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Downy mildew resistance evaluation in 28 grapevine hybrids promising for breeding programs in Trentino region (Italy)

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Abstract Downy mildew is a major grapevine disease caused by the biotrophic oomycete, *Plasmopara viticola*. Numerous disease resistance studies of diverse *Vitis* germplasm have been previously carried out to identify downy mildew resistance sources; however, ratings were mainly reported using leaf disc in vitro testing and foliage field assessment, or upon leaf and cluster field evaluations. In the current study, 28 grapevine hybrid cultivars were screened using leaf disc bioassay, for disease resistance characterization of both existing and wild-collected materials. 16 hybrids were identified as highly resistant or resistant, and will serve as relevant resistance donors in future pre-breeding and breeding programs. All grapevine hybrids were evaluated for foliar and cluster downy mildew resistance in an untreated field trial over three successive years. This study showed that the leaf disc bioassay provided some information on the resistance level of the genotypes under scrutiny, but it was a weak predictor of their resistance level under field conditions on leaves and even more on bunches. These findings are relevant to future applications in both traditional and marker-assisted breeding programs which promote sustainable viticulture.

Keywords *Plasmopara viticola* · *Vitis* spp. · Disease symptom assessment · Weather effect

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Introduction

Downy mildew (DM) is a major grapevine disease caused by the biotrophic oomycete, *Plasmopara viticola* (Berk. and Curt.) Berl. & de Toni. The classic cultivars for wine, table grape and raisin production belong to the *Vitis* species widely spread in Europe and Asia Minor, *Vitis vinifera* L., and are susceptible to this pathogen (Deglène-Benbrahim et al. 2010). *P. viticola* infects all green parts of the vine, leaves and bunches in particular (Ingram 1981). In favourable weather conditions, the pathogen causes numerous infection cycles, which are responsible for both quantitative and qualitative yield reductions (Toffolatti et al. 2012). Not only is control of DM a past and present problem for viticulture. In fact a recently published infection model due to projections of climate change on air temperature and leaf wetness data, and parameterized with the thermal and moisture requirements of DM, foresee an increase in DM disease pressure throughout Europe (+ 5 to + 20%) by 2030 (Bregaglio et al. 2013).

Control of DM often requires frequent fungicide treatments, especially in temperate rainy regions, to prevent severe DM epidemics and obtain acceptable quality grapes (Gisi and Sierotzki 2008). For a

sustainable viticulture, nowadays there is a need to reduce fungicide usage. A solution is the development of new varieties with innate disease resistance from crossing between *V. vinifera* cultivars and resistant *Vitis* species. Resistant or partially resistant hybrids have the potential to greatly reduce the application of plant protection compounds and thereby lead to a substantial contribution to viticulture sustainability; in particular, treatments can be limited during unfavourable vintages (highly rainy) or crucial phenological stages (i.e. flowering and berry set). Several breakthroughs have been achieved in grapevine resistance breeding during the twentieth century when over 6000 hybrids were registered in Europe. Unfortunately the offspring of most of these varieties did not succeed in the market due to poor wine quality or other factors (Pacifico et al. 2013). Despite evidence by 1960 that disease resistance did not necessarily mean a decrease in wine quality in these cultivars, including even the best French hybrid cultivars, they were not accepted in the European market (Topfer et al. 2011). However, newly bred wine grape cultivars showing good field disease resistance and high wine quality have been entering the market over the past few decades particularly outside of Europe. Still these tolerant or resistant cultivars produce wine with some characteristics which are not appreciated by European consumers (Guedes de Pinho and Bertrand 1995).

More recent blinded tastings of such wines have demonstrated that it is possible to make wine with these cultivars of equal quality compared with traditional ones (Basler and Pfenninger 2003). The recent registration of twenty grapevine hybrid cultivars (e.g. Regent, Bronner, Solaris) in the National Grapevine Variety Catalogue of Italy (Catalogo Viti) may signal a new recognition of the results of such studies and a move toward acceptance of non-traditional varieties.

The evaluation of resistance of *Vitis* germplasm or progeny individuals to downy mildew has been evaluated in field conditions under natural infections (e.g. Eibach et al. 1989; Pavlousek 2012; Wan et al. 2007) or in laboratory and greenhouse by means of artificial infections (inoculations) on leaf discs (e.g. Bellin et al. 2009; Boso et al. 2006; Kennelly et al. 2007), detached leaves (e.g. Boso and Kassemeyer 2008; Kiefer et al. 2002) and entire plants (e.g. Brown et al. 1999; Gindro et al. 2006; Malacarne et al. 2011). Field evaluation is a reliable method of assessing DM resistance that is representative of field conditions and allows to process efficiently large amount of plants (Kono et al. 2015).

The present work aims at assessing and comparing the phenotypic variability of grapevine hybrids upon *P. viticola* infection under natural conditions, namely in untreated vineyard, and under controlled (in vitro) conditions. Combining leaf disc bioassay with leaf and cluster DM evaluation, we examine the correlation between field and laboratory assessments of DM. These findings can provide a valuable starting point both for breeders and researchers who seek novel grapevine genetic resources to enhance disease resistance and preserve good fruit quality traits.

Materials and methods

Plant material

The evaluated plant material consisted of 28 grapevine hybrids, from various European institutions or nurseries located in France, Germany, Austria, Hungary and Czech Republic, and *V. vinifera* cv Chardonnay as a positive control (Table 1 of Supporting Information). The genetic characterization of this overall plant material has been performed and for most hybrids the trueness-to-type has been validated against the VIVC database (www.vivc.de) and/or through pedigree analysis (Vezzulli et al. 2015). All hybrids and the positive control cv Chardonnay were cultivated in an untreated vineyard in San Michele all'Adige (TN, Italy). Each genotype was represented by one single plot of 25 plants cultivated and managed since 2009 using a Guyot (cane based) training system with planting density of 6250 plants/ha (0.8 m × 2 m).

DM propagation and leaf disc inoculation

Leaf disc bioassay was performed twice per year, in June 2011 and 2012, for a total of four experiments. With the aim to propagate and collect a large amount of fresh inoculum the abaxial leaf surfaces of ten *V. vinifera* cv Pinot gris potted plants were sprayed with a distilled water suspension of 5×10^5 freeze stored sporangia ml^{-1}, and kept under high relative humidity (RH > 90%) overnight at 21 °C for 5–6 dpi (days post-inoculation). Freeze stored sporangia derived from a collection of *V. vinifera* susceptible varieties in an untreated field. After incubation, the plants were again maintained under moist conditions overnight to induce sporulation. Sporangia were recovered by soaking infected leaves

in cold (4 °C) distilled water and adjusting to a dilution of 5×10^5 sporangia ml^{-1}.

In each experiment, leaves were collected when the shoots reached the stage of 5–6 unfolded leaves and before the occurrence of weather conditions suitable for DM primary infections. For each genotype, the fourth and fifth leaves beneath the shoot apex were detached from three chosen plants (for a total of six leaves) and rinsed with water. Eight leaf discs of 2.5 cm diameter were excised from the six bulked leaves, paying attention to avoid veins, with a cork borer and plated onto wet paper in a Petri dish with the abaxial side up. Discs were sprayed using a micro-sprayer (Ecospray®, Bluestar, MonteCarlo, Monaco) with 125 μl of *P. viticola* inoculum suspension at about 1×10^5 sporangia ml^{-1} (corresponding to 1 ml per Petri dish), the inoculum was prepared as previously described. Petri dishes were incubated in a growth chamber at 21 °C in the dark for 6 days. Drops were removed from the discs with sterile filter paper after 24 h to avoid development of moulds or bacteria. Leaves of the susceptible *V. vinifera* cv Chardonnay were used as a positive control.

Upon leaf disc inoculation, the level of resistance of all genotypes was scored at 6 dpi for: disease severity (DS) (percentage of the disc area showing symptoms of sporulation) and disease incidence (DI) (number of discs with sporulation/total number of discs), according to OEPP/EPPO (2001). Moreover, the degree of resistance to *P. viticola* was evaluated by visual observation using OIV descriptor 452-1 recommended for leaf disc testing by the Organisation Internationale de la Vigne et du Vin (2009), adapted according to Bellin et al. (2009) (Fig. 1).

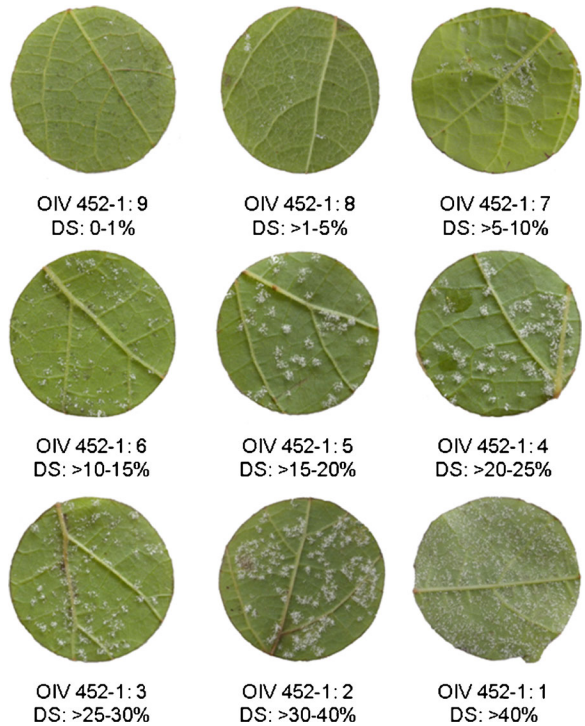

Fig. 1 OIV 452-1 classes, indicating the downy mildew resistance level, coupled with the OEPP/EPPO sporulation percentage at 6 days post-inoculation

DM symptom assessment in untreated vineyard

For 28 European hybrids and the positive control grown in the untreated vineyard, symptoms of DM natural infections on both leaves and clusters were assessed weekly from onset of flowering until veraison over three growing seasons (April–October of 2011, 2012 and 2013), according to OIV descriptor 452 and OIV descriptor 453 (OIV 2009). These descriptors provide an international reference for the assessment of DM resistance on leaf and cluster based on symptom visual observation. The considered data corresponded to the maximum level of symptom expression (lower OIV scores) that occurred at 3–5 weeks after the onset of flowering, depending on each genotype. Weather conditions including rainfall, temperature and RH values were recorded over every growing season at the weather station situated at San Michele all'Adige (TN, Italy) about 1.5 km from the vineyard and managed by the Geographical Information System of the Technology Transfer Center at FEM.

Statistical analysis

All statistical analyses were performed with the statistical software SPSS 17 (SPSS, Chicago, USA). DS and DI percentage data from the in vitro bioassay were arcsine transformed and subjected to analysis of variance (ANOVA); mean comparisons were made by Students Newman Keuls (SNK) post-hoc test. Experiment, year and hybrid were considered as fixed factors. OIV scores (categorical variables rating: 1, 3, 5, 7 and 9) from field assessment were subjected to non-parametric test (Kruskal-Wallis H test) with the aim to evaluate the year effect. For relationships between different pairs of OIV scores a non-parametric test (Spearman's rank correlation coefficient, ρ) was used. For statistical analyses the maximum score obtained during the weekly

assessments in the field was used, as the best indicator of the resistance level.

Finally, the quantitative relationship between DS and OIV scores was analysed by means of a simple linear regression.

Results

Leaf disc bioassay (in vitro assessment)

For leaf discs, significant differences upon SNK test in both DS ($F = 20.78$, $P < 0.001$) and DI ($F = 3.009$, $P < 0.001$) were seen among genotypes, while the year effect was not significant ($F = 0.413$, $P = 0.521$ for DS and $F = 2.52$, $P = 0.116$ for DI). Because of the non-significant difference observed over the 2 years, leaf discs data were pooled. Out of all studied 29 genotypes, Bronner resulted to be totally resistant, showing no DM symptoms. Moreover, Muscaris, Cabernet Cortis, SV023, and Solaris revealed a high resistance level with DS ≤ 5%, a mean DI value of 61%, and an OIV 452-1 = 8. Furthermore, 11 hybrids showed to be resistant with 5% > DS ≤ 15% and DI values ranging from 62% (Cabernet Carbon) to 100% (Johanniter) with a OIV 452-1 of 6 and 7, with the only exception of the value 8 in Nero. Out of the remaining 13 genotypes with DS > 15%, 8 hybrids presented values of DS > 15% and ≤ 25%, coupled with a high DI of between 92% (Aromera) and 100% (Leon Millot, 29-2-85, 30-4-87, Lidi) and a OIV 452-1 range from 4 to 6. Moreover, besides these low susceptible hybrids, 30-4-154, MW14, Fanny and 16-02-102 showed a higher level of susceptibility, with 25% > DS ≤ 60%, mean DI of 94% and OIV 452-1 of 3 and 4. Finally, the positive control Chardonnay (DS = 61% and DI = 100%) showed a very high susceptibility (Table 1).

A significant correlation ($r = -0.939$, $P < 0.001$) was found between the OIV 452-1 and DS on leaf discs (Fig. 2).

Table 1 Mean Disease Severity, Disease Incidence and OIV 452-1 scores on leaf discs of the entire genotype set

Genotype name	Disease Severity (%) (DS)[a]	Disease Incidence (%) (DI)[a]	OIV 452-1
Bronner	0.0a	0.00a	9
Muscaris	2.9ab	50.0b	8
SV023	3.7ab	60.4b	8
Cabernet Cortis	3.4bc	68.7b	8
Solaris	5.0bcd	64.6b	8
Cabernet Carbon	6.4bcd	62.5b	7
Nero	5.8bcde	91.7b	8
Regent	7.1bcde	84.4b	7
Phoenix	8.5bcde	83.3b	6
Johanniter	7.2bcdef	100.0b	7
29-2-187	9.0bcdefg	91.7b	7
Esther	10.5bcdefg	87.5b	7
30-3-040	12.1bcdefg	70.8b	6
Poloskei muskotaly	12.2bcdefgh	89.6b	6
Prior	12.0cdefgh	92.7b	6
30-4-190	13.3cdefgh	91.7b	6
Leon Millot	15.2defghi	100.0b	5
29-2-85	17.9efghi	100.0b	5
Aromera	18.3efghi	91.7b	6
Bianca	19.6efghi	95.8b	5
30-4-87	19.5fghi	100.0b	5
24-02-112	20.2fghi	95.8b	5
30-4-154	25.2ghi	83.3b	4
Lidi	23.4hi	100.0b	4
Palatina	23.9hi	95.8b	4
MW14	28.0i	95.8b	3
Fanny	28.7i	91.7b	4
16-02-102	43.8l	93.7b	3
Chardonnay	61.0m	100.0b	1

[a] Means with the same letter are not significantly different at SNK test ($P \leq 0.05$)

Natural field infection trial

In 2011, 16 hybrids showed a high level of resistance on leaves (OIV 452 scores 7–9), while during 2012 and 2013 only six and eight hybrids respectively showed such scores. Over the evaluated 3 years a consistent level of DM resistance on leaves was observed in four hybrids: Bronner, Solaris, Prior and Muscaris. Indeed, regarding disease resistance on clusters, 21 hybrids showed a high level (OIV 453 scores 7–9) in 2011, while during 2012 and 2013 the number of hybrids showing such scores decreased to 14 and 8, respectively. Over the evaluated 3 years a consistent level of DM resistance on clusters was observed for four hybrids: Bronner, Solaris, Prior and Bianca. Considering the level of DM resistance both on leaves and clusters over the 3 years, the best results were achieved by Bronner,

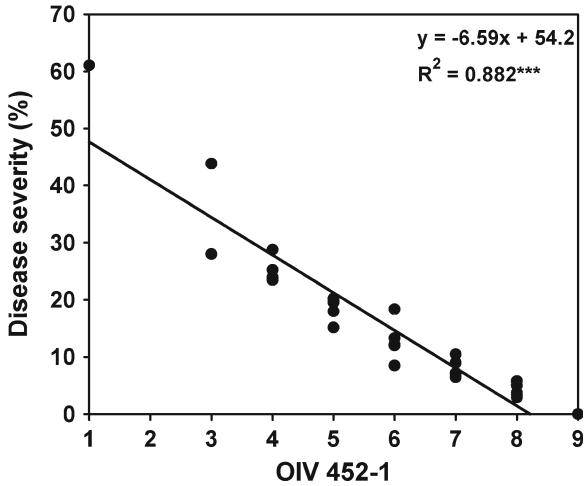

Fig. 2 Regression between Disease Severity and optimized OIV 452-1 on leaf discs of the 29 genotype set. Pearson's correlation significance was indicated by *** (significant at $P < 0.001$)

Solaris and Prior (Table 2 of Supporting Information). Chardonnay (positive control) showed a high level of symptoms both on leaf and cluster (OIV 452 and OIV 453 = 1).

Significant ($P < 0.001$) correlations were found between leaf and cluster OIV descriptors all over 3 years (Table 2).

The experimental vineyard is located in a region characterised by humid-temperate summers. The mean seasonal temperatures (during growing season) for this area were 18.5 °C for 2011, and 18.3 °C for both 2012 and 2013 years (three-year mean temperature: 18.4 °C). The total seasonal rainfalls were 617, 891 and 900 mm for, respectively, 2011, 2012 and 2013 (3-year mean rainfall: 803 mm). A significant difference between years was recorded for both OIV 452 ($\chi^2(2) = 6.052$, $P = 0.049$) and OIV 453 ($\chi^2(2) = 17.155$, $P = 0.00019$) (Table 2 of Supporting Information). In both clusters and leaves the disease was less severe in 2011 than 2012 and 2013. The pairwise correlation between the same OIV 452 on leaves assessed in successive years (Fig. 3a and b) revealed a higher value of Spearman's rank correlation coefficient ($\rho = 0.815$, $P < 0.001$) for 2012–2013 than for 2011–2012 ($\rho = 0.636$, $P < 0.001$).

Table 2 Spearman's coefficient of rank correlation for OIV 452 on leaves and OIV 453 on clusters of the 29 genotypes present in the untreated vineyard over 3 years

OIV Descriptor/Year		
OIV 452/2011 vs OIV 453/2011	ρ	0.666
	P	< 0.001
OIV 452/2012 vs OIV 453/2012	ρ	0.614
	P	< 0.001
OIV 452/2013 vs OIV 453/2013	ρ	0.714
	P	< 0.001

Comparison between in vitro and field DM assessment

DM symptoms assessed by the OIV descriptors on the 29 genotypes evaluated both under controlled (bioassay) and natural (untreated vineyard) conditions showed significant ($P < 0.01$) correlations in both 2011 (ρ = 0.544) and 2012 (ρ = 0.557) between leaf disc

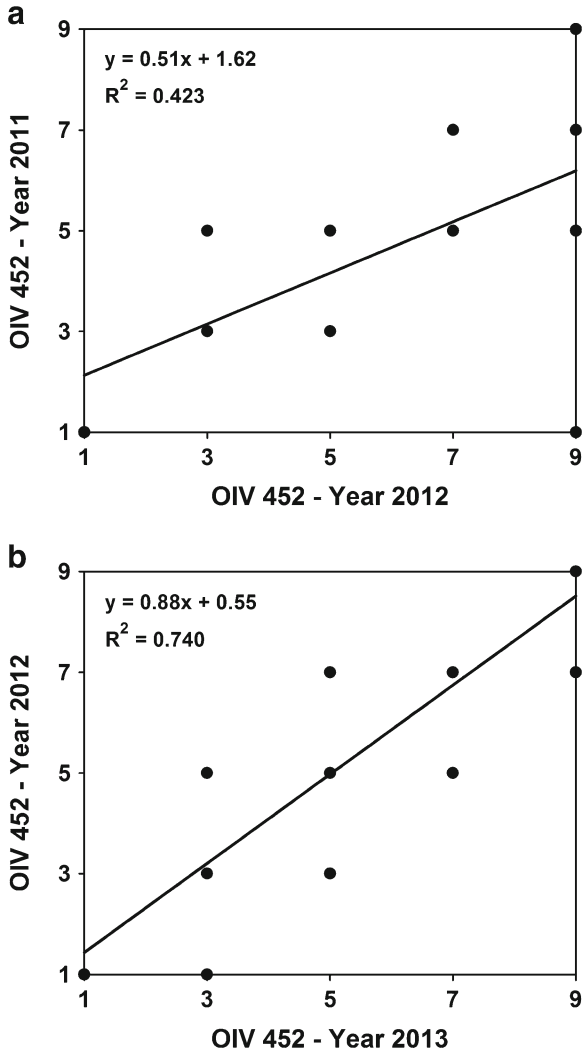

Fig. 3 Regression between OIV 452 on leaves of the 28 hybrids evaluated in the untreated vineyard in successive years: 2011–2012 (**a**) and 2012–2013 (**b**)

assays and leaves (Fig. 4a). The correlation between disease assessments on leaf discs and on clusters resulted statistically not significant in 2011, while was found statistically significant ($\rho = 0.575$, $P < 0.01$) in 2012 (Fig. 4b).

Discussion

The importance of grapevines with DM resistance has long been recognized and breeding for grapevine resistance to DM was initiated in the first half of the nineteenth century. Even when variability in susceptibility toward *P. viticola* was present in the available *V. vinifera* cultivars, breeders focused on hybridization of this species with selections from American species with high levels of DM resistance, or with the American hybrids that displayed good field disease resistance (Alleweldt et al. 1990; Millardedt 1885). The advent of rootstock resistant to phylloxera and advances in the chemical control of DM reduced the demand for cultivars with resistance to *P. viticola*. Resistance breeding was no longer a priority in most European countries. Eventually, the first-generation DM-resistant hybrids mostly disappeared from commercial vineyards, except for few well-accepted ones such as Chambourcin released only in 1963. Since the late 1980's breeding of disease resistant cultivars (second-generation hybrids) has become a priority once again in some new wine regions and in regions in which pesticide applications have been intensive and expensive (Gessler et al. 2011). The recent emergence of a DM strain which has adapted to overcome a newly introduced resistant hybrid (Peressotti et al. 2010) highlights the urgent need to identify different mechanisms of resistance. This may be achieved through the phenotypic screening of large populations of resistant wild grapes or of particularly resistant hybrids as sources of novel resistances. The construction of a "library" of resistant plant genetic sources will enhance the possibilities for gene pyramiding, which relies on the combination of multiple genes in a variety to reach broad spectrum and potential durable resistance (Joshi and Nayak 2010). Although the genetic basis of the current grapevine breeding germplasms is narrow, the screening of underexploited available and newly wild-collected resources for disease resistance traits is uncommon; this may be of fundamental importance to the development of sustainable viticulture in increasing regions of the world. In fact, a renewed interest in interspecific varieties has emerged, due mainly to increased awareness of producers and consumers of the positive contributions of organic farming and the negative impact of some fungicides on the environment (Pacifico et al. 2013).

In our study, 29 grapevine genotypes were rated for DM resistance by the combination of three assessments: leaf disc bioassay and both foliar and cluster resistance evaluation in an experimental untreated vineyard. These assessments contribute to the phenotyping know-out and are preparatory to the detection of new genomic intervals associated to DM resistance as well as negative quality traits which can be exploited for respectively positive and negative selection in Marker-Assisted Breeding programs.

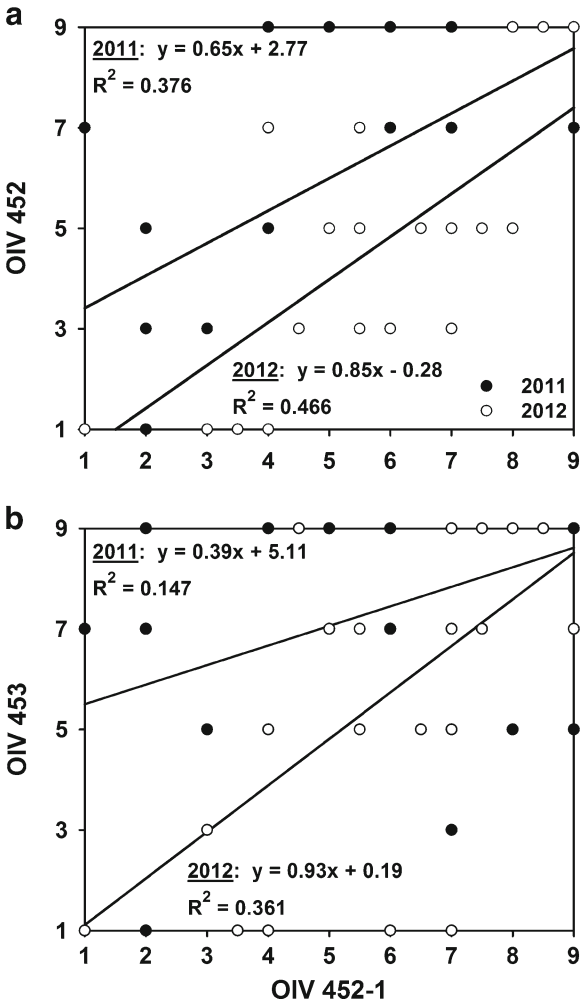

Fig. 4 Regression between OIV 452-1 on leaf discs and: OIV 452 on leaves (**a**) and OIV 453 on clusters (**b**) of the 28 hybrids evaluated

The hybrid in vitro phenotypic variability

The present inoculation experiments carried out on leaf discs revealed a different degree of susceptibility and resistance to *P. viticola* among hybrids. This observation is in agreement with a number of previous studies focused on the in vitro (or ex-planta) screening of wild species and inter-specific hybrids, confirming that these non-*vinifera* materials are not all fully resistant to mildews (e.g. Prajongjai et al. 2014; Staudt and Kassemeyer 1995). All the 28 studied hybrids are currently under deep genetic characterization though their pedigree validation or reconstruction. Such characterization will help to understand which genome portions of the resistant ancestor or which already known specific *P. viticola* resistance (*Rpv*) locus have been inherited (Peressotti et al. 2015; Zini et al. 2015). In particular, eight hybrids (SV023, 29-2-187, 30-3-040, 30-4-190, 29-2-85, Aromera, 30-4-87, 24-02-112), resulted unknown and related to known genotypes present in the international grapevine variety databases (Vezzulli pers. comm.), represent new relevant resources for resistance/low susceptibility - coupled with quality traits.

Even though both DS and DI were statistically affected by the genotype factor, we found an higher genotype effect on DS compared to DI as revealed by the smaller *F* value for DI. In fact, regarding DI, the complex German hybrid Bronner (Merzling x Gm 6494), descending from *V. rupestris, lincecumii,* and *amurensis*, only significantly differentiated from all other studied hybrids showing an absolute degree of DM resistance as confirmed by DS score. Moreover, the German Solaris and its derived hybrids Muscaris and Cabernet Cortis resulted to be highly resistant. These findings are consistent with Boso and Kassemeyer (2008), who examined Solaris (Merzling x (Severnyi x Muskat Ottonel). Indeed SV023 represents a novel genotype to be employed for inbreeding or mapping family creation. Two recently introduced half-sibling hybrids (Cabernet Carbon and Prior) derived from Bronner were found to be resistant. Besides this, it is relevant to underline that the German hybrid Regent, well-characterized for its partial DM resistance (Spring et al. 1998; Zamboni et al. 2009), showed only the 7% of DS. In fact, the effect of pathogen source on Regent resistance ratings, might reflect genetic variation in *P. viticola* in overcoming race-specific resistance (Cadle-Davidson 2008).

The same reasoning may apply to the Hungarian hybrid Bianca resulted a low susceptible hybrid. Bianca was initially characterized as resistant to DM (Kozma and Dula 2003); subsequently, findings by Peressotti et al. (2010) suggested that a *P. viticola* isolate may have evolved to overcome Bianca resistance.

Moreover, we identified low susceptible genetic backgrounds that ranged from *V. riparia* and *rupestris* (most hybrids, including well-known hybrids such as Leon Millot and less well known such as Lidi) and four novel hybrids (number series and Aromera) derived from a breeding private platform. Concerning the four hybrids that showed susceptibility, excluding the positive control, they corresponded to DS values comparable to *V. vinifera* varieties, resulting in a not interesting material for disease resistance breeding, but in a valuable tool to study mechanisms of resistance and race specificity.

Considering that 96% of the studied genotypes showed DM symptoms, those which revealed a DI rate = 100% presented a DS range from 7 to 61%. Moreover, the assessment of DI reflected DS only for distribution tail values of the latter, and does not seem to be a good indicator of the intermediate resistance/sensitivity levels to DM. Since for leaf excision we bulked the two most responsive leaves to DM (Calonnec et al. 2013), we overcome the leaf-age dependant resistance issue (Steimetz et al. 2012). While the production of sporangia derived from the artificial inoculation are widely accepted as good criteria for estimating the resistance/sensitivity to pathogens (Alonso-Villaverde et al. 2011), our results suggest the need to combine DI measurement with a parameter describing disease severity such as DS as we report here.

This phenotypic variability might correspond to different response patterns as observed by Jürges et al. (2009), who proposed that the interaction between host and pathogen is under control of specific signals that have been subject to evolutionary diversification. Moreover, the development of the pathogen can be stopped or reduced by different defense mechanisms, such as the synthesis and deposition of callose, the production of reactive oxygen species (ROS), hypersensitive responses, peroxidase activity and the synthesis, and accumulation and conversion of phenolic compounds (e.g. Gindro et al. 2003, 2006; Toffolatti et al. 2012; Yu et al. 2016).

The hybrid field DM resistance level on leaf and cluster

Unlike for leaf disc bioassay where it is usually possible to rely on the DS to compare current results with

previous findings such a comparison is not so straightforward for field evaluation. In fact, OIV descriptors are employed mainly in Europe and rarely in the USA but they are often not uniquely recorded. Although the UPOV (the International Union for the Protection of New Varieties of Plants; French: Union internationale pour la Protection des Obtentions Végétales) system is widely known and adopted, in some European studies a key scale (ranging from 1 to 9) developed by Mohibullah (1991), and derived from bulb vegetables, is adopted with opposite meaning (sensitivity corresponds to higher scores) respect to OIV 452 and 453 (e.g. Boso et al. 2011). Investigations conducted in Asia rated from 0 (high resistance level) to 7 (high susceptibility level) based on the percentage of lesion over the whole leaf (e.g. Wan et al. 2007).

Various study comparisons have been performed in attempt to develop a uniform disease susceptibility rating system, despite the fact that studies were often conducted under different climate and pedological conditions (*terroir*) or with diverse vineyard management systems. For instance, Pacifico et al. (2013) monitored 19 inter-specific grape varieties in northeastern Italy, treated four times during the growing season over two seasons. The estimation of disease resistance resulted in a great discrepancy into two observed years, largely due to different weather conditions: during 2004 the antifungal treatments limited disease damage, while in 2005 high rainfall occurred causing more severe disease damage. In the study by Lisek (2010), 23 hybrid cultivars were evaluated for several years in Poland carrying out just one or two antifungal treatments each season while in field surveys performed in Italy (Zulini et al. 2008), Serbia (Cindric et al. 2003), Switzerland (Basler and Pfenninger 2003) and Germany (Schwab et al. 2000) no fungicide treatments were applied. The results of these studies have generally supported the propagation of hybrids in an effort to reduce the application of plant protection compounds such as fungicides and reduce production costs, with important ecological benefits (Zulini et al. 2008).

At both leaf and cluster level for the hybrids Bianca, Bronner, Regent and Leon Millot our results support the findings of others about high DM resistance levels (Basler and Pfenninger 2003; Cindric et al. 2003; Schwab et al. 2000; Spring 2001; Zulini et al. 2008). However, Johanniter and Palatina showed a DM susceptibility level contrasting the high resistance level both on leaves and/or clusters compared to what has been described in the literature (Basler and Pfenninger 2003; Schwab et al. 2000; Zulini et al. 2008). The high cluster resistance in Solaris is in accordance with other studies (Basler and Pfenninger 2003; Spring 2001), while its foliar resistance is not entirely confirmed in the same reports. This discrepancy can be explained with the variability of the plant-pathogen interaction phenomenon. Besides the environmental factors listed above, plant can be affected by rootstock, while for the pathogen the existence of various and specific *P. viticola* genotypes may not be excluded (Gessler et al. 2011). Finally, Fanny and Lidi confirm the DM susceptibility level reported by Zulini et al. (2008).

Among the remaining partially resistant/resistant hybrids (Muscaris, Prior, Cabernet Carbon, Cabernet Cortis, Phoenix, 30-3-040) it is not possible to make a valid comparison to existing literature. Indeed, we think they hold a great potential for study in our future prebreeding and breeding programs lately oriented to mildew resistance introgression (Bavaresco et al. 2015). So far four varieties for wine quality traits only have been released (Tomasi et al. 2014).

The comparison between the effects of maximum level of DM assessed on leaves and on clusters resulted in a significant positive linear correlation over the 3 years of observation of our study. The present findings are in agreement with results reported by Calonnec et al. (2013), who found similar correlation values in a 2 year survey on both resistant and susceptible genotypes. On the other hand, Boso et al. (2011) did not find significant correlation between leaf and cluster considering both disease incidence and disease severity on 44 *V. vinifera* grapevine varieties, as well as Savary et al. (2009) which reported non-linearity of foliage-cluster severity relationship.

As concerning the between-years variability in the disease rating and hybrid ranking, we found a significant effect on the development of DM infections on grapevine leaves and clusters. The effect of year can be mainly associated with differences between weather patterns, particularly rainfall (Savary et al. 2009). Actually, a rain event can trigger germination of the part of the oospore population which has broken dormancy at the time of rain (primary infections); this oospore cohort will produce sporangia sometime after (secondary infections), depending on temperature and the availability of water (Rossi et al. 2008). Moreover, Burruano et al. (1987) reported that oospores always kept in dry conditions cannot germinate, and Zachos (1959) found a

relationship between germination course and the amount of rainfall oospores receive between December and March. Subsequent studies revealed the role of rainfall distribution rather than its total amount (Serra and Borgo 1995; Tran Manh Sung et al. 1990; Vercesi et al. 1999), and that long dry periods in spring stop oospore maturation, but the process recovers when rainfall returns (Rouzet and Jacquin 2003). As a consequence, dry periods in spring delay the time of disease onset (Rossi et al. 2002).

In our studied vineyard, the mean temperatures registered during the three considered growing seasons were very similar, while the total rainfalls were quite different during 2011 (617 mm), compared to 2012 (891 mm) and 2013 (900 mm). This different annual amount of rainfall might explain the significant difference in the disease symptoms found on leaves and clusters in 2011 (less severe) than both 2012 and 2013 (more severe) as well as the best fit for years 2012–2013 than years 2011–2012 in the correlation between DM symptoms assessed on leaves in successive years.

The in vitro and field DM assessment correlation

In this work, the correlation between pairs of DM assessments performed on leaf disc bioassay with the OIV 452-1 and on natural field infections on leaves with the OIV 452 were significant, but the variability accounted for by the regression was lower than 50% in both years of the study. Therefore, the leaf disc bioassay provided some information on the resistance level of the genotypes under scrutiny, but it was a weak predictor of their resistance level under field conditions. Based on these results, the leaf disc bioassay could be used for a first screening of the resistance/susceptibility of genotypes, being more practical than the greenhouse and hotbed provocation method for the screening of large populations (Boso et al. 2014; Brown et al. 1999; Sotolář 2007). Moreover, the laboratory screening assay, from field grown plants, is also reported as efficient for the rapid, reliable and economical identification of resistant hybrids in grapevine breeding programs (Prajongjai et al. 2014) and helps in the accurate selection of resistant genotypes (Calonnec et al. 2013). By contrast, our observed in vitro-field correlation is not consistent with lack of correlation between leaf disc inoculation and natural infection in the vineyard reported by Cadle-Davidson (2008). This fact can be explained by their in vitro use of single-isolate inoculations compared to the actual inoculum derived from a mix of isolates collected in untreated vineyards.

The detected between-years difference in correlation of DM symptoms on leaf discs vs clusters supported that cluster sensitivity to DM is more affected by the year effect than leaf susceptibility. Besides year, the cluster susceptibility and symptoms also vary with berry development, genotypes and site (Kennelly et al. 2005).

This finding demonstrates that the leaf disc bioassay is not a robust indicator of field resistance/susceptibility on clusters. Although barely comparable, this result is in agreement with Calonnec et al. (2013), who reported on the reliability of grapevine leaf bioassays for predicting DM resistance on fruit in the field only above the threshold of OIV 452-1 = 5.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical statement All authors have contributed significantly to this manuscript, and they agree with its content.
We declare that the paper has not been published previously or submitted for publication elsewhere and will not be submitted for publication while the acceptance by your Journal is under consideration.

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