

Evaluation of intra-varietal variability and selection for resistance to downy mildew: The case of Antão Vaz variety in Portugal

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Abstract. Antão Vaz is a Portuguese white grapevine variety grown mainly in the wine-growing regions of southern Portugal. It is highly susceptible to downy mildew, caused by *Plasmopara viticola*, a destructive disease that occurs in years with rainy springs. Therefore, evaluating downy mildew and selecting for greater resistance to this disease are important objectives for this variety, aiming to reduce the number of fungicide sprays needed for disease control. A field trial established with a representative sample of the intra-varietal variability of Antão Vaz (110 clones), according to a resolvable row-column design, was evaluated for symptoms of downy mildew over 2 years. The field was naturally infected, with no treatments for the disease applied during the study, and a scale was used to assess downy mildew severity. A generalized linear mixed model with a cumulative probit link function was fitted to the downy mildew severity data. Significant intra-varietal variability for disease severity was detected ($p < 0.001$), indicating that selection for higher resistance to downy mildew is feasible even in highly susceptible varieties. This work confirms the importance of intra-varietal variability in ancient varieties for contributing to a more sustainable viticulture.

1. Introduction

In Portugal, since the 1980s, intensive research has been carried out on the conservation and valorisation of intra-varietal variability and selection (both polyclonal and clonal) within ancient grapevine varieties [1]. High levels of intra-varietal variability for quantitative traits have been reported for numerous Portuguese grapevine varieties, not only for yield, but also for other traits such as soluble solids content, acidity, anthocyanins [2] and, more recently, for abiotic stress [3, 4]. Intra-varietal variability also exists for other important traits, such as disease resistance, but it has not yet been sufficiently explored by breeders. However, an important contribution to a more sustainable use of autochthonous varieties in viticulture, is the search for intra-varietal variability in these traits. With this aim in mind, field trials with representative samples of intra-varietal variability, located at the Experimental Centre for the Conservation of Grapevine Diversity of the Portuguese Association for Grapevine Diversity (PORVID), have recently began to be evaluated for disease resistance in untreated plots. Among the field trials conducted, the symptoms of downy mildew, caused by

Plasmopara viticola (Berk. and Curt.) Berl. & de Toni, were assessed in the Antão Vaz.

Antão Vaz is a Portuguese white grapevine variety grown mainly in the wine-growing regions of southern Portugal, particularly in the Alentejo, Lisbon, and Setúbal Peninsula regions. It is a very vigorous and productive variety, giving the wines a strong identity. It needs heat and sunlight and prefers deep and dry soils, which makes it tolerant to scald caused by the high summer temperatures of southern Portugal. However, this variety is very susceptible to downy mildew, caused by *P. viticola*, a very destructive disease in years with rainy springs. Under such favourable conditions, and to prevent downy mildew from reaching epidemic proportions, disease management still largely relies on the intensive use of synthetic and/or copper-based fungicides, leading to environmental, human health and economic impacts.

From previous results obtained in field selection trials, it is known that the Antão Vaz variety has a high level of intra-varietal variability for yield and must quality traits [1]. Therefore, evaluation for downy mildew and selection for greater resistance to this disease is a very

important objective for this variety, aiming to reduce the use of fungicides.

The main objective of this study is to outline experimental strategies for utilizing intra-varietal variability in the direction of selection that helps to promote the sustainable development of the vine and wine sector, consolidating a competitive advantage offered by the originality and history of ancient grapevine varieties.

2. Material and methods

The study was conducted in a field trial located in the experimental Center of PORVID in Pegões (Palmela district, Southern Portugal). This field trial contains a representative sample of the intra-varietal variability of Antão Vaz (110 clones sampled in old vineyards of Alentejo region) and was established according to a resolvable row-column design with 6 resolvable replicates (with 11 rows nested within a resolvable replicate and 10 columns nested within a resolvable replicate) and 3 plants per experimental unit.

The training system was a vertical shoot position, the pruning system was a bilateral Royat cordon system, and the planting density was 2.50 m × 1.20 m. All the plants of the field trial were grafted onto a single clone of the 1103 Paulsen rootstock. This field trial was evaluated for symptoms of downy mildew, under natural infections, over 2 years. No treatments for this disease were applied during the study. The weather conditions observed in the region of the field trial in the 2 years of the study are described in Table 1.

Table 1. Influence of the weather conditions on downy mildew of grapevine over the 2 years of the study in the region of Palmela.

Year	Weather conditions	Downy mildew
First year (2022/ 2023)	Concentrated rainfall in December, with almost no rainfall between February and August.	Low disease incidence and severity.
	Temperature amplitude was normal from December to May.	Only highly sensitive grapevine varieties, such as Antão Vaz, showed disease symptoms.
Second year (2023/ 2024)	Very high rainfall from January to March, accompanied by high minimum temperatures and low temperature amplitude. These conditions favoured oospore maturation.	High disease incidence and severity. All grapevine varieties were infected, due to highly favourable conditions for disease development.

In the first year, a scale adapted from the European and Mediterranean Plant Protection Organization (EPPO) [5] was used to assess the percentage of the leaf surface and bunch area affected, as follows 1 = no disease; 2 = < 25%; 3 = 25-50%; 4 = 50-75%; 5 = > 75% (therefore, lower categories, higher disease resistance). In the second year,

the full scale of EPPO (2020) was applied: 1 = no disease; 2 = < 5%; 3 = 5-10%; 4 = 10-25%; 5 = 25-50%; 6 = 50-75%; 7 = > 75%. Assessments were made for each plant in the experimental unit.

As several categories with a logical order (disease score) were considered, the data (the response variable with an ordinal multinomial distribution) were analysed using a generalised linear mixed model (GLMM) with a cumulative probit link function (i.e. a model with a probit link for the cumulative probabilities). This model estimates the probabilities of resistance levels corresponding to lower ordered values (indicating higher resistance) on the scale used.

The genotypic effects of the clones and the experimental design effects (resolvable replicates, rows within replicates, and columns within replicates) were treated as random effects.

For the generalised linear mixed model described, parameter estimation was performed using maximum likelihood via Laplace approximation [6]. A likelihood ratio test was performed on the genotypic variance component, and the p-value for this test was derived from a mixture of chi-squared distributions, following the approach of Self and Liang [7]. A generalised measure of broad-sense heritability [8] was calculated to quantify the proportion of observed variability in disease resistance attributable to genotypic effects. In addition, clones were ranked based on empirical Bayesian estimates of clonal effects, and the results are presented in terms of the estimated probability of a clone falling into lower categories on the scale of resistance used. The GLIMMIX procedure of SAS version 9.4 [9] was used for these analyses.

3. Results and discussion

In the first year of evaluation (a year with a low downy mildew incidence and severity), all levels of the resistance scale were observed for leaf surface and bunch area. As a result, the generalised linear mixed model described in the previous section was fitted to the data, and the results for within-variety variability for the level of resistance observed in leaves and bunches are shown in Table 2. In the second year of the evaluation (a year with a high incidence and severity of downy mildew), the statistical analysis was not carried out because only one level of resistance was observed: for leaves, all observations were classified as level 6 and for bunches as level 7 of the EPPO scale [5].

According to the results presented in Table 2, significant intra-varietal variability for the level of resistance was found ($p < 0.001$), using both leaf and bunch symptom data. The values of broad-sense heritability, though moderate, were important, reaching nearly 0.50 for the bunch data (i.e., almost 50% of the variability in resistance levels can be attributed to genotypic causes). These values for broad-sense heritability are very interesting, aligning with the range typically observed for other quantitative traits in grapevine selection field trials

of Portuguese varieties [1, 3, 4, 10]. It is important to note that this quantitative genetic approach is uncommon for the analysis of this type of data and was only possible due to the use of a probit link function (based on the Gaussian distribution) [6]. This study demonstrates that a quantitative genetic interpretation of the data is feasible, which is crucial when accounting for random genotypic effects.

Another important aspect of the analysis is the role of the experimental design in modelling the variability of inoculum presence in the field trial. According to the results presented in Table 2, the effects associated with the experimental design were significant, indicating that they all contributed to modelling the variability in resistance levels in the field trial. Among the design effects, whether in leaf or bunch data, the percentage of variability attributed to the effect of columns within replicates was over 55%, followed by the percentage attributed to rows within replicates. The least important design effect (although still significant) was the effect of resolvable replicate. These results highlight the influence of pathogen spread over short distances from one plant to its neighbour. In the columns of the design (corresponding to vineyard rows), the distance between plants is smaller, making inoculum spread easier. This type of variability, unrelated to genotypic effects but related to the higher possibility of inoculum dissemination (and therefore different inoculum pressures affecting different clones), was modelled by the experimental design of the field trial. The importance of using a resolvable row-column design in grapevine selection field trials for evaluating resistance levels is also demonstrated in this work, which is consistent with previous results for yield [10].

Table 2. Results for the intra-varietal variability (genotypic variance estimate, $\hat{\sigma}_g^2$, and respective estimated asymptotic standard error, SE), broad-sense heritability (H^2) obtained for the level of resistance observed in leaves and bunches in the first year of evaluations, and the effects of the experimental design expressed as the percentage of each design effects variance in the total variance associated to the experimental design (resolvable replicate, $\hat{\sigma}_r^2$; columns within replicate variance estimate, $\hat{\sigma}_{col(r)}^2$; rows within replicate variance estimate, $\hat{\sigma}_{row(r)}^2$)

	$\hat{\sigma}_g^2$ (SE)*	$\hat{\sigma}_r^2$ (%)	$\hat{\sigma}_{col(r)}^2$ (%)	$\hat{\sigma}_{row(r)}^2$ (%)	H^2
Leaf surface affected	0.201 (0.071)	9.39	55.62	34.99	0.415
Bunch area affected	0.231 (0.071)	18.55	59.33	22.12	0.492

*Likelihood ratio test for the genotypic variance component ($H_0: \sigma_g^2 = 0$ vs $H_1: \sigma_g^2 > 0$), p-value<0.001 (for both cases, leaf surface and bunch area affected). All variance components associated with the experimental design were also significant (p-value<0.01).

As mentioned above, in the second year of the evaluation (a year with a high incidence and severity of downy mildew) all clones were classified as having the same low level of resistance. It is important to note that no treatments were applied during the trial. Therefore, in a year typically characterised by a highly destructive epidemic of downy

mildew, such a result was expected. It is important to understand that there are several levels of resistance, but no complete resistance. Even for genotypes that are considered to be resistant to grapevine downy mildew, it is advisable to combine disease resistance genes with other means of disease control (e.g. intermittent applications of fungicides), to increase the durability of these genes [11, 12].

In the first year, which was a year with a low incidence of downy mildew, and with no treatment against the disease, Antão Vaz, a very susceptible variety, showed intra-varietal variability, as already mentioned in Table 2. However, it is interesting to analyse the different levels of resistance among the 110 clones studied. The ranking of these clones, based on their estimated probability of falling into lower resistance categories, is presented in Tables 3 and 4 for the resistance observed in leaves and bunches, respectively (only the results for the 10 most and least resistant clones are shown).

Table 3. The ranking of the clones, based on their estimated probability of falling into lower categories on the resistance scale; ranked from the clones with the highest level of resistance to those with the lowest, according to observations made on the leaves.

Rank	Clone	Estimated probability
1	AN0121	0.742
2	AN0185	0.730
3	AN0225	0.718
4	AN0177	0.704
5	AN0205	0.698
6	AN0446	0.691
7	AN0549	0.689
8	AN0306	0.687
9	AN0143	0.686
10	AN0348	0.672
...	...	...
101	AN0506	0.356
102	AN0137	0.349
103	AN0134	0.338
104	AN0303	0.332
105	AN0424	0.319
106	AN0130	0.315
107	AN290081	0.287
108	AN290071	0.259
109	AN0507	0.235
110	AN0123	0.195

Using the leaf symptom data (Table 3), the estimated probability of a clone falling into lower categories on the resistance scale (i.e., the probability of having higher resistance) ranged from 0.742 to 0.195. For example, the clones identified as AN0121, AN0185, and AN0225 were the top three with the highest resistance to downy mildew, while the clones AN290071, AN0507, and AN0123

showed the lowest resistance to downy mildew. For the bunch symptom data (Table 4), the range of the estimated probabilities of falling into lower categories on the resistance scale was higher, varying from 0.82 to 0.09. In this case, the clone identified as AN0177 showed the highest resistance to downy mildew, while the clone AN0123 showed the lowest resistance to downy mildew. When analysing the list of clones ranked by their level of resistance, some of them have common results for both leaf and bunch symptom data, but there is not a complete overlap.

This study should be continued and there is a need to cross these results with those from selection field trials on the same clones already evaluated for other traits such as yield and must quality. However, the results obtained for intra-varietal variability have shown that it is possible to select for higher downy mildew resistance within an old variety that is highly susceptible to downy mildew.

Table 4. The ranking of the clones, based on their estimated probability of falling into lower categories on the resistance scale; ranked from the clones with the highest level of resistance to those with the lowest, according to observations made on the bunches.

Rank	Clone	Estimated probability
1	AN0177	0.820
2	AN0205	0.740
3	AN0185	0.725
4	AN0306	0.714
5	AN0121	0.696
6	AN0244	0.684
7	AN0211	0.681
8	AN0143	0.679
9	AN0354	0.669
10	AN0416	0.668
...	...	...
101	AN0195	0.360
102	AN0315	0.323
103	AN0110	0.311
104	AN0507	0.295
105	AN290030	0.292
106	AN0137	0.274
107	AN0502	0.273
108	AN0303	0.242
109	AN0245	0.232
110	AN0123	0.092

4. Conclusions

This work confirms the importance of genetic resources, particularly those within ancient varieties, as a complementary tool to reduce pesticide use and contribute to more sustainable viticulture. The methodology

presented (type of experimental design, and model for data analysis) proved to be a powerful tool for dealing with downy mildew data.

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6. References

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