

Genetics-assisted breeding for downy/powdery mildew and phylloxera resistance at fem

S. Vezzulli, L. Zulini, and M. Stefanini^a

Research and Innovation Centre, Fondazione Edmund Mach, via E. Mach 1, 38010 San Michele all'Adige, Italy

Abstract. The genetics-assisted program for resistance to biotic stresses began at the Edmund Mach Foundation in 2007 and has developed on two lines. The first line was based on obtaining resistant materials with parents the historical varieties of Trentino. During the three-year period 2015–2017, 3 Teroldego X Merzling genotypes, 3 Marzemino X Merzling genotypes, 1 Nosiola X Bianca genotype and 1 Nosiola X Kulneany genotype were collected the data related to resistance to various fungal diseases both on the leaf and on the bunch. At harvest the plant production data and the must characteristics were recorded; the grapes were vinified and for each year the wines were subjected to sensorial analysis. 5 resistant selections were considered interesting for all the characteristics found and therefore the data for the registration to the National Register of Grapevine Varieties will be collected. The second line of research was based on the retrieval and both genotypic and phenotypic characterization of potentially parental lines acquired from foreign breeding programs and of wild materials. Once the crossings have been planned and obtained, the evaluation of the progeny takes place following a process of Marker-Assisted Selection optimized in order to maintain a compromise between efficiency and cost containment.

1. Introduction

The cultivation of the vine in the world requires more and more attention in the choices in order to make the cultivation more sustainable. Climate change and the spread of numerous diseases, which can only be dealt with until now by chemical interventions, ask to find alternative solutions in the field of biodiversity present in the genus *Vitis* (1). Historically, on the vine, biotechnological solutions have been applied by researchers such as the identification of phylloxera tolerant or resistant rootstocks or the creation of hybrids between *Vitis vinifera* and the species that showed resistance to fungal diseases such as downy and powdery mildew. The organoleptic quality of the products obtained from these hybrids, defined as “direct producers”, did not meet the needs of the market. The repeated backcrossing between these genotypes and different varieties of *V. vinifera* led to the selection of genotypes with high levels of disease resistance, percentages of *V. vinifera* in the genome of more than 95% and a qualitative level of wine comparable to wines from European varieties. Many European Research Centers have dedicated themselves to this type of activity, so much so that today there are different resistant varieties registered in the national vine registries (2). The sensitivity to making viticulture more sustainable has always been present in the projects of genetic improvement of the Lives developed at the Edmund Mach Foundation (FEM). The breeding program envisaged the use of several resistant genotypes in the attempt to obtain mainly downy and powdery mildew resistant genotypes. The first crosses were made between the typical varieties of Trentino

and genotypes carrying resistance sources from different European Research Centers. Different populations of crossing have been obtained, in particular from the parental varieties Teroldego, Marzemino, Nosiola and the resistant strains Merzling, Bianca and Kunleany.

From 2007 at the FEM the activity of crossing and gathering new sources of resistance to downy and powdery mildew from new genotypes has resumed with more conviction, as it has been confirmed the possibility of cultivating these new varieties in important European countries such as Germany. Today the selection of genotypes requiring a reduced number of phytosanitary interventions and that do not reduce the quality of the final product, i.e. wine, is a necessity to respond to the requests arising from the application of standards that require areas of respect for water courses, public and private buildings as well as cycle lanes, called sensitive areas. In some cases, as in Trentino, the reduction of viticulture could reach 30% of the current area cultivated with grapevines, if the law were to enter strictly into force (3).

2. Materials and methods

2.1. Obtainance of resistant varieties from landraces

2.1.1. Experimental field and crosses

In the three-year period 2015–2017, data were collected for 5 genotypes of the Teroldego X Merzling cross, 2 genotypes of Marzemino X Merzling, 1 genotype of Bianca X Nosiola and 1 genotype of Kunleany X Nosiola, located in the Girelli field in San Michele all'Adige in the province of Trento to 200 m asl. The new varieties derived from the Teroldego X Merzling cross F22P09, F22P10 and

^a e-mail: marco.stefanini@fmach.it

F22P134 are black berried, while the genotypes F22P94 and F22P113 are white berried. The genotypes obtained from the Marzemino X Merzling cross are white berried F23P65, while the F22P121 genotype is black berried. The two genotypes with Nosiola F26P92 and F25P87 are white berried. For each genotype 25 plants were planted in rows with a planting density of 6,250 plants/ha. All the plants were grafted onto Kober 5BB rootstock. The annual management includes Guyot pruning with a branch with 8–10 buds renewed and during the vegetative period all the practices of choking, shearing and peeling were carried out. No antifungal treatment has been carried out to protect these plants, even if 1–3 treatments are considered plausible in practice only if necessary.

2.1.2. Data collection and analysis

The annual surveys were addressed to the level of resistance to various fungal diseases both on the leaf and on the bunch, as well as the presence of phylloxera and erinosis attacks. For the collection of these data, a card was used, showing the different OIV descriptors (452; 453; 455; 456; 459; 461) (4) referring to pathologies, in addition to the level of attack of the erinosis and damage from leafhoppers and the phenological status according to Eichhorn and Lorenz. The main phenological phases of flowering, veraison and ripening have been correlated with the climatic index of the sum of the Degrees Day above 10° accumulated from the beginning of the year to the flowering, between the flowering and the veraison, and between the veraison and the ripening. The survey was carried out on a weekly basis (5).

From the veraison onwards it was followed by the accumulation of sugars in the berries with a measurement of the Brix Grade (°Brix) at least weekly with a digital refractometer. At harvest the data of the production by plant, the number of clusters and the number of shoots were recorded in order to derive the average weight of the bunch and the fertility of 5 representative plants. At harvest the grapes of all the production were collected for microvinification. On the pressed homogeneous mass a sample of 100 cc of must was taken and analyzed by FT-IR spectrophotometer (FOSS Analytical), determining the following parameters: must sugar content (°Brix), titratable acidity (g/l of tartaric acid), pH, malic acid (g/l), tartaric acid (g/l), potassium (g/l), readily assimilable nitrogen (g/l).

Wines obtained following different vinification for white wines and red wines were subjected to FOSS analysis to determine the content of alcohol (% vol), pH, total acidity (g/l of tartaric acid), volatile acidity (g/l), dry extract (g/l), reducing sugars (g/l), ash (g/l), malic acid (g/l), tartaric acid (g/l), lactic acid (g/l), glycerin (g/l), potassium (g/l) and methanol (g/l).

The wines were subjected to a spectrophotometric analysis to define their anthocyanic profile, in particular the parameter 3–5 malvidin diglucoside, a parameter that the OIV regulations limit to 15 g/l to define the product as a marketable wine (6).

The wines were subjected to a sensorial evaluation of a trained panel, using an unstructured parametric data sheet to describe the product and its relative pleasantness.

2.2. Retrieval of new genetic material and its employment

2.2.1. Parental lines and progenies

Parental lines. A total of 264 grapevine accessions acquired from (non-)European breeding programs, an Italian private breeding platform and wild-collected in north-eastern America during 2011 were studied. Most were phenotyped for DM and PM resistance, while all were genetically characterized.

Progenies. During the last 6 years a total of 86 different crossing combinations (*V. hybrid* × *V. hybrid*) were performed aiming at pyramiding *R*-loci. A total of 8793 progeny individuals were submitted to the MAS workflow, namely firstly disease resistance screening and secondly *R*-loci characterization.

2.2.2. Phenotypic screening

Parental lines. Within the overall genetic material, 100 accessions present at FEM were evaluated for their degree of resistance against DM and PM, through *in vitro* leaf disc bioassay and *in vivo* pathogen inoculation on potted plants respectively. DM symptom annotation was performed based on three parameters: Severity (percentage of the disc area showing symptoms of sporulation), Incidence (number of discs with sporulation/total number of discs), according to OEPP/EPPO (7), and the OIV 452-1 descriptor (4). PM symptoms were evaluated based on the foliar OIV 455 descriptor.

Progenies. All progeny individuals were evaluated for their degree of resistance against downy and powdery mildew. Firstly, *P. viticola* spores were inoculated on potted seedlings following a well-established protocol (8); secondly, prior plant treatment and recovery, *E. necator* artificial infection was caused by naturally infected plants adjacent to the potted seedlings. The assessments were recorded respectively at 7 and 14 day post-inoculation (dpi) based on the OIV 452 and 455 descriptors.

2.2.3. Genotypic characterization

Parental lines. Following a detailed literature search, 11 reliable *R*-loci were analyzed in the entire parental sample set: i) 6 associated to PM resistance, *Run1*, *Run2*, *Ren1*, *Ren2*, *Ren3*, *Ren9*; ii) 5 associated to DM resistance, *Rpv1*, *Rpv3*, *Rpv10*, *Rpv12*, *Rpv14*. The applied PCR conditions were those reported in the overall literature, with some modifications (9).

Progenies. The progeny individuals maintained downstream of the phenotypic screening and performing well were finally characterized at the expected *R*-loci based on the parent combinations.

3. Results and discussion

3.1. Novel resistant varieties ready-to-market

The genotypes of the study present a different Degrees Day request to reach the phenological phases reported; F22P09 and F22P10, with 218 Degrees Day, have the lowest request to go from the budding to flowering, while the most demanding, with 301 Degrees Day, is the crossing Marzemino X Merzling F23P65. In the phase between flowering and fruit set the requirements

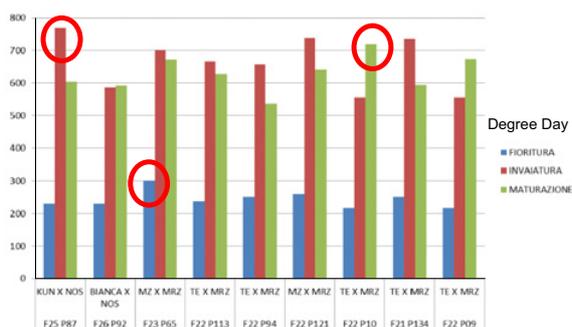


Figure 1. Phenology of the selections under study with the different needs of Degree Day for the three most relevant stages of budding-flowering, flowering-veraison and veraison-ripening. Similar to the reference wines such as Teroldego and Marzemino.

of Degrees Day vary between the 554 required of the Merzling X Teroldego F22P09 and F22P10 and 769 of the Kunleany X Nosiola F25P87 selections.

For the ripening phase, the least demanding variety in terms of Degrees Day to reach the sugar content of 21 °Brix is the Marzemino X Merzling F22P94 with white berries with 537, while the most demanding is the F22P10 selection with 719 to reach the 22 °Brix concentration (Fig. 1).

The different genotypes object of comparison have different behaviour both productive and qualitative, and they introduce variation of productivity also tied to the year like the year 2017 for the fertility and the productivity, that they have low values because of the consequences of the frozen one found in the night between April 20 and 21 (Fig. 2). The values of the OIV descriptors – related to resistance to downy and powdery mildews on leaves and bunches and to botrytis on leaves – are all 9 and 7. The values of resistance to phylloxera, erinosis and leafhoppers also reach maximum values.

The wine tasting over the years has different profiles with the Kunleany X Nosiola genotype characterized by a profile with citrus, spicy and fruity notes. The F26P92 genotype resulting from the Bianca X Nosiola cross presents a profile with spicy and thiol-like notes with a good structure. The white berried genotypes obtained from the Teroldego X Merzling and Marzemino X Merzling crosses present wines with more neutral profiles, but with high levels of structure and sapidity that can be oriented towards the production of “neutral” white wines or sparkling base wines. In the tasting of wines, these white berried selections were examined together with wines of varieties already known for their resistance and registered in the National Register of Wine Grape Varieties such as: Helios, Bronner, Johanniter, Muscaris and Solaris (10), coming from plants grown in the same vineyard. The vines selected at San Michele resulted more pleasant than those of the varieties already registered (Fig. 3).

The red berried genotypes obtained from the Teroldego X Merzling F22P09, F22P10, F21P134 selections have a good structure and interesting spicy aromas. The F21P134 selection is characterized by floral notes and black fruit and good flavor. The F22P10 selection is the most complex profile with notes of structure, red fruit and higher sapidity than the average of the other genotypes. The profile of



Figure 2. Summary table of the quantitative parameters of the grape, the must and the wine, as well as the table of the different resistances with reference to the OIV descriptors for a selection of white and black berried selections.

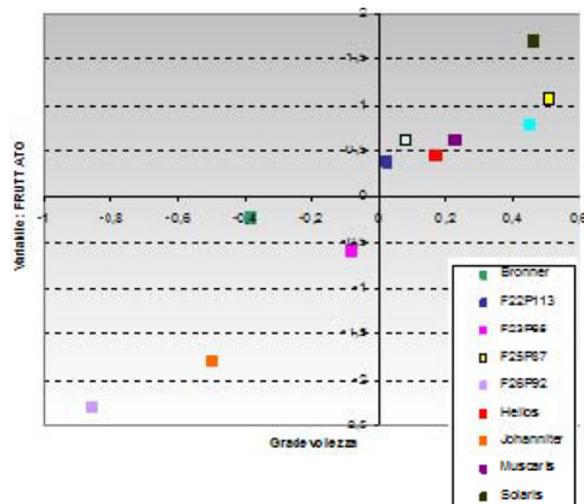


Figure 3. Evaluation of the pleasantness of the white wines of the resistant varieties in selection compared to the varieties already registered in the RNVV.

the F22P09 selection has a high astringency, but with balsamic notes of spices and flowers higher than the other genotypes. The Marzemino X Merzling selection is very interesting, with high aromas of red and black fruit besides the floral note. In comparison, the new resistant genotypes selected by FEM compared to the red berried resistant

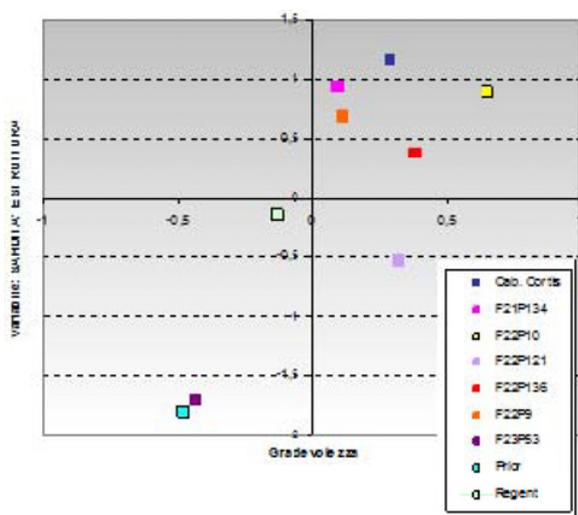


Figure 4. Evaluation of the pleasantness of red wines of the resistant varieties in selection compared to the varieties already registered in the RNVV.

Table 1. Table showing the values of the presence of the diglucoside anthocyanins detected in the wines of the selected red varieties.

Sigla	Anno	MV 3,5-diglucoside	DP 3,5-diglucoside	CN 3,5-diglucoside	PT 3,5-diglucoside	PN 3,5-diglucoside
L.O.Q		0.1	0.1	0.1	0.1	0.1
F22P134		8.7	0.9	0.2	1.3	1.5
F22P10	2017	3.8	0.3	0.1	0.5	0.7
F22P10	2016	5.4	0.4	0.1	0.6	0.6
Pinot nero		0.1	—	0.1	—	0.1
F22P121		3.0	0.4	0.1	0.7	0.4
Regent		78.2	8.1	2.1	22.5	19.0
F22P09		3.1	0.2	0.1	0.2	0.3

varieties registered in the RNVV such as Cabernet Cortis, Prior, Regent (10) are positioned with a higher degree of pleasantness compared to the comparison selections (Fig. 4).

It is important to underline that the red berried genotypes selected in FEM result with values of malvidine 3–5 diglucoside lower than the maximum value agreed by the European regulations (15 g/l); contents were more similar to the values found in the varieties registered in the RNVV, as reported in Table 1 where the Regent variety has levels of these anthocyanins 10 times higher than those found in the FEM varieties.

3.2. Novel pyramided resistant donors

Parental lines. Within the 100 physically available accessions, the *in vitro* evaluation demonstrated a wide phenotypic variability in terms of DM resistance. In particular, 41 accessions resulted highly resistant (OIV scores 7–9) and will be employed as a direct source of resistance in breeding sub-programs with the objective of releasing novel sustainable grapevine cultivars presenting also good fruit/wine quality. Regarding PM, only 18 out

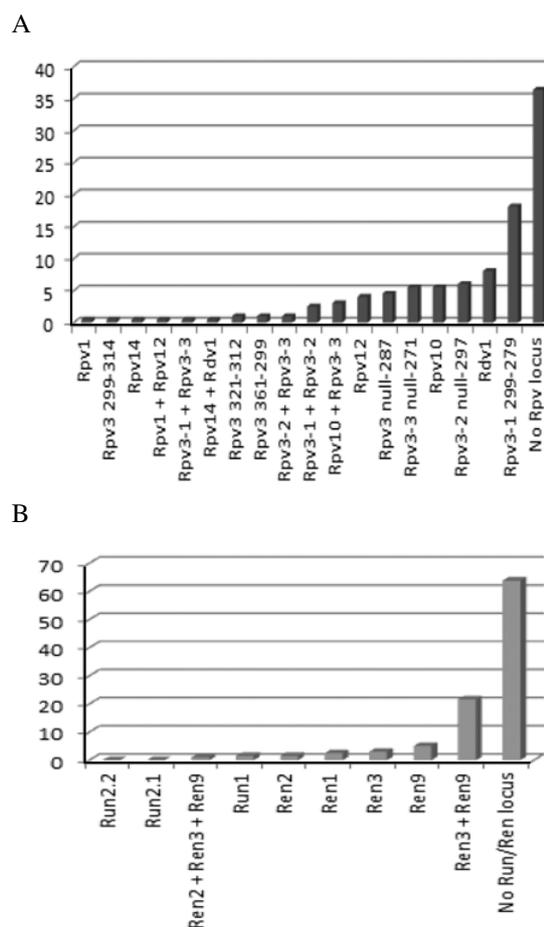


Figure 5. *Rpv* (A) and *Run/Ren* (B) loci distribution in the overall parental genetic material.

of the 100 *in vivo* tested accessions resulted resistant. This demonstrates that PM is rarer than DM resistance and highlights the importance of discovering new genetic sources. Among these accessions, a deep phenotypic characterization has recently been reported for 28 resistant varieties (2).

The following genetic study allowed the identification of already pyramided parents obtained in the past by traditional breeding activities. Moreover, most genotypes were found with no presence of any 11 reliable and actually analysable *R*-loci, representing potentially novel and exclusive genetic resources (Fig. 5).

Progenies. Out of the total 8793 plants subjected to DM and PM resistance screening, 6182 were eliminate due to their high susceptibility level to both disease while 2611 (29.7%) were maintained in order to perform the *R*-loci characterization. Table 2 shows the specific classes, based on the OIV scores (1–9), in which the progeny individuals maintained fell.

Out of the 2611 progeny individuals retained upon the phenotypic screening, 963 plants showing the highest degree of resistance and the best agronomic performance were subjected to the genotyping analysis in order to define both the number and the typology of *R*-loci. Besides 11.1% of genotypes carrying a single *R*-locus associated to DM or PM resistance, our findings highlighted the pyramidation of *R*-loci against DM in 48.4% and against PM in 59.5% of the analysed progeny individuals. In

Table 2. Efficiency of the phenotypic screening for DM and PM resistance in progenies derived from *V. hybrid* × *V. hybrid* during 2013, 2014, 2015 and 2017.

Total of inoculated plants	8793	100%
Total of discarded plants DM 1–3 PM 1–3	6182	70.3%
Total of maintained plants	2611	29.7%
DM 9 PM 9	623	7.1%
DM 9 PM 7–9	1771	20.1%
DM 9 PM 5	31	0.4%
DM 5–7 PM 9	44	0.5%
DM 5–7 PM 7	123	1.4%
DM 5–7 PM 5	19	0.2%

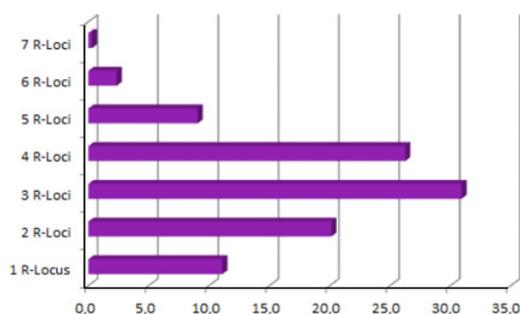


Figure 6. Level of *R*-loci pyramiding in the analyzed progeny individuals.

particular, 30.3% of genotypes resulted pyramided for *R*-loci to both mildews. Overall, the most abundant class (30.9%) was represented by genotypes carrying 3 *R*-loci, while a maximum level of pyramiding with 7 *R*-loci was reached in the 0.3% of cases (Fig. 6).

4. Conclusions

Progress in the selection of new genotypes will lead to the identification of increasingly resistant and qualitatively matching genotypes to different needs. The different varieties can present an adequate solution to the problem arising with the definition of the constraint related to the “areas of respect” that for the viticultural reality of Trentino can affect up to 30% of the wine-growing area. The selections presented had a good impact from the sensorial point of view. The genotypes F23P65 and F22P113 were interesting for characters related to the sparkling bases. The F25P87 genotype, on the other hand, is particularly interesting for producing wine instead of the Nosiola cv for “areas of respect”. The F22P09, F22P10 and F22P121 selections are suitable for areas of production of red wines, as they have levels of malvidine

3–5 diglucoside similar to those present in the varieties of *V. vinifera* in addition to sensory characteristics similar to the reference wines such as Teroldego and Marzemino. From the genetic point of view, the performed survey allowed the identification of already pyramided parents obtained in the past by traditional breeding activities; this supports the role that genetics, coupled with genomics, plays in assisting genetic improvement for resistance. Moreover, novel and exclusive genetic resources were identified, providing peculiar and preparatory information to ongoing and forthcoming Marker-Assisted (pre-)Breeding programs. Following a MAS workflow, nowadays a relevant level of *Rpv* and *Run/Ren* loci pyramiding has been reached in the obtained selections. In the next coming few years, FEM is going to launch eight new varieties – carrying from 1 to 3 *R*-loci – which are at least mid-resistant to DM and PM.

The authors are grateful to Erhard Tutzer (Innovitis, IT) for providing already known and recently developed grapevine genotypes. The authors also thank M. Stella Grando and S. Lorenzi for their initial contribution to the work.

References

- [1] M. Stefanini, S. Vezzulli, S. Clementi, C. Dorigatti, A. Vecchione, L. Zulini, Proceedings del IV Congresso Internazionale sulla Viticoltura di Montagna e in forte pendenza, Conegliano (TV), Italy, 29 March–April 1 (2017)
- [2] S. Vezzulli, A. Vecchione, M. Stefanini, L. Zulini, Eur. J. Plant Patol. **150**, 485 (2018)
- [3] D. Merdinoglu, S. Wiedemann-Merdinoglu, P. Mestre, E. Prado, C. Schneider, PAV **126**, 290 (2009)
- [4] OIV 2009. Descriptor list for grape varieties and Vitis species, 2nd edn. Office International de la Vigne et du Vin, Paris, <http://www.oiv.org>
- [5] R. Zorer, G. Nicolini, R. Larcher, M. Malacarne, F. Marincoz, D. Porro, M. Stefanini, Italian J. Agrometeorol. **13**, 50 (2008)
- [6] U. Vrhovsek, G. Malacarne, D. Masuero, L. Zulini, G. Guella, M. Stefanini, R. Velasco, F. Mattivi, Australian J. Grape Wine Res. **18**, 11 (2012)
- [7] OEPP/EPPO, Guidelines for the efficacy evaluation of fungicides, OEPP/EPPO Bulletin, **31**, 313 (2001)
- [8] D. Buonassisi, L. Cappellin, C. Dolzani, R. Velasco, E. Peressotti, S. Vezzulli, Scientia Horticulturae **236**, 79 (2018)
- [9] VIVC 2018, Vitis International Variety Catalogue. <http://www.vivc.de/index.php>
- [10] PIWI, <https://www.piwi-international.de/it/>