < 20 %: M22E ('Kamuri Shavi N'), L22A ('Mgaloblishvili N') and O22B ('Ubakluri Rs'). On the contrary, I%I typical of resistant individuals were found on most of the accessions (75 %) in 2012 and in half of the samples in 2013. These differences could be due to a different aggressiveness of the pathogen inoculum: the I%I values of the susceptible reference varieties in this study N21B ('Tita Kartlis B') and 'Pinot noir N', were 98 and 86 % respectively in 2011, 45 and

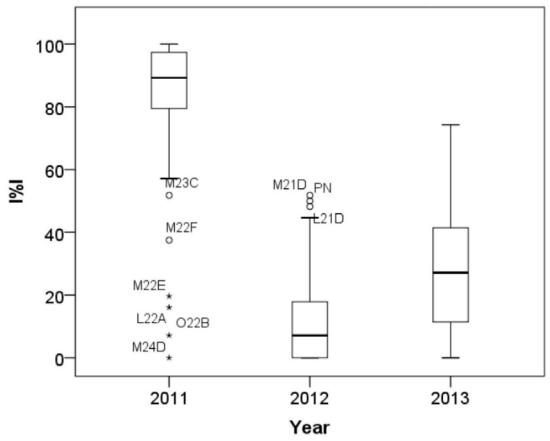

Fig. 1: Box-plot distribution of the I%I values recorded on the leaves collected from the grapevine varieties cultivated in open field from 2011 until 2013. PN and M24D indicate the susceptible 'Pinot noir N' and resistant *Vitis* x *labruscana* L.H. Bailey references respectively.

50 % respectively in 2012 and both 60 % in 2013. According to statistical analysis, the I%I obtained on 'Pinot noir N' in 2012 and 2013 were significantly lower than those obtained in 2011 ($F = 25.8$; $df = 2,6$; $P = 0.001$). The three inocula caused different degrees of damage to the host, leading to the different distribution observed in the three years (Fig. 1).

Based on the hair density, the plants were divided in three groups (Tab. 1): weak (OIV score = 3), medium (OIV score = 5) and high (OIV score = 7) hair density. The rank-transformed I%I values of the accessions with weak, medium and high levels of hairiness were significantly different in 2012 ($H = 9.04$; $df = 2$; $N = 90$; $P = 0.011$) and 2013 ($H = 10.7$; $df = 2$; $N = 92$; $P = 0.005$), whereas no differences could be found between the three categories in 2011 ($H = 1.15$; $df = 2$; $N = 93$; $P = 0.56$). In detail, in 2012 the group with high levels of hairiness showed significantly reduced values of I%I from the low level group, whereas the medium level group was not significantly different from the previous ones (Tab. 2). In 2013, the medium and low level of hairiness groups were significantly different from the high-level group, which showed the lowest I%I. These results could indicate that, in experimental inoculations, the degree of hairiness of the host plant plays a role in the colonization potential of pathogen only in presence of a less aggressive pathogen inoculum. On the contrary, it does not influence the colonization of the leaf tissues in presence of a highly aggressive inoculum.

Table 2

Average values of rank-transformed I%I of the accessions between 2001 and 2013 in relation to hairiness levels and results of statistical analysis*

Hairiness level	Year		
	2011	2012	2013
Weak	50.9 a	54.0 a	56.1 a
Medium	45.3 a	45.7 ab	46.7 b
High	44.0 a	33.3 b	33.1 b

*Mean values within the same column followed by the same letter are not significantly different at 0.05 significance level.

Sporulation and experimental inoculations on leaves collected from the greenhouse: Significant differences were found between the rank-transformed values of sporangia/cm^{-2} of the different accessions ($H = 25.8$; $df = 12$; $N = 39$; $P = 0.011$). The post-hoc test showed that the production of sporangia by the pathogen was significantly reduced on N21F and L22A compared to O23D and L22B (Tab. 3). The other accessions are not significantly different from these two groups.

Table 3

Average values of sporangiax10^3/cm^{-2} of the accessions and results of statistical analysis*

Accession	Sporangia/cm^2
N21F - Buza	1.2 a
L22A - Mgaloblishvili	1.5 ab
M22F - Jani Bakhvis	2.2 abc
L23A - Khushia Shavi	2.3 abc
M22E - Kamuri Shavi	2.4 abcd
N23E - Imeruli Shavi	2.6 abcd
M24C - Argvetula	3.2 abcd
O22B - Ubakluri	3.3 abcd
L23F - Vertkvitchalis Shavi	3.9 abcd
N24F - Acharuli Tetri	7.3 bcd
O23D - Didshavi	18.7 cd
L22B - Marguli Sapere	25.9 d

*Mean values within the column followed by the same letter are not significantly different at 0.05 significance level.

A significant positive correlation was found between the I%I values and number of sporangia/cm^{-2} of each accession: Spearman's Rho $r(16) = 0.938$, $P < 0.0001$; Kendall's Tau $t(16) = 0.805$, $P < 0.0001$.

No significant correlation was, on the contrary, found between the results of the experimental inoculations carried out on the cultivars cultivated in open field and in greenhouse (Fig. 2): Spearman's Rho $r(16) = 0.167$, $P = 0.538$; Kendall's Tau $t(16) = 0.138$, $P = 0.467$. Only two *V. vinifera* varieties, L22A and M22A, confirmed the resistant behaviour in both the inoculations, together with *V. x labruscana*.

Field evaluation: The different weather conditions occurring in vineyard (Fig. 3) led to different disease

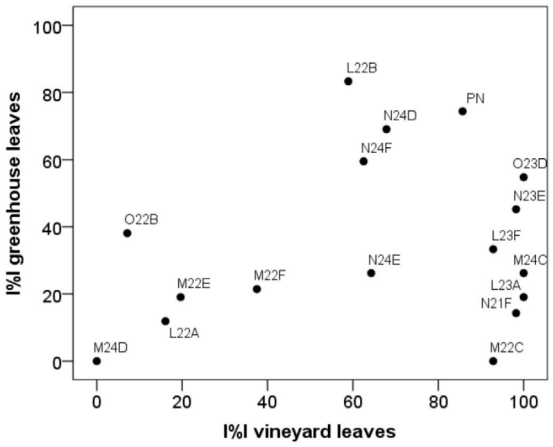

Fig. 2: Scatter plot showing the I%I of the same accessions collected from the field and from the greenhouse. PN and M24D indicate the susceptible 'Pinot noir N' and resistant *Vitis* x *labruscana* L.H. Bailey references respectively.

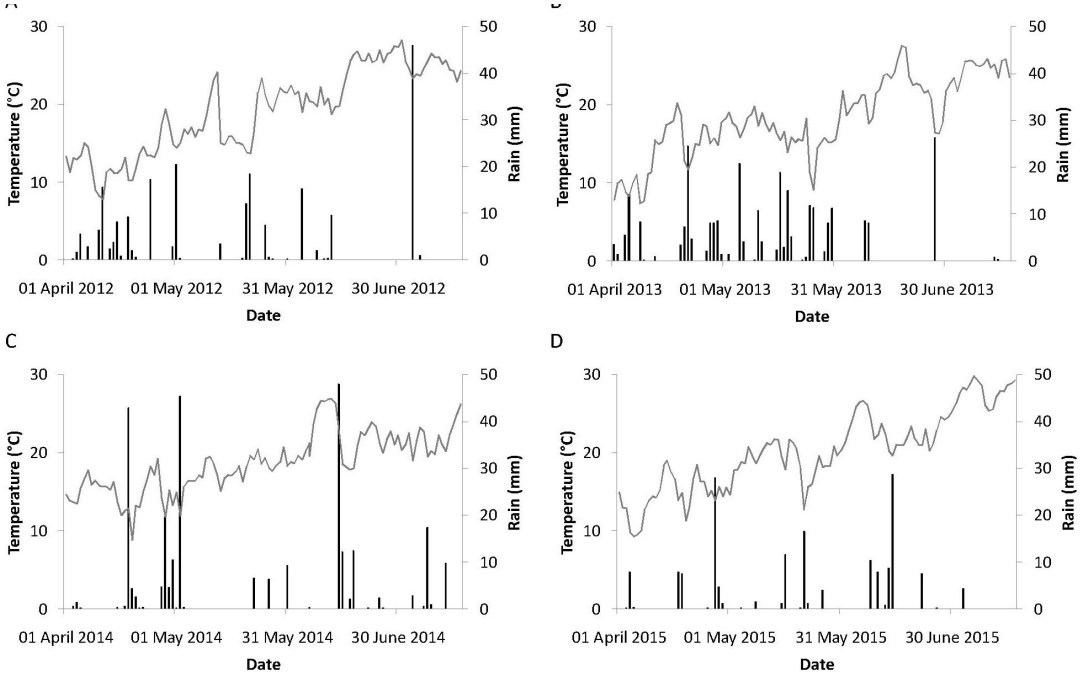

Fig. 3. Average temperatures (line) and sum of rain (bars) daily recorded in vineyard in 2012 (**A**), 2013 (**B**), 2014 (**C**) and 2015 (**D**).

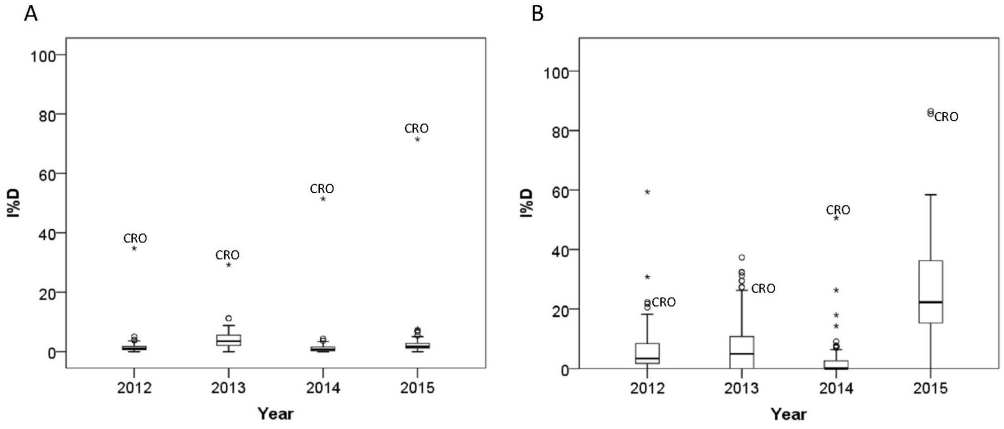

Fig. 4: Box-plot distribution of the I%D on leaves (**A**) and bunches (**B**) of the Georgian varieties. The I%D of the 'Croatina N' plot (CRO) are also indicated.

intensities in the untreated plot of *V. vinifera* 'Croatina N' during the 4-years study (Fig. 4).

In 2012 and 2013 the rainy events mainly occurred between April and the beginning of June and were almost completely absent in the following period. The first downy mildew symptoms were observed in the middle of May in both years. The I%D showed an increasing trend until the end of June, but it didn't increase further as a consequence of the dry period. In the 'Croatina N' plot at berry touch, 35 % of the leaves and 22 % of the bunches were affected by the disease in 2012. Analogous I%D were observed on leaves in 2013, whereas only 2 % of the bunches were affected by the disease. This difference could be due to the lower temperatures occurring in vineyard at the end of May. The rainy events were more evenly distributed throughout the April-July period in 2014 and 2015, leading to more severe downy mildew epidemics. The I%D on leaves and bunches were slightly higher than 50 % in 2014 and than 70 % in 2015. The downy mildew incidence on Georgian accessions was generally very low on leaves, with I%D beneath 12 % (Fig. 4A). Also on bunches the pathogen diffusion was reduced, with some exceptions. A few cultivars showed I%D close or higher than those observed on 'Croatina N ' in 2012 and 2013. In the following season, the Georgian varieties generally showed I%D lower than 10 %, despite the weather conditions particularly conducive to the pathogen. Even in 2015, when the disease pressure was particularly high, 50 % of the Georgian accessions showed a reduced percentage of infected bunches with I%D lower than 22 % whereas the remaining ones exceeded 20 % (Fig. 4B). Particularly interesting are 23 accessions with I%D lower than 14 %, among which there is L22A. The second accession that showed a constant resistant behaviour during the experimental inoculations with *P. viticola* (M22E), was characterized by I%D on bunches analogous to those of the 'Croatina N' variety in 2015 (86 %): this could be due to the particularly low number of bunches present on the plants, therefore this result is not reliable for the purpose of the study. No symptoms of the disease were observed on *Vitis* x *labruscana* during the whole period of investigation.

Conclusion

In this multi-year study, the level of susceptibility to *P. viticola* of Georgian *V. vinifera* varieties was assessed by combining data obtained in bioassays with the disease incidence in the field. Using this approach it is possible not only to get a more reliable evaluation of the plant behaviour but also to get insights on the durability of resistance. *Plasmopara viticola* has been shown, in fact, to undergo differential adaptation to host cultivars, sometimes leading to erosion of partial resistance (Delmotte *et al.* 2014).

Vitis x *labruscana*, the resistant control accession, did not show any disease symptoms in the assays confirming its resistant behaviour, whereas the susceptible control 'Pinot noir N' was heavily contaminated by the pathogen during the assays. The great majority of the 94 Georgian varieties tested were susceptible to the pathogen. Two varieties, 'Kamuri Shavi N' (M22E) and 'Mgaloblishvili N' (L22A), showed the capability to contain the infections during the experimental inoculations with different *P. viticola* populations, both when grown in the field and in greenhouse. On the contrary, the 'Ubakluri Rs' variety (O22B), that in 2011 showed a resistant behaviour, was heavily infected by the pathogen in the following tests.

The different I%I distributions obtained in the three experimental inoculations carried out on the leaves collected from the field highlight the importance of repeating the tests with different populations of *P. viticola.* This is necessary to confirm the obtained results and particularly important when the screening activity is carried out on plant material collected from the field, where the environmental conditions are not controlled as in the greenhouse, and when the pathogen can be characterized by different levels of aggressiveness. Despite belonging to the same vineyard, the *P. viticola* populations used in this study showed different aggressiveness levels. The existence of variability in the pathogen populations isolated from the same vineyard in different years could be due to several factors, among which are the occurrence of sexual reproduction at the end of each grapevine growing season and the migration of strains from surrounding vineyards. It has to be pointed out that the vineyard from which the inocula were sampled is located in an area with a high level of viticultural activity. Indeed, different compositions of *P. viticola* populations were found between years in the same field while monitoring QoI resistance (Toffolatti *et al.* 2011) and among subpopulations of the same plot in microsatellite analyses (Rumbou and Gessler 2006).

No significant correlation was found between the disease severity indexes of the leaves collected from the field and from the greenhouse in 2011. Of the 15 accessions tested, 5 (33 %) showed analogous I%I when inoculated with two different inocula, and 10 (67 %) showed different I%I with different inocula, leading to an overall absence of significant correlation. Several factors, related to the pathogen and the plant and their interaction, could explain this result. The pathogen inocula used for the experimental inoculations of the leaves collected from the field and greenhouse were actually different, being the first one a field population and the second one a strain derived from a single germinating oospore. However, the susceptible and resistant controls ('Pinot noir N' and M24D *Vitis* x *labruscana*) showed analogous I%I with the two inocula, suggesting that the environmental conditions of the field could have more likely influenced the response of some accessions and in particular of M22C, O22B, N21F and L23A, that showed a resistant or susceptible behaviour in the two assays. The different susceptibility of some accessions could be due to the different physiological state of the plant leading to different capability of synthesizing the compounds that are involved in the response to the pathogen. Since no symptoms associated with hypersensitive response could be observed in the accessions, but the plant response allowed a reduction of the pathogen colonization, it is likely that quantitative resistance occurr in the Caucasian grapevine accessions. It has been already demonstrated that also in this case the response to the pathogen may vary (StClair 2010, Toffolatti *et al.* 2012).

In parallel, the downy mildew incidence in vineyard was assessed on an untreated plot of cv 'Croatina N' and on the Georgian varieties. Due to the weather conditions, the plants were exposed to variable levels of disease pressure during the four grapevine growing seasons. The Georgian plants always showed a reduced downy mildew incidence on leaves, also when the disease pressure was particularly high as in 2014 and 2015. On bunches a more variable situation could be found, with some varieties showing a disease incidence similar to that observed on the 'Croatina N' plot. It has to be pointed out that in the Georgian plot only five plants per variety were available, therefore the reduced number of bunches of the Georgian varieties could negatively influence these results, as observed in 2015 with 'Kamuri Shavi N' (M22E). 'Kamuri Shavi N' (M22E) and 'Mgaloblishvili N' (L22A), that showed a resistant behaviour during the experimental inoculations, also showed a reduced disease incidence in the field, whereas 'Ubakluri Rs' (O22B) showed a more variable behaviour. Moreover, a reduced number of sporangia was differentiated by the pathogen on 'Kamuri Shavi N' (M22E) and 'Mgaloblishvili N' (L22A). The significant reduction in *P. viticola* sporulation, an important component of the pathogen fitness, observed in the latter accession is particularly important at the epidemiological level because it could help reducing the entity of secondary infection cycles in field conditions.

Among the factors that influence the early interaction between the pathogen and its host, the structural defence mechanisms of the host can have a considerable role. A high level of leaf hairiness can constitute a physical barrier to the pathogen penetration in the stomata and affect the infection process (Kortekamp and Zyprian 1999). Based on the experimental inoculations carried out on the leaves collected from the field, a high leaf hair density did not prevent from heavy *P. viticola* infections when an aggressive pathogen strain was inoculated, but significantly reduced the disease severity in presence of a less aggressive inoculum. Therefore, this kind of preformed barrier does not completely prevent the pathogen infection but in some particular conditions limits it. Indeed, 'Kamuri Shavi N' (M22E) and 'Mgaloblishvili N' (L22A) are characterized by high trichome density leaves. However, the trichome density levels can not be clearly related to the resistant levels of the different varieties. This result is in line to the observations of Boso *et al.* (2010).

Therefore, resistance could be more likely the result of the pathogen recognition by the plant.

The overall data obtained in the study strongly indicate that among the tested Georgian varieties, 'Mgaloblishvili N' (L22A) has a good capability to contain *P. viticola* colonization, both in natural and in experimental conditions, and to limit the pathogen sporulation. 'Kamuri Shavi N' (M22E) showed an analogous behaviour. However, due to the results obtained on bunches in 2015, further investigations should be carried out to confirm the resistant attitude of the accession. The progenies of these two cultivars, obtained by self pollination, open pollination and pollinated with pollen of 'Pinot noir N', are currently under investigations in order to get insights on the resistance mechanism at the genetic and histochemical levels.

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