Breeding new resistant grapevine varieties

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Introduction

Grapevine breeding has entered a new era in terms of providing techniques for developing and selecting new varieties which are resistant to downy (*Plasmopara viticola*) and powdery (*Erysiphe necator syn. Uncinula nector*) mildew. Downy mildew requires high humidity and rainfall to germinate and grow, whereas powdery mildew develops under a wider range of climatic conditions. The organisms causing downy and powdery mildew are therefore often referred to as 'bad' and 'good' weather fungi, respectively. The aims of breeding disease resistant varieties of grapevines include lowering production costs by reducing spray applications and thus the need for labour, chemicals and fuel, improving the microbial activity of the soil in the vineyard by reducing the compaction caused by tractor usage, and to provide a healthier environment for humans and animals around vineyards.

The Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia, French National Institute for Agricultural Research (INRA) in France, The University of California, Davis in USA and Julius Kühn-Institut (JKI) in Germany are all important research organisations breeding new disease resistant varieties. Important aspects of this work include studying genotype behaviour and characteristics under differing climatic conditions, as well as the potential of the different genotypes to produce quality wines.

Species and evolution

While different grapevine species can be found around the world, *Vitis vinifera* originated in Europe and central Asia. *Vitis vinifera* is the main species used to produce wines. Wild North American species (*Muscadine*) contain resistant genes for many diseases but present inferior quality fruit compared to *Vitis Vinifera* (Donald et al. 2010). Therefore, American species are not widely used for wine production. Crossing disease resistant grapevine genotypes with disease susceptible *Vitis vinifera* varieties could allow the creation of new varieties which produce quality fruit with disease resistance. North American species such as *Muscadinia rotundifolia* have long been considered to be an important source of resistance against pathogens such as nematodes and mildews (Olmo 1986). However, recent research has shown that Chinese species such as *Vitis romanetii* also carry genes which confer promote resistance to fungal diseases (Ramming et al. 2011).

Vitis vinifera varieties do not contain genes which promote resistance to mildews, most likely because the introduction of the pathogens to Europe has only been recent, in the 19th century. On the other hand, American and Chinese species have been exposed to these pathogens over a much longer period and have developed resistance against mildews over time.

Natural selection

Grapevines naturally have female, male or hermaphroditic (containing both male and female structures) flowers. However, the cultivated varieties of *Vitis Vinifera* have nearly always hermaphroditic flowers (Boursiquot et al. 1995).

Natural crossings have been occurring long-term via the intervention of insects and wind, both distributing pollen over considerable distances. More recently, humans have been crossing different grapevines to reproduce their individual characteristics (Figure 3 and Figure 4). Some success was initially achieved from purely observational work, which led to the selection of some disease resistant grapevine varieties based on their ability to maintain a healthy status in the vineyard.

An excellent example of a successful 20th century breeding program is that of Professor Alleweldt who created Regent from a cross between Diana (Silvaner x Müller-Thurgau cross) and Chambourcin (interspecific hybrid) at JKI in 1967 (Eibach and Töpfer 2003). Regent performs well in north European climates and is currently the fifth most planted cultivar in Germany. It also performs well in wine competitions.

Recently, DNA technology has enabled researchers to identify resistant grapevines by screening the genotypes and assessing the origin of the genes. Consequently, progeny generated can be checked for the presence of mildew resistant genes at seedling stage and then planted in the field for evaluation of grape attributes. This leads to considerable savings in time and effort in the breeding program.

Figure 3. Fertilisation of flowers with pollen. Photo: E. Ruehl, HGU.

Figure 4. Fertilisation of flowers with pollen. Photo: E. Ruehl, HGU.

Advanced methods for breeding selection

Field assessment and selection

New genotypes originating from crossings need to be assessed for their disease resistance. Plant evaluation is time consuming as the plants need to be evaluated under environmental conditions to determine the characteristics of the phenotype and the sustainability of resistance. Resistance depends on gene interactions, which can enhance or reduced the resistance to some degree. Field evaluation can determine the physiological ability of genotypes to repress mildew infection. Therefore, plants in the field may be inoculated and disease parameters such as sporulation, germination and appearance of necrotic spot assessed using scales of incidence and severity.

However, in order to reduce the cost and time requirements related to the evaluation of numerous new seedlings, the leaf disk technique can be used where leaf disks are placed in petri dishes to enable rapid assessment of disease resistance. These results can then be correlated with the results of field and greenhouse evaluations. This new technique allows a primary selection of promising resistant genotypes to allow only the best seedlings to progress to field evaluation.

Robotics for field assessment

Once in the field, phenotyping of seedlings is labour intensive and robotic technology has been designed to speed up the evaluation and simplify the selection process. PHENObot (Figure 5) was developed by the Federal Ministry of education and research (BMBF) in Germany and is equipped with different sensors, cameras and GPS technology, allowing the robot to conduct independent phenotyping.

The robot is able to assess phenological development (from bud burst to ripening), yield parameters (berry size, number of berries per cluster, number of cluster per shoot, yield per vine), and resistance characteristics (e.g. powdery and downy mildew resistance efficiency).

The data collected is directly stored for each grapevine into a computer system and can subsequently be used to determine the performance of the plant.

DNA technologies

Studies of grapevine DNA have recently enhanced our understanding of the resistance mechanisms associated with different genes. Analysis of the genome of resistant genotypes enables the region (locus) responsible for conferring specific disease-resistance traits to be identified through comparisons to the genome of susceptible genotypes. Once the resistance gene is localised to a chromosome (linkage group), it can be fine mapped with specific markers such as single sequence repeats (SSRs) or single nucleotide polymorphisms (SNPs). These DNA markers can then be used for rapid screening of breeding populations to identify resistant progeny. This technique is referred to as marker assisted selection (MAS; Dalbò et al. 2001). While the methods used to identify these DNA markers are costly and require numerous genotypes to ensure they are highly specific, they enable rapid selection. Only progeny carrying these markers will be planted and used for further crossing or evaluation.

Figure 5. The PHENObot robot in a vineyard being used to assess the characteristics of new varieties of grape vines. Photo: P. Rüger, DLR RLB.

New disease-resistant varieties for Australian vineyards

New disease-resistant varieties have been bred by the CSIRO and evaluated for Australian conditions. The first generation of mildew-resistant varieties have been crossed to integrate Run1 (Resistance *Uncinula necator* 1) and Rpv1 (Resistance *Plasmopara viticola* 1) genes from *Muscadinia Rotundifolia*, thereby breeding resistance to powdery and downy mildew, respectively. The Run1/Rpv1 locus was initially introgressed into *V. vinifera* by the French breeder Alain Bouquet using a backcrossing procedure (Bouquet 1986; Pauquet et al. 2001). After only four back crossings from the first filial (F1) generation, more than 95% of genes coming from the premium variety are retained and the resistant genes are found in the 3–5% originating from the wild species genome. The CSIRO used a resistant BC5 progeny plant generated by Alain Bouquet and crossed it with eight premium white varieties including Chardonnay and Riesling as well as eight premium red varieties, to generate a range of first generation mildew-resistant premium wine grape varieties.

More recently, it has been discovered that wild Chinese *Vitis* species also exhibit powdery mildew resistance with different specificity to the Run1 locus, thereby raising the possibility of combining or pyramiding the different resistance genes within the same variety to enhance the durability of the resistance in the vineyard.

Compared to annual crops, grapevines exhibit a perennial structure, meaning the breeding process is more complex. Single gene resistance is usually sufficient for annual crops because if the resistance is broken by the pathogen, the crop can be replaced with alternative genotypes containing a different resistance gene in the following season. With perennial crops such as the grapevine, uncertainty surrounding the duration of pathogen resistance can present a challenge. It is important to assure grape growers that the new varieties present long-term disease resistance, and that combining more than one resistant gene boosts the potential for durable resistance against mildews. Different types of grapevine powdery and downy mildew are found around the world and individual resistance genes are unable to protect the plant against all strains of the pathogen. With this in mind, CSIRO is now breeding the second generation of mildew resistant wine grape varieties that will contain multiple resistance genes to powdery and downy mildew.

From the first generation crosses, a total of 20 white and 20 red varieties that exhibit promising viticultural and winemaking characteristics have been selected. These selections have been planted in diverse grape growing regions around Australia and are under evaluation. Commercial winemaking will be conducted and assessed to see if they can be suitable for the

Australian market. Ultimately these varieties have the potential to reduce the production costs of wines exhibiting characteristics similar to Chardonnay and Shiraz. This would then enable cost effectiveness of production and international competitiveness for Australian wines.

Genetically modified organisms

Genetically modified organisms (GMOs) are used in research to better understand the functioning of the genes responsible for disease resistance. Genetically modified mildew-resistant versions of premium wine grape cultivars such as Shiraz and Tempranillo, containing the Run1 and Rpv1 resistance genes, have been successfully created by the CSIRO in collaboration with INRA (Feechan et al. 2013). Furthermore, some grapevine genes have been identified which are thought to increase the susceptibility of the plant to mildew (Dry et al. 2010) and there could be future targets of gene manipulation approaches.

Nevertheless, long-term studies will be required to evaluate grapevine developmental responses to genetic modifications prior to any commercial establishments. Currently, despite their potential to reduce fungicide usage for grape and wine production, public opposition to GMOs impedes further commercial development at this time.

A future in the hand of the consumer

Consumer acceptance could be a potential drawback in terms of market establishment of new disease resistant varieties. Even if the resistant varieties provide quality assurance, climate adaptability and reduced spray application requirements, consumer acceptance may be limited because these varieties will have different names to the well-known French varieties. Research will continue into new varieties because alternatives to fungicide applications are needed. Similar to other grapevine diseases requiring fungicide use (e.g. trunk diseases), current control methods present public health concerns, environmental contamination issues and potential wine residues. Ensuring consumer awareness of the advantages of these varieties should be prioritised.

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