

Evaluation of resistance to downy mildew in grape varieties grown in a Spanish collection

L. GAFORIO, F. CABELLO and G. MUÑOZ ORGANERO

Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Alcalá de Henares, Spain

Summary

The genotypic diversity maintained in grapevine repositories can provide an invaluable source of resistance to diseases. Resistance to downy mildew (*Plasmopara viticola*) of most main and minor grapevine varieties grown in Spain was evaluated in the germplasm collection at "El Encín" (IMIDRA, Madrid). Resistance assessment using the leaf disc technique was compared with the results of field evaluations of natural infected plants according to OIV452-1 and OIV452 descriptors, respectively. A good correlation was found among both methods. Results ranged from very low to high levels of resistance to fungal infection, with most varieties showing high levels of susceptibility, as expectable in *Vitis vinifera* varieties, but some of them (usually those originally collected from humid temperate regions in Spain) were found to be less susceptible.

Key words: *Plasmopara viticola*; Spanish varieties; OIV descriptors; tolerance; biodiversity.

Introduction

Downy mildew, caused by the oomycete *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni, is one of the most destructive diseases of grapevine, particularly in areas with warm and humid climate. The disease is distributed in all the viticulture regions (BARRAU and ROMERO 1996) and has caused enormous losses in Europe since 1845, when it first appeared, after it was carried in from America (HESLER 2008). In Spain *P. viticola* is an endemic pathogen in almost all grape-growing areas, and it causes important economical losses when climatic conditions are favourable to its development (BARRAU and ROMERO 1996). Under these conditions losses due to premature defoliation and berries spoilage can reach 50–100 % (AGRIOS 1988, THIND *et al.* 2004). Agrochemicals are intensively used in the control of the disease. These treatments increase economic and environmental costs of vineyards and induce the appearance of pesticide-resistant pathogen strains (LEROUX and CLERJEAU 1985, GISI *et al.* 2006). Thus, alternative management strategies, compatible with a sustainable viticulture, should be adopted. One of these alternatives is cultivating varieties with natural genetic resistance. To that end, the screening of grape varieties for the resistance to *P. viticola* is an indispensable resource of information. Resistance to downy

mildew has been analyzed in different genera of Vitaceae (PATIL *et al.* 1989), including *Vitis* (DEMAREE *et al.* 1937, PATIL *et al.* 1989, STAUD and KASSEMMEYER, 1995, REISCH and PRATT 1996, BROWN *et al.* 1999a and 1999b, KORTEKAMP and ZIPRIAN 2003, YIZHEN *et al.* 2007, CADLE-DAVIDSON 2008). Although no *Vitis vinifera* variety is notably resistant under all conditions (HESLER 2008), differences in susceptibility have been found among varieties (DEMAREE *et al.* 1937, PATIL *et al.* 1989, KORTEKAMP and ZYPRIAN 2003, BOSO and KASSEMMEYER 2008, BOSO *et al.* 2011, BITSADZE *et al.* 2014).

Grapevine collections are valuable resources for large screening of germplasm in order to identify varieties less susceptible to fungal diseases, as seen for powdery mildew (GAFORIO *et al.* 2011). One of the main uses of these repositories is breeding (DUDNIK and THORMANN 2001), but alternative uses comprise helping growers to search for useful germplasm and reintroducing *in situ* diversity (VISSER and ENGELS 2003). The germplasm collection at "El Encín" (IMIDRA, Madrid) is the largest grapevine repository in Spain. It holds about 3,600 *Vitis* accessions. In the case of *V. vinifera*, the collection includes a wide range of autochthonous varieties collected throughout Spain since the late 19th century and currently grown under the same field conditions. The aim of this study was to evaluate the susceptibility to *P. viticola* of 158 grape varieties grown at "El Encín" collection and prospected in different Spanish regions time ago.

Material and Methods

Plant material. Vines were located in the Vitis Germplasm Bank "Finca El Encín" (IMIDRA, Alcalá de Henares, Spain). A total of 158 *V. vinifera* varieties (Table) coming from all over Spain, with up to 8 replications randomly planted and 5 plants per replication were evaluated. In addition, 'Müller Thurgau' and 'Regent' were used as references for very low and high resistant varieties, respectively.

Culture conditions: This study was conducted under field conditions in 2011, when climatic parameters were suitable for the development of downy mildew. All the varieties were subjected to the same edaphoclimatic conditions and traditional management practices. Vines had been planted in 2002, grafted onto 110 Richter, simple cordon pruning, eight buds per vine, planting density 4.808 vines·ha⁻¹ (0.80 m x 2.60 m), drip irrigation and no chemical treatment during the study. A further evaluation

Table

Varieties tested in the study and their resistance to *Plasmopara viticola*. The varieties are ranked based on their resistance to *Plasmopara viticola* under field conditions, and then listed by alphabetical order

Variety	Resistance		Variety	Resistance		Variety	Resistance	
	Field ^a	Leaf disc ^b		Field ^a	Leaf disc ^b		Field ^a	Leaf disc ^b
Caiño Tinto	7	9	Blanquiliña	1	1	Mansés de Tibbus	1	1
Ferrón	7	3	Bobal	1	3	Manto Negro	1	3
Folle Blanch	7	7	Borba	1	1	Mantúo	1	5
Hondarrabi Beltza	7	7	Cabernet S.	1	3	Mantúo de Pilas	1	1
Sousón	7	9	Cagarrizo	1	7	Marfal	1	7
Batista	5	7	Caiño Bravo	1	1	Mazuela	1	1
Brancellao	5	5	Callet	1	5	Miguel de Arco	1	3
Carrasquín	5	7	Cañorrojo	1	1	Monastrell	1	5
Chasselas Doré	5	5	Cariñena Blanca	1	7	Mondragón	1	1
Hondarrabi Zuri	5	1	Castañal	1	1	Morate	1	1
Lado	5	5	Castellana Blanca	1	1	Moravia Dulce	1	1
Loureiro Blanco	5	9	Cayetana Blanca	1	1	Morenillo	1	3
Maturana Blanca	5	9	Chenín Blanc	1	1	Morisca	1	1
Mencía	5	5	Cherta	1	5	Moristel	1	1
Merseguera	5	7	Coloraíllo	1	5	Moscatel de grano gordo	1	3
Palote	5	5	Cuatendrá	1	1	Moscatel de Angüés	1	5
Pampolat Girat	5	7	Derechero de Muniesa	1	1	Moscatel de Grano Menudo	1	7
Parellada	5	7	Doña Blanca	1	7	Moscatel de Grano Menudo Rosa	1	3
Pedro Ximénez	5	1	Doradilla	1	1	Moscatel de Grano Menudo Rosa	1	3
Pedrol	5	5	Eperó de Gall	1	1	Muller Thurgau	1	1
Planta Nova	5	5	Espadeiro	1	3	Palomino	1	1
Quigat	5	5	Excursach	1	1	Palomino Fino	1	1
Regent	5	9	Fogoneu	1	3	Pampolat de Sagunto	1	5
Rufete	5	1	Forastera	1	1	Parduca	1	3
Sumoll	5	9	Fumat	1	1	Parraleta	1	5
Treixadura	5	5	Gabriela	1	1	Pensal Blanco	1	3
Vijariego	5	5	Garnacha Blanca	1	1	Perruno	1	1
Xarello Rosado	3	9	Garnacha Gris	1	5	Petit Bouschet	1	1
Xarello	3	7	Garnacha Peluda	1	1	Picapoll Blanco	1	5
Caiño Blanco	3	7	Garnacha Tinta	1	1	Planta Fina	1	7
Torrontés	3	5	Garnacha Tintorera	1	1	Puerto Alto	1	1
Moravia Agria	3	5	Garrido Fino	1	1	Puesto Mayor	1	1
Albariño	3	5	Garrido Macho	1	1	Rayada Melonera	1	1
Albarín Negro	3	5	Giró	1	1	Rey	1	1
Cabernet Franc	3	3	Giró Blanco	1	1	Rocía	1	1
Albarín Blanco	3	3	Godello	1	3	Rojal	1	1
Negramoll	3	3	Gorgollasa	1	1	Sabaté	1	7
Merenzao	3	1	Graciano	1	1	Sabro	1	1
Forcallat Tinto	3	1	Gran Negro	1	3	Salvador	1	3
Prieto Picudo	3	1	Grumet	1	1	Tarragoní	1	1
Pardillo	3	1	Gualarido	1	1	Tempranillo	1	1
Verdejo	3	1	Hebén	1	1	Tinto de la Pámpana Blanca	1	1
Airén	1	3	Jaén Rosado	1	1	Tinto Velasco	1	1
Alarije	1	1	Jaén Tinto	1	5	Tortosí	1	3
Albillo de Albacete	1	1	Juan García	1	1	Trepat	1	1
Albillo de Granada	1	1	Legiruela	1	1	Trobat	1	5
Albillo Mayor	1	5	Listán del Condado	1	5	Valenci Tinto	1	1
Alcañón	1	1	Listán Negro	1	3	Verdejo de Salamanca	1	1
Allarén	1	1	Listán Prieto	1	1	Verdil	1	5
Beba	1	1	Macabeo	1	5	Verués de Huarte	1	7
Beba Roja	1	3	Malvar	1	1	Vidadillo	1	1
Benedicto	1	5	Malvasía Aromática	1	1	Zalema	1	1
Benedicto de Aragón	1	1	Malvasía Volcánica	1	1			
Bermejuela	1	1	Mandón	1	1			

a) Degree of resistance in the field according to OIV descriptor 452. 1: very low; 3: low; 5: medium; 7: high; 9: very high or total.

b) Degree of resistance (leaf disc test) according to OIV 452-1. 1: very little; 3: little; 5: medium; 7: high; 9: very high.

was carried out during 2012 using the leaf disc methodology to confirm the field results.

Field evaluation: Evaluation of downy mildew natural infection was visually recorded 3 weeks after the onset of flowering and before veraison, according to OIV descriptor 452 (degree of resistance on leaves) of the International Organization of Vine and Wine (OIV, 2009), using a 1 to 9 scale (1 = very low resistance, 9 = very high or total resistance). A total of ten leaves were randomly selected among all the shoots.

Leaf disc test: Evaluation following the OIV descriptor 452-1 (leaf disc test) modified according to previous studies (STAUD and KASSEMAYER 1995, RUMBOLZ *et al.* 2002, BOSO and KASSEMAYER 2008) was conducted during 2012 to confirm the field results obtained during 2011. Leaf samples were collected from the grapevine collection when shoots length ranged from 20 to 30 cm (phenophase BBCH scale: 1-1-0). Twenty leaf discs (16 mm diameter) per variety were sampled from mature leaves (5th to 6th position on the shoot), surface sterilized with 70 % ethanol and rinsed in distilled water. Leaf discs were placed upside down in Petri dishes with water agar (0.8 %), inoculated with a sporangial suspension and incubated for 5 d (25 °C, > 95 % RH) under white illumination on a 16 h photoperiod. Inoculum was obtained by rinsing infected leaves from the Misión Biológica de Galicia (CSIC) research centre with distilled water and adjusting the concentration to 25.000 spores·mL⁻¹. Leaf discs were inoculated with a droplet (40 µL) of this suspension. Downy mildew infection incidence (number of leaf discs with sporulation per total number of discs) was rated and subsequently transformed to a 1 to 9 scale depending on the infection incidence (9 = 0-20 %, 7=25-40%, 5 = 45-60 %, 3 = 65-80 %, 1 = 85-100 %).

Statistical analysis: Spearman Rho coefficients for ranked data and coefficients of determination were calculated to detect the correlation between results obtained in the field and obtained using the leaf disc tests. All statistical analyses were performed with the statistical program SPSS v.19.

Results and Discussion

Climatic conditions affecting the infection had a decisive influence on the development of the disease during 2011. A rainfall of 121 mm from May 26th to June 6th and average temperatures from 16.1 to 23.3 °C made the strong fungal attack and disease development inevitable over the whole collection.

Resistance degree to downy mildew varied among the 158 tested varieties although none showed very high or total resistance. No differences were found in the field between different accessions from the same variety. A total of 117 varieties of the 158 tested (74 %) showed low or very low resistance level as expected on *V. vinifera* varieties, while 15 (9.5 %) and 21 varieties (13.3 %) were scored with low and medium level of resistance, respectively (Figure and Table). Only 5 varieties (3.2 %) showed a high level of resistance. All the varieties belonging to this last group ('Caiño Tinto', 'Ferrón', 'Folle Blanch', 'Hondarrabi

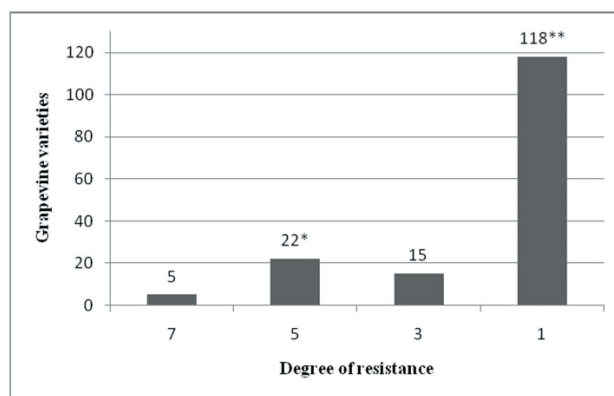


Figure: Degree of resistance to *Plasmopara viticola* in the field for the 158 tested varieties. 1: very low; 3: low; 5: medium; 7: high; 9: very high or total. *: resistant control 'Regent' belongs to this group. **: susceptible control 'Müller-Thurgau' belongs to this group.

Beltza' and 'Sousón') had been collected in Northern Spain, where climatic conditions are favourable to downy mildew development regularly. In these varieties only some bunches were slightly attacked without further consequences for the vintage. In these cases the use of agrochemicals may be minimized, thus reducing production costs and health risks and improving the environment. Further studies could corroborate this hypothesis.

The group with medium level of resistance also includes many varieties from Northern Spain ('Brancellao', 'Carrasquín', 'Chasselas Doré', 'Hondarrabi Zuri', 'Lado', 'Loureiro Blanco', 'Maturana Blanca', 'Mencia', 'Palote', 'Pedrol', 'Rufete' and 'Treixadura') as well as varieties from Mediterranean regions such as Catalonia, Valencia and Balearic Islands. This may point out to a regional origin trend in the resistance to this disease. The varieties with medium level of resistance on leaf also showed a high percentage of bunches attacked but with many whole grapes remaining, therefore resulting only in distinct consequences for the vintage. Treatments against downy mildew are needed in this case but the number of treatments and the dose of agrochemicals may be revised towards its reduction.

Some of the 158 varieties included in this study had also been tested by Boso *et al.* (2011), who found similar results in the field for 'Albarín Blanco', 'Caiño 'Castañal', 'Chenin Blanc', 'Morratrel Bouschet', 'Tempranillo' and 'Torrontés'. Nevertheless, we have found 'Godello', 'Garnacha Tintorera' and 'Moscatel de Grano Menudo' being more susceptible than shown by these authors, while 'Brancellao', 'Loureiro Blanco' and 'Prieto Picudo' showed more resistance in the present study. Differences in field evaluation for a given variety are not unusual. CADLE-DAVIDSON (2008) reported over 30 % of inconsistencies between different studies. Cultivar, berry development, site and year factors influence susceptibility to downy mildew (KENNELLY *et al.* 2005). *In vitro* techniques are particularly useful for resistance screening where field natural infection occurs sporadically (LIU *et al.* 2003), like in the present study. The most reliable method used to assess resistance in the laboratory is the leaf disc procedure (LIU *et al.* 2003), which has been used by several authors (DENZER *et al.*

1995, STAUDT and KASSEMAYER 1995, RUMBOLZ *et al.* 2002; LIU *et al.* 2003, BOSO *et al.* 2006, BOSO and KASSEMAYER 2008, BITSADZE *et al.* 2014). This technique appears to be a good predictor of field resistance and it is more practical than the greenhouse method for screening large populations (BROWN and MOORE 1999).

Leaf disc evaluations of the 158 tested varieties showed intraspecific variation in the susceptibility to downy mildew: very little (score = 1) or little (score = 3) in 65 % of varieties and high (score = 7) or very high (score = 9) in 10 % and 4.4 % of them, respectively. The results showed a good correlation ($r = 0.55$, $p < 0.01$) between the degree of disease resistance assessed on leaf discs and the same degree assessed in the field. This result is similar to that obtained by BROWN and MOORE (1999), who reported correlation coefficients ranging from 0.47 to 0.67 between sporulation in leaf discs and sporulation *in vivo* using a different inoculation method, since they sprayed the entire surface of the leaf disc with the sporangial suspension. These results confirm that the leaf disc method may be used in screening for susceptibility to downy mildew of large amounts of germplasm although potential sources of natural resistance must be confirmed in the field.

On the other hand, four varieties ('Ferrón', 'Pedro Ximénez', 'Hondarrabi Zuri' and 'Rufete') with high or medium level of resistance to natural infection in the vineyard, showed a lower level of resistance on leaf discs than expected. On the contrary, 11 varieties (7 %) showed more resistance in the inoculated leaves (scores of 7 or 9) than in the vineyard (scores of 1 or 3). These results may be related to differences in pathogen strain between natural and *in vitro* inoculations and strain-specific resistances, and to randomly failed inoculation (CADLE-DAVIDSON 2008). Infection of plants with leafroll virus may also modify the resistance to the fungus since some degree of defence activated against virus may reduce susceptibility to *Plasmopara* attacks in virus-infected plants as seen by REPETTO *et al.* (2012); however after checking the presence of viruses into the plants no relation was found between presence/absence of viruses and degree of susceptibility to *Plasmopara*, maybe because the recorded downy mildew infection in the vineyard was earlier than infection cited by these authors. These preliminary results may facilitate the selection of varieties of interest for further studies in which more detailed analysis could be considered.

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