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Development of a novel phenotyping method to assess downy mildew symptoms on grapevine inflorescences

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ABSTRACT

Grapevine downy mildew (DM), caused by the oomycete *Plasmopara viticola* (Berk. & Curt.) Berl. & De Toni, is one of the most important plagues affecting viticulture, especially in temperate rainy climates. *P. viticola* reduces fruit quality and yield, either by direct infection of berries or as a result of the reduction in photosynthesis and plant vigor caused by leaf infections. DM control is based on the repeated and massive use of fungicides, leading to problems such as environmental pollution, development of resistance and residual toxicity. The use of varieties showing durable resistance to DM is an alternative and promising strategy to control the disease. Nevertheless, most of the lab tests developed so far for DM resistance assessment are focused on leaf disc bioassays. This led us to consider that these tests might not always represent a proper evaluation and prediction of the disease symptom extent on inflorescences/bunches and therefore on final yield and grape/wine quality. Therefore, based on the screening of nine *Vitis* hybrids, we developed a new lab phenotyping method to assess the disease extent on inflorescences at flower button phenological stage, along with a novel annotation descriptor. Secondly, we combined this approach with the optimized leaf disc bioassay and found a general positive correlation between organ DM resistance phenotypes. Finally, we found that Cabernet Cortis could be a model to study DM divergent dual (on leaf and inflorescence) epidemics.

1. Introduction

The evaluation of germplasm collections is a prerequisite for their employment in crop improvement. Vast genetic resources are available for crop plants, although to date few of them have been phenotypically well characterized. Precise and standardized phenotyping procedures of morphological and physiological - as well as abiotic/biotic stress tolerance and quality - traits have been always playing a crucial role in traditional breeding activities. Up to know, robust phenotypic data represent the major limiting resource to complement the current wealth of genomic information. The promise of using inexpensive sequencing technology to speed up plant breeding is being achieved with a vision of genomics-assisted breeding that will lead to hasty genetic gain for money-consuming and complex traits (Poland, 2015). Bust in plant phenomics, namely the study of plant growth, performance and composition, can sort out the phenotyping bottleneck. As regards herbaceous species, a wide range of tools is now accessible for high-throughput, fully automated and low-resolution phenotyping, facilitating the process of trait characterization, gene tagging and genotype development essential to release a new crop variety. By contrast, within woody species lower-throughput measurements with higher-resolution are feasible, affordable and thus desirable (Furbank and Tester, 2011).

Among fruit trees, grapevine is mainly cultivated worldwide for the production of wine, fresh fruit and raisins, and thus plays a pivotal role in the economy of many countries. Unfortunately, viticulture is endangered by numerous pathogens. Among those of primary importance, *Plasmopara viticola* (Berk. & Curt.) Berl. & De Toni, is an obliged biotrophic pathogen which causes downy mildew (DM) to members of the *Vitaceae* family, in particular to the most cultivated species *Vitis vinifera* L. It was introduced in Europe in the 1870s, probably with the acquisition of American rootstocks resistant to *Phylloxera* used for grafting the susceptible European varieties (Viennot-Bourgin, 1949). Since then, grapevine DM has expanded across European regions and it is currently present in grape growing areas around the world, especially in temperate-humid climates.

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All green plant tissues can be attacked. First symptoms generally come out as green-yellow lesions (also called oil spots) on the leaf surface. Suited conditions for sporulation are saturating humidity (> 93%) and temperatures of 18–20 °C. Sporulation can be observed on the abaxial side of the leaf and on the surface of tendrils, inflorescence and young berries. The oomycete overwinters as sexually produced oospore in fallen leaves and berries. In spring, with temperature above 10 °C and rain precipitation, the oospores germinate and produce macrosporangia which release zoospores. Generally, 5 – 10 days after the infection, depending on the temperature, *P. viticola* produces sporangia containing asexually produced zoospores. Secondary disease cycles can take place under appropriate infection conditions which are similar to those suitable for primary infections (Gobbin et al., 2003). Depending on the environmental conditions, numerous clonal cycles may occur in one season leading to abrupt increase in disease severity with a disastrous impact on the yield. The organism is diploid in both sexual and asexual stages (Rumbou and Gessler, 2004).

Worldwide, the predominant strategy to control the disease is based on the use of pesticides. The repeated and massive application of chemical products not only entails huge expenses to grape production, but also leads to problems such as environmental pollution, development of resistance and residual toxicity. All these aspects foster human health concerns. Thus, the search for alternative approaches in the DM management is of paramount relevance for viticulture (Peressotti et al., 2010). The deployment of resistant *Vitis* hybrids showing durable resistance is a promising strategy to control the pathogen (Topfer et al., 2011). Nevertheless, most of the lab tests developed so far for DM resistance assessment are focused on leaf disc bioassays (e.g. Staudt and Kassemeyer, 1995; Cadle-Davidson, 2008; Prajongjai et al., 2014) but not always represent a proper evaluation and prediction of the disease extent on grapevine inflorescence/bunch and therefore on final production and quality. Indeed the organ-specific nature of susceptibility to DM in some cultivars makes it complicated to deduce resistance in foliage to fruit and vice versa (Kennelly et al., 2005).

In this work we firstly developed a new lab phenotyping method (from inoculation to symptom evaluation) for DM resistance assessment on grapevine inflorescence, considering three different phenological stages. To obtain a practical and reliable assay to be employed mainly for breeding purposes, we compared this method with field performance under *P. viticola* natural infection of several genotypes. Thus, we identified the E-L 17 stage as the most reliable and suitable for lab evaluations. At this stage we screened all genotypes, in parallel with the established leaf disc bioassay (e.g. Peressotti et al., 2011), to compare the different pathogen responses between leaf and inflorescence collected from plants in an untreated field at Edmund Mach Foundation (FEM).

2. Materials and methods

2.1. Grapevine materials

The studied genotypes were chosen on the basis of their susceptibility, tolerance and resistance to DM as determined in previous field observations using the OIV 452 and OIV 453 descriptors for leaves and inflorescences, respectively (OIV, 2009). These two organs were collected from three plants of nine *Vitis* hybrids and a *V. vinifera* variety grown in an untreated experimental field at FEM. In particular, inflorescences were harvested at three phenological stages (17, 25 and 29) of the Eichhorn-Lorenz (E-L) scale (Eichhorn and Lorenz, 1977); the phenological stage term is peculiar of grapevine and refers to the developmental stage (Mullins et al., 1992). In addition, for one relevant *Vitis* hybrid and one reference *V. vinifera* variety, the two organs were detached from fruiting cuttings grown under controlled conditions in phytotron (Table 1).

Table 1
The 11 studied grapevine genotypes, followed by their taxon, releasing country, FEM origin and preliminary level of downy mildew (DM) organ response upon natural infection in untreated field (mean of each studied phenological stage during 2012–2013).

Genotype (clone)	Taxon	Releasing country	FEM origin	E-L 17 stage		E-L 25 stage		E-L 29 stage		Preliminary DM response level upon natural infection				
				L	I	L	I	L	I	Leaf (L)		Inflorescence (I)		
				OIV 452	OIV 453	OIV 452	OIV 453	OIV 452	OIV 453	Average OIV 452	Definition	Average OIV 453	Definition	
Pinot Gris (SMA514)	*Vitis vinifera*	Italy	Field	3	4	3	1	2	1	2.67	Susceptible	2		Susceptible
MW14	*Vitis* hybrid	Austria	Field	5	3	3	3	3	1	3.67	Susceptible	2.33		Susceptible
16-02-102	*Vitis* hybrid	Italy/Austria	Field	3	5	3	1	1	1	2.33	Susceptible	2.33		Susceptible
Aromera	*Vitis* hybrid	Italy/Austria	Field	7	8	5	6	5	6	5.67	Tolerant	6.67		Tolerant
Bianca	*Vitis* hybrid	Hungary	Field	7	6	7	7	7	5	7	Tolerant	6		Tolerant
Bronner	*Vitis* hybrid	Germany	Field	9	9	9	9	9	8	9	Resistant	8.67		Tolerant
Jasmin8/1	*Vitis* hybrid	Hungary	Field	8	9	9	9	9	9	8.67	Resistant	9		Resistant
Muscaris	*Vitis* hybrid	Germany	Field	8	7	7	6	7	7	7.33	Tolerant	6.67		Tolerant
Regent	*Vitis* hybrid	Germany	Field	7	7	7	8	7	7	7	Tolerant	7.33		Tolerant
Cabernet Cortis	*Vitis* hybrid	Germany	Field/Phytotron	7	3	7	5	7	5	7	Tolerant	4.33		Susceptible
Pinot Noir (ENTAV115)	*Vitis vinifera*	France	Phytotron	–	–	–	–	–	–	–	–	–	–	–