

The FEM grapevine crossbreeding program for resistance to the main ampelopathies: Towards climate-resilient varieties

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Abstract. The technique of crossing, whether free or controlled, has always been a source of variability allowing the selection of new varieties with improved fitness. Therefore, one promising strategy towards a sustainable viticulture is crossbreeding for resistance traits to biotic stresses under climate change conditions to reduce pesticide usage and improve resilience. With this awareness and vision, the Edmund Mach Foundation (FEM) began its grapevine genetic improvement program in the 1990s. Later, in 2010 with the exploration of the genetic pool of resistance loci to downy and powdery mildew, a group of accessions was selected as donors. Next, genotypes with stacked (“pyramided”) loci were generated through marker-assisted parental selection (MAPS) with up to seven loci combining resistance to both mildews. Then, upon protocol optimization a highly efficient marker-assisted seedling selection (MASS) was established, allowing since 2019 to overcome phenotypic screening and revealing inter- and intra-population effects. Upon multi-year agronomic surveys, grape quality composition and wine tastings, in 2018 four new varieties were registered for their novel organoleptic characteristics and tolerance to grey mould. In 2020 four (mid)-resistant varieties to mildews were patented and in 2025 other four are in the process. At the same time, the resistance to other “emergent” ampelopathies, as black rot, is being introgressed. Various collaborations are in place across the national territory for the exploitation of the superior parental lines. Lately, the genetic and phenotypic characterization of the FEM germplasm (ca. 3,000 accessions) has been completed, so that the scouting process within such biodiversity is continuously ongoing towards the development of varieties coupling disease resistance with climate resilience.

1. Introduction

Viticulture and winemaking have significant socio-economic roles worldwide. In Italy, grapevine cultivation is a cornerstone of the agricultural landscape, significantly contributing to the economy and cultural identity, with considerable contribution to the rural tourism. However, climate change and the increasing prevalence of diseases pose substantial challenges to sustainable vineyard management. Moreover, the regulatory frameworks within the European Union are progressively tightening constraints on the use of synthetic plant protection products, prompting a necessary paradigm shift toward alternative phytosanitary strategies (1). Notably, in 2023, viticulture faced unprecedented stress: global wine production reached its lowest level since 1961, with

Italy—long the world’s leading producer—experiencing a 23% decrease, the most severe decline since 1950 (2). Besides damage from floods and hail, significant rainfall promoted downy mildew in central and southern regions, resulting in losses of 30-70%, peaking at 90-100% in organic vineyards (2). In response, the cultivation of resistant grapevine varieties presents a promising solution to reconcile the dual objectives of reduced pesticide dependency and effective disease control.

2. Disease Resistance: The “Roots”

The strategic use of interspecific hybridization for disease resistance in grapevine has been ongoing in Europe since at least 1863, when the rapid advent of steam

navigation facilitated the introduction of phylloxera (*Daktulosphaira vitifoliae*) from the United States. This pest devastated European viticulture, prompting a dichotomy among vine growers between advocates of chemical control called "sulfurists", and "Americanists". The latter ultimately prevailed through the implementation of rootstock grafting techniques employing resistant American *Vitis* species, notably *Vitis riparia*, *V. rupestris*, *V. berlandieri*, and *V. cinerea* (3). Concurrently, other diseases such as powdery mildew and black rot (causal agent the ascomycetes *Erysiphe necator* and *Phyllosticta ampellicida*, respectively) and downy mildew (causal agent *Plasmopara viticola* later reclassified as an oomycete), were introduced from North America. While European *V. vinifera* varieties were found to be highly susceptible, American species demonstrated inheritable genetic resistance, shaped by co-evolutionary dynamics in their native environments (4). This marked the beginning of a persistent reliance on copper- and sulphur-based phytosanitary treatments in European viticulture—chemicals persisting today.

The historical trajectory of hybridization between American and European grapevines can be traced back already in the United States, where indigenous *Vitis* species were initially utilized for winemaking purposes. However, results were unsatisfactory due to their poor organoleptic qualities, notably characterized by excessive foxy aromas and poor balance in phenolic and aromatic profiles (Koyama et al., 2022). Conversely, European cultivars encountered significant adaptation challenges in the North American environment, particularly due to climatic constraints and the high prevalence of endemic pathogens. This prompted pioneer early breeders to initiate interspecific crosses, but it was only after the transcontinental introduction of "American diseases" into European vineyards that systematic genetic improvement programs based on controlled hybridization for disease resistance were established. The first-generation direct-producer hybrids, mainly of French origin, were interspecific crosses between *V. vinifera* cultivars and American species such as *V. labrusca* and *V. aestivalis*. These hybrids inherited approximately 50% of American genetic heritage, which, while conferring resistance traits, also imparted limited oenological aptitude. To mitigate these drawbacks, breeding strategies subsequently focused on recurrent backcrossing with elite *V. vinifera* cultivars, thereby reducing the proportion of non-vinifera genome while preserving resistance loci. In the latter half of the 20th century this led to the development of a second generation of resistant cultivars, primarily in Germany, known under the acronym PIWI (Pilz-Widerstands-fähig: literally "fungus-resistant"). These cultivars are currently promoted by the association "PIWI international", which advocates for the sustainable cultivation and valorisation of resistant grape varieties. Despite the existence of more than 60 *Vitis* species and approximately 6,000 registered *V. vinifera* varieties globally, just 33 cultivars account for over 50% of the total vineyard area worldwide. Critically, none of these dominant varieties possess genetic resistance to major fungal pathogens, making conventional viticulture heavily reliant on chemical pesticides (5).

3. Scientific Research as an Ally: Marker-Assisted Breeding

Over the past 25 years, considerable research efforts have focused on the identification and functional characterization of genomic regions, referred to as resistance loci, underpinning grapevine defence mechanisms against major fungal ampelopathies. To date, 31 loci associated with resistance to downy mildew, 14 to powdery mildew, and 3 to black rot have been identified, respectively known as Resistance to *P. viticola* (Rpv), to *E. necator* or syn. *Uncinula necator* (Ren or Run), and to *Guignardia bidwellii* (Rgb) (4), the latest being the name of the teleomorph form of *P. ampellicida*. Some of these loci are associated with markers (polymorphic DNA sequences identifiable via PCR-based assays) which enable to track the inheritance of resistance alleles from donor parents to progeny in breeding populations. This approach, known as marker-assisted selection (MAS) or more broadly marker-assisted breeding (MAB), improves the accuracy of early-stage selection by circumventing the limitations of phenotype-based screening. Moreover, MAS accelerates the breeding cycle by facilitating the identification of desirable genotypes during the early developmental seedling stage, thereby obviating the need to await full vine maturation for phenotypic trait expression and evaluation. This methodology is especially important in advanced breeding programs aiming to combine ("to pyramid") multiple resistance loci against a single pathogen, therefore developing new-generation selections. In fact, while such pyramiding may not necessarily produce enhanced phenotypic resistance, it constitutes a critical strategy to enhance the durability of resistance by counteracting the adaptive potential of pathogen populations through multilayered genetic barriers (6).

4. Resistant Grapevine Varieties in Italy: The Experience at FEM

Since 2009, Italy has officially registered 36 fungus-resistant grapevine cultivars in the National Registry of Grapevine Varieties. This is of particular importance, as since 2019, Protected Geographical Indication (PGI) wines have been authorized to use resistant cultivars, provided they are listed in the corresponding product specification (Italian Republic law No. 238/2016). To date, nine Italian regions have approved the cultivation of such varieties: the autonomous province of Bolzano (the pioneer in 2009), followed by Abruzzo, Emilia-Romagna, Friuli-Venezia Giulia, Lombardy, Marche, Veneto, and the autonomous province of Trento. More recently, Lazio, Piedmont, and Campania have joined the initiative, while Apulia is still awaiting regulatory approval.

The first resistant varieties, registered between 2009 and 2014, originated from Germany and were developed at the Freiburg State Viticulture Institute (WBI) during the 1970s and 1980s. These include white-berried cultivars such as 'Bronner', 'Johanniter', 'Helios', 'Solaris', and 'Muscaris', as well as red-berried cultivars like 'Cabernet

cortis', 'Cabernet carbon', and 'Prior', along with 'Souvigner gris'.

Since 2015, Italian breeding programs have significantly expanded the portfolio of resistant cultivars, with more than 20 novel genotypes now registered, selected specifically for Italian viticultural production and local pedoclimatic conditions (7). Indeed, shaping tomorrow's viticulture requires genetic improvement aimed at targeting optimal genotype $\times$ environment $\times$ oenology interactions. The University of Udine, in partnership with the Institute of Agricultural Genetics (IGA-Tech) and the Rauscedo Cooperative Nurseries (VCR, Friuli-Venezia Giulia), was among the first to contribute to this effort. Utilizing resistance donors previously selected in Hungary, they conducted targeted crosses with elite *V. vinifera* varieties of national and international relevance, including 'Tocai Friulano', 'Sauvignon Blanc', 'Pinot Blanc', 'Cabernet Sauvignon', 'Merlot', and 'Pinot Noir'. This led in 2015 to the registration of ten new resistant varieties: five red-berried ('Cabernet Eidos', 'Cabernet Volos', 'Merlot Khorus', 'Merlot Kantus', 'Julius') and five white-berried ('Fleurtaï', 'Soreli', 'Sauvignon Kretos', 'Sauvignon Nepis', 'Sauvignon Rytos'). A second group of four cultivars was registered in 2020, resulting from backcrosses with 'Pinot Blanc' ('Pinot Iskra' and 'Kersus') and 'Pinot Noir' ('Pinot Kors' and 'Voluturnis'). In 2018 the *V. vinifera* $\times$ *V. vinifera* crossbreeding program at the Edmund Mach Foundation (FEM, Trentino-Alto Adige) led to the registration of four varieties ('ECO IASMA' series 1-4) selected for their improved organoleptic characteristics and tolerance to *Botrytis* bunch rot, while the crossbreeding program for biotic stress (mainly downy and powdery mildew) resistance led to the registration of its first (mid-)resistant selections in 2020. These included crosses between the local varieties 'Teroldego' and 'Nosiola' and the resistance donor 'Merzling', resulting in two red-berried varieties ('Nermantis' and 'Termantis') and one white-berried variety ('Valnosia'). Additionally, the program targeted the increasingly appealing sparkling wine base market, by crossing two pre-existing resistant hybrid lines to obtain a new white-berried cultivar ('Charvir'), the most resistant also to black rot. Thanks to the Consortium for Grapevine Innovation (CIVIT), a joint venture between FEM and the Associazione Vivaisti Viticoli Trentini (AVIT), two further cultivars developed in Hungary by the University of Horticulture and Food Industry were evaluated and subsequently registered in Italy: 'Pinot Regina' and 'Palma'. In 2024, four additional grapevine cultivars—two white-berried, intended for sparkling wine base, and two red-berried, selected for late ripening and international relevance—were expected to enter the national registry. The resistant varieties patented by CIVIT are listed in Figure 1 together with the R-loci arrangement of the resistance donors.

However, their registration has been delayed due to regulatory amendments introduced by Legislative Decree No. 16 of 2 February 2021, which mandates that the required four-year Distinctness, Uniformity, and Stability (DUS) evaluations be conducted by the official examining authority of the Community Plant Variety Office (CPVO)

at CREA-VE, Conegliano, rather than by the proposing entity. This procedural change not only extends the registration timeline by an additional four years for genotypes that had already completed DUS trials in 2023 but also imposes substantial financial burdens on breeding programs for varieties whose registration is not guaranteed. Despite these constraints, the increasing national interest in fungus-resistant grapevine cultivars is evidenced by FEM's active breeding collaborations with both public and private institutions. These initiatives aim to introgress resistance traits into regionally significant cultivars, including 'Lambrusco' in Emilia-Romagna, 'Verdicchio' in Marche, 'Chardonnay' in Lombardy, 'Corvina' in Veneto, 'Sangiovese' in Tuscany (in collaboration with CREA-VE Arezzo), and various table grape genotypes in Apulia (CREA-VE Turi, BA), thus promoting a decentralized strategy for developing climate-resilient, locally adapted resistant varieties.

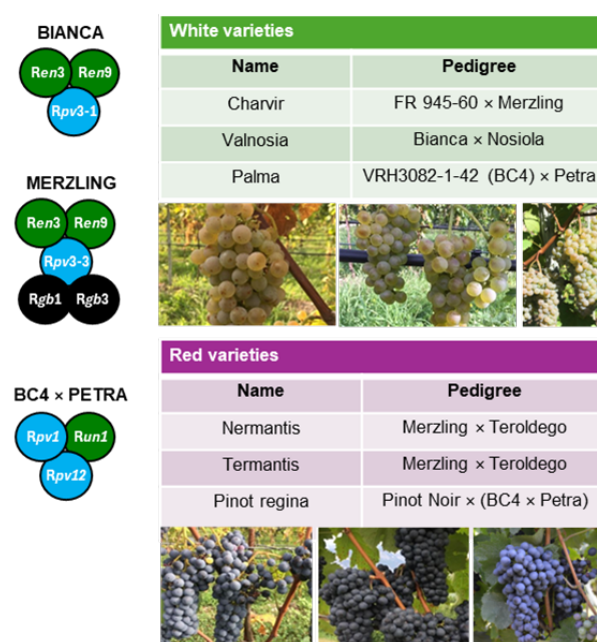


Figure 1. Disease resistant (PIWI) varieties registered by CIVIT, and the R-loci arrangement of the resistance donors.

All of this contributes to the ongoing development of modern resistant varieties in the context of climate change. While Italy's total vineyard area exceeds 600,000 hectares, only slightly more than 2,000 hectares are currently declared as planted with resistant varieties (2). Nevertheless, the number of wineries producing wine from resistant varieties already exceeds 220 companies, with a growing number of labels—from 364 in 2023 to over 400 in 2024. Many of these are evaluated annually by a panel of 30 judges during the "PIWI Wine Contest" organized by FEM. Clearly, the increase in wine labels is directly related to the expansion of vineyard area and the approval of new varieties across different regions.

5. Disease Resistant Grapevines: The Protection

A critical yet frequently misinterpreted issue pertains to the management and potential reduction of phytosanitary

interventions. Notably, these resistant varieties are not immune to pathogens; rather, they display differential defence responses upon pathogen recognition, which manifest as variations in activation timing and the associated metabolic profiles. Consequently, protective strategies must be adapted to the specific phenological phase of the vine and the prevailing pathogen pressure.

The flowering stage represents a critical phenological window of heightened susceptibility to infection. In the absence of prophylactic treatment applications administered immediately prior to and following anthesis, the host tissue is extremely vulnerable to pathogen ingress. Notably, *P. viticola* may persist in a latent state and later manifest in an aggressive form on berries, leading to substantial reductions in vine productivity. Similarly, early-season fungicide applications targeting *E. necator* are warranted to suppress initial inoculum pressure, which may otherwise exceed the containment threshold of even genetically resistant cultivars. Implementing these preventive measures optimizes the plant's capacity to mitigate subsequent infection waves during later phenological stages (8).

It has been estimated that with the cultivation of fungus-resistant varieties the extent of phytosanitary intervention can be substantially reduced by 30% to 70%, depending on the applied protection strategy, the phenological development stage, and the prevailing intensity of pathogen pressure throughout the growing season (9). Nonetheless, a baseline level of treatment remains indispensable. These prophylactic measures are critical for disrupting the reproductive cycles of pathogenic organisms, thereby mitigating the emergence and establishment of novel, resistance-breaking strains that could compromise the durability and efficacy of the resistance traits deployed in grapevine cultivars.

6. Biodiversity: the Green Gold to Preserve and Enhance

FEM conserves and manages a grapevine germplasm repository consisting of approximately 3,000 accessions differentiated by provenance and/or unique phenotypic attributes (10). Each genotype is maintained through 1 to 20 plants. Recent acquisitions from global repositories and wild collections comprise predominantly *Vitis* hybrids and species, which serve as a valuable reservoir for the identification of novel alleles in genetic diversity analyses and for their use for different breeding purposes (wine/table grapes and rootstocks). The accessions have been genotyped through the reference set of 9 microsatellite markers proposed by the International Organisation of Vine and Wine (OIV) (11) to confirm varietal identity in accordance with national and international reference databases and revealing more than 2,200 genetically distinct profiles. Furthermore, comprehensive phenotyping has been conducted over three consecutive years to evaluate a broad spectrum of traits, including ampelographic descriptors, developmental parameters (e.g., phenology, fertility), biotic stress responses, and compositional attributes of berries and derived wine (e.g., chemical composition).

Biodiversity, in this context, constitutes not only a biological asset but also a strategic resource, here referred to as "Green Gold". It encapsulates the intrinsic evolutionary potential of natural systems to respond and adapt to environmental perturbations. Conceptually, biodiversity may be regarded as the nature's toolbox, a repository of adaptive traits: the greater its extent, the more options are available to face emerging challenges such as novel pathogens, climate variability, and evolving market demands. Conversely, erosion of this diversity constrains adaptive capacity. The existence of disease-resistant grapevine cultivars underscores this principle, as genetic resistance to fungal pathogens has naturally evolved in American, Asian (e.g., *V. amurensis* and, more recently, other *Vitis* spp. From China), and some *V. vinifera* lineages. Visionary breeding efforts have capitalized on this genetic heritage through introgressive hybridization with elite European cultivars.

In recognition of the strategic value of genetic diversity, the European Union's NextGenerationEU initiative—implemented via the National Recovery and Resilience Plan (PNRR)—has directed financial support toward the National Centre for Agricultural Technologies (AgriTech), specifically within research domains focusing on the study and valorization of genetic resources to enhance the climate resilience of agriculture.

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