Assisting a Portuguese grapevine breeding program focused on the development of oidium and mildew resistant cultivars – the interest of molecular markers linked to resistant *loci*

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INTRODUCTION

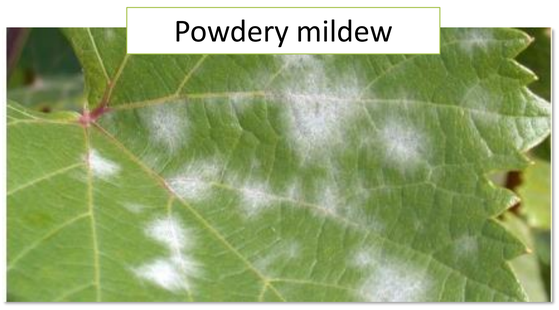
Plasmopara viticola and *Erysiphe necator* are two pathogens able to infect *Vitis vinifera* plants. The diseases caused by them (downy mildew and powdery mildew, respectively) lead to huge economic losses in wine-producing companies. Thus, to fight these pathogens high amounts of fungicides have to be applied to plants, which has negative impacts on the environment and food safety, creating an urgency to find alternative ways to control the damages caused by these diseases.

The breeding program created by Viveiros PLANSEL, Lda, a Portuguese company located at Alentejo region (Montemor-o-Novo, Portugal) alongside the University of Évora (UÉvora) aims to develop resistant cultivars of commercial grapevines. If these resistant varieties are achieved, it will be possible to reduce the number of fungicides sprayed on the crops each year which will have great economic and public health benefits, contributing therefore to a more sustainable agricultural system. The program used Portuguese elite cultivars, used for red and white wine production, on controlled crosses with already established resistant hybrid genotypes (pollen gently provided by Dr. Oliver Trapp from the Julius Kühn-Institut, Germany), in the hope of eventual introgression of these resistance-associated genes into Portuguese grapevines.

The importance of molecular markers on assisting this breeding program is highlighted by showing the results achieved in the offspring generation.

Downy mildew

Powdery mildew



INTRODUÇÃO

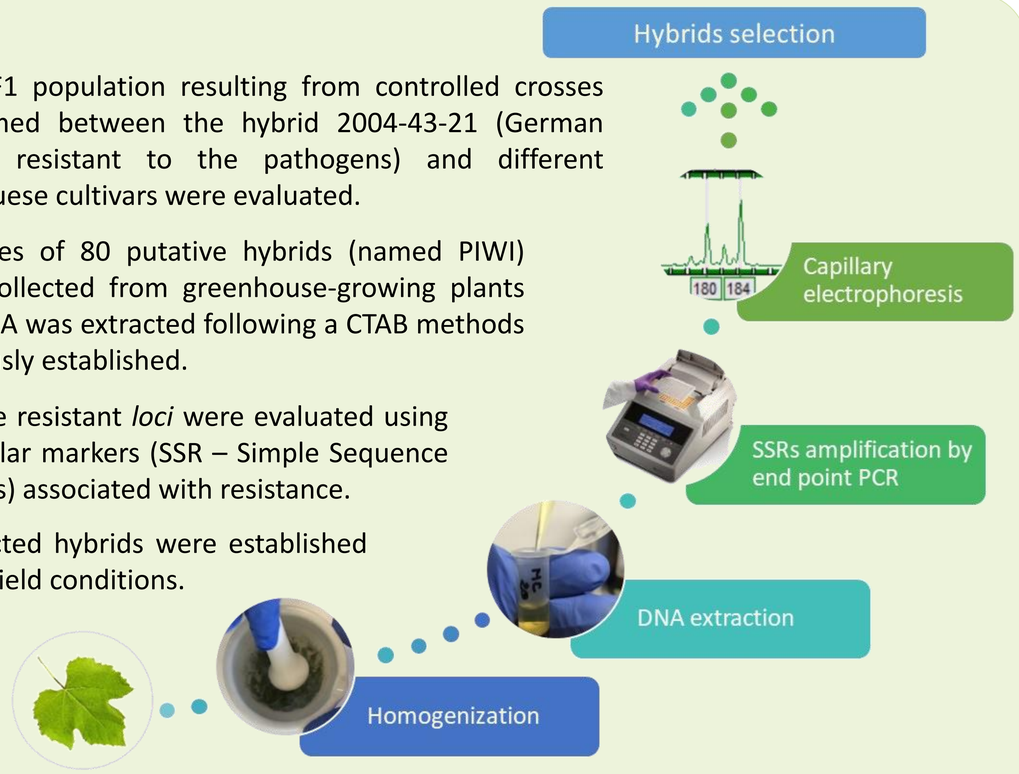
Plamospara viticola e *Erysiphe necator* são dois agentes patogénicos capazes de infetar plantas das diversas cultivares de *Vitis vinifera*. As doenças causadas por estes, conhecidas como míldio e oídio, respetivamente, estão associadas a prejuízos económicos bastante avultados. Para controlar estes agentes patogénicos são aplicados nas vinhas quantidades elevadas de fungicidas, o que tem um impacto extremamente negativo no ambiente e na segurança alimentar. Perante esta situação é muito importante encontrar formas alternativas de controlar estes agentes patogénicos, com um impacto menos negativo para o ambiente.

O programa de melhoramento desenvolvido pela empresa Viveiros PLANSEL Lda., uma empresa Portuguesa localizada na região do Alentejo (Montemor-o-Novo, Portugal), em parceria com a Universidade de Évora, visa desenvolver variedades Portuguesas resistentes. Se esse objetivo for alcançado será possível reduzir o volume de fungicidas aplicados à vinha, contribuindo para o estabelecimento de um setor vitivinícola mais sustentável. No programa de cruzamentos controlados foram utilizadas variedades de elite Portugueses e genótipos híbridos resistentes (pólen gentilmente fornecido pelo Dr. Oliver Trapp do Julius Kühn-Institut, Alemanha), para que através da segregação os genes associados à resistência possam ser integrados nos híbridos da F1.

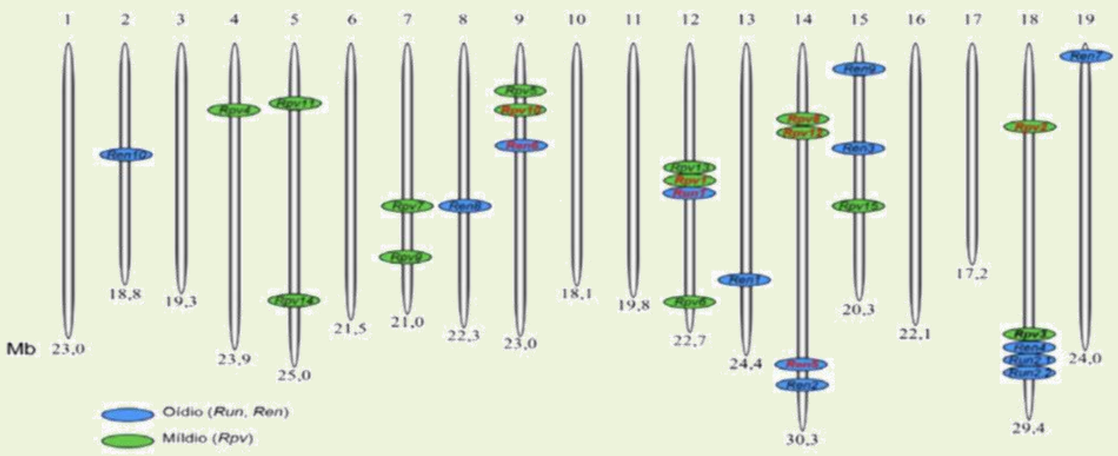
A importância da utilização de marcadores moleculares na seleção dos híbridos de interesse será realçada através da apresentação dos resultados obtidos na genotipagem de plantas F1.

METHODOLOGY

- An F1 population resulting from controlled crosses performed between the hybrid 2004-43-21 (German Hybrid resistant to the pathogens) and different Portuguese cultivars were evaluated.
- Leaves of 80 putative hybrids (named PIWI) were collected from greenhouse-growing plants and DNA was extracted following a CTAB methods previously established.
- Three resistant *loci* were evaluated using molecular markers (SSR – Simple Sequence Repeats) associated with resistance.
- Selected hybrids were established under field conditions.



Distribution of the resistant *loci* are nowadays very well known (see figure below). Putative hybrids were genotyped for the loci **Run1-Rpv1**, **Rpv3.1**, and **Ren3/9**.



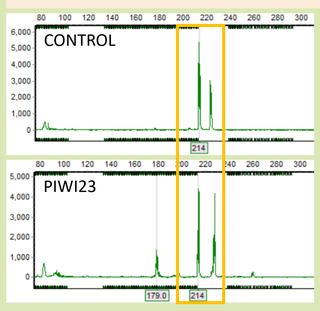
Adapted from Eibach, R., 2017 (in red are the most efficient *loci*).

RESULTS

From the 80 plants genotyped, 27 did not show any of the three *loci*, 17 showed the introgression of a single *locus*, 33 showed the introgression of two *loci* (different combinations were detected), and 3 genotypes showed the introgression of the three *loci* (see plants under field conditions on the right).

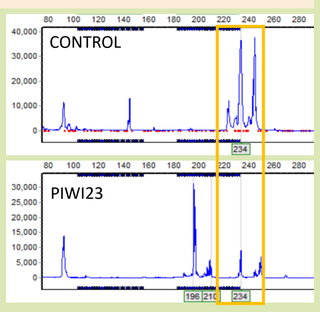
Below is shown the genotype PIWI 23, exhibiting introgression of the three *loci.* The figure shows the size of the resistant allele identified for each marker in comparison with the resistant progenitor (named control).

Locus Run1-Rpv1

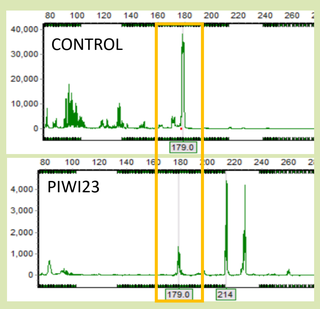


Locus Run1-Rpv1 was detected by markers Sc34-8 (HEX - 214 bp) and Sc35-2 (FAM - 234 bp) previously developed by Li *et al*. (2013).

PIWI23 is a offspring plant.

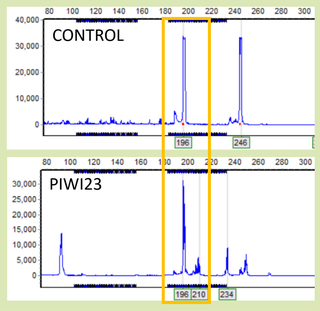


Locus Rpv3.1

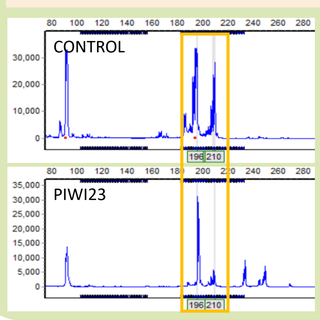


Locus Rpv3.1 was detected by markers Indel-26.032 (HEX - 179 bp) and Indel-25.941 (FAM - 196 bp) previously developed by Foria *et al*. (2018).

PIWI23 is a offspring plant.

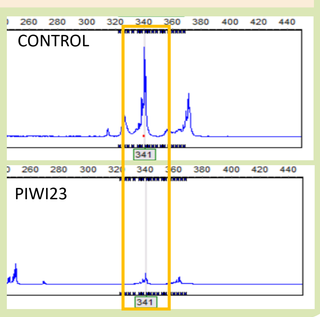


Locus Ren3/9



Locus Ren3/9 was detected by markers GF15-39 (FAM – 210 bp) and GF15-28 (FAM - 341 bp) previously developed by Zendler *et al*. (2017).

PIWI23 is a offspring plant.



CONCLUSIONS

- This study showed that the controlled pollination procedure was effective, as 62% of the F1 population showed at least one *locus* for resistance, 35% had at least two *loci* and 5% had the three screened *loci*. The presence of each *locus* was confirmed by using markers located at 5'-end and at 3'-end.
- In *Locus* **Ren3/9**, the resistance alleles with 209 bp and 340 bp were introgressed in 30% of the F1 genotypes.
- *Locus* **Run1/Rpv1** with 213 bp and 233 bp alleles size showed a similar introgression rate with 37% of the desired alleles being inherited.
- The **RPV3.1** *locus* showed an inheritance rate of 41%.

This research highlights the importance of MAS (Marker Assisting Selection) in assisting breeding programs by showing that from the 80 plants genotyped, only three plants should be considered to proceed with further analysis, which includes the analysis of the most important agronomical traits in viticulture.

References

Foria, S. *et al.* (2018) 'InDel markers for monitoring the introgression of downy mildew resistance from wild relatives into grape varieties', *Molecular Breeding*, 38(10).

Li, C. *et al.* (2013) 'Selection for Run1-Ren1 Dihybrid Grapevines Using Microsatellite Markers', *American Journal of Enology and Viticulture*, 64(1), pp. 152–155.

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Acknowledgments

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