Polyclonal selection for abiotic stress tolerance in Arinto: Implications in yield and quality of the must

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Abstract. The valorisation of genetic variability through the identification suitable genotypes for traits such as yield and must quality is an effective strategy used for grapevine selection. Currently, climate change-driven heat waves and drought affect plant growth and wine quality, but little information is available on intravarietal variability regarding responses to stress. In the current work, the intravarietal genetic variability of the Portuguese variety Arinto was studied for yield, must quality, and tolerance to abiotic stress. An innovative approach using rapid, and nondestructive measurements of surface leaf temperature (SLT), Normalized Difference Vegetation Index (NDVI), Photochemical Reflectance Index (PRI), and chlorophyll content (SPAD), was used in an experimental population of 165 clones of Arinto installed according to a resolvable row-column design with 6 replicates. Also, yield and quality characteristics of the must were quantified. Linear mixed models were fitted to the data, and the empirical best linear unbiased predictors (EBLUPs) of genotypic effects for each trait were obtained as well as the coefficient of genotypic variation (CVG) and broad sense heritability. The results enabled the selection of a group of genotypes with increased tolerance to stress, which maintained the must quality of Arinto.

1 Introduction

Grapevine is one of the most economically important crop species in Portugal. Its main product, grapes, is mostly used for the production of wine, highly significantin the national economy and a major export. In 2021Portugal was the fourth European country in terms of vineyard area (194,000 thousand ha), the fifth wine producer in the EU (6.6 million hl), and the tenthworldwide [1]. Portugal also has a very high grapevine biodiversity with 265 officially described Portuguesevarieties in 2020 [2], that are made up of hundreds of genotypes (clones). These clones are the natural reservoir of the genetic and phenotypic variability of the varietiesand are different from one another regarding the most important economic traits (quality of the must and yield). In the Mediterranean area, climate change is driving temperatures towards higher values and increasing the frequency of extreme weather events (such as severe drought and heat waves), that will affect viticulture [3]. On the long term one of the most effective strategies to overcome these changes is to use the biodiversity that exists within each variety (intravarietal variability) and choose the genotypes that are best adapted to withstand climate extremes.

Grapevine selection methods involve the comparative analysis of hundreds of genotypes, represented by several biological replicates in an experimental field. When yield and quality of the must is analysed, possible changes due to the time of day of sampling are not significant and will not alter the results. However, when analysing the plant's response to an environmental challenge, that response isin itself dependent on an environmental condition that is not stable for a long period during the day. Therefore, oneof the most challenging aspects of selection to characterize abiotic stress response is plant phenotyping work. This makes the measurement of some important physiological parameters impractical and sometimes even unfeasible. It is thus paramount to develop expedite and accurate measurements for field phenotyping.

Infrared thermography or canopy temperature shows some potential for plant phenotyping in the field andassess response to drought [4-6]. Stomatal behavior is correlated with the plant water status and is also responsible for plant evaporative cooling, because when stomata are closed leaf temperature increases. When this happens CO_2 intake for photosynthesis becomes

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minimal and carbohydrate production is affected. Temperature analysis can be performed using cameras recording electromagnetic radiation in the infrared region [7], where most of the heat electromagnetic spectrum is located. Thermal imaging has proven to be adequate for different plant species and in different conditions [6], especially when a stress treatment is applied. When the objective is to assess the response of many genotypes to the same environmental conditions, the sensitivity of the method is not enough to achieve an accurate characterization. Therefore, another leaf temperature related parameter, Surface Leaf Temperature (SLT) has been developed [8], which has shown good results in genotypes of the variety Aragonez.

In this work, a collection of Arinto clones in a grapevine selection experimental field was evaluated. This field is established in the Palmela District, in the Experimental Centre for the Conservation of Grapevine Intravarietal Diversity of PORVID (Portuguese Association for Grapevine Diversity) and comprises 165 genotypes of this variety. These genotypes were used for stress tolerance monitoring based on the identification of genotypes with lower SLT, better values of Normalized Difference Vegetative Index (NDVI), Photochemical Reflectance Index (PRI), and chlorophyll content through the SPAD index. These were complemented with analyses of yield and berry characteristics. SLT and the stress indices were used as non-invasive and expedite indicators of abiotic stress tolerance.

2 Material and methods

2.1 Experimental design and location of the field trial

An experimental population of 165 clones of the variety Arinto, containing representative samples of the intra-varietal diversity of the variety, was used. This field trial is located in the experimental centre of PORVID in Pegões (38°38'54.9"N 8°38'38.2"W; Palmela district, Southern Portugal). It is laid out in a resolvable row-column design consisting of six resolvable replicates (165 genotypes × 3 plants per plot (experimental unit) × 6 replicates), with incomplete blocks in row and column directions within each resolvable replicate (complete block). All plants were grafted on the same clone of 1103P rootstock and were free fromgrapevine leafroll associated virus type 3 and grapevine fanleaf virus. The training system was a vertical shoot position and the pruning system was a bilateral Royat cordon system.

2.2 Abiotic stress evaluation

The evaluations were conducted in 2019, in a non-irrigated field. Water stress conditions were quantified through the measurement of pre-dawn leaf water potential (pressure chamber, Model 600, PMS Instruments Company, Albany, OR, United States) in the field, and the average values obtained were -0.7 MPa.

For all evaluations, measurements were taken on peak heat hours on leaves exposed to the sun. The experimental design comprised the control of environmental effects such as the effects of the day and hour of evaluation. The resolvable replicate (complete block) comprised the effect of the original experimental design and the effect of the day. The incomplete blocks in column direction within a complete block comprised the effect of the original experimental design and the hour of measurement in each day. In each plot, three measurements were performed in three different leaves. For SLT, each measurement had ten technical replicates, to overcome any possible instrumental error.

Chlorophyll content was indirectly measured with the portable chlorophyll content meter CL-01 (Hansatech Instruments Ltd, Pentney, King's Lynn, Norfolk, UK). Chlorophyll content is expressed in logarithmic scale through the SPAD index. The photochemical reflectance index (PRI) was measured using a Plant Pen PRI 200 (Photon System Instrument, Drásov, Czech Republic). Normalized Difference Vegetative Index was measured with the Plant Pen NDVI 300 (Photon System Instrument, Drásov, Czech Republic), individual surface leaf temperature (SLT) was measured using a non-contactinfrared thermometer, SCANTEMP 440 (Dostmann Electronic, Wertheim-Reich•olzheim, Germany). All data obtained were exported to a spreadsheet for analysis.

2.3 Yield and quality traits evaluation

Berry quality traits (soluble solids, acidity, and pH) were analysed in the must, as well as berry weight. Berry collection was performed for all genotypes in three complete blocks. A sample of 60 berries per plot (experimental unit) was collected the day before harvest. In the laboratory, the berries from each plot were counted and weighted. The analyses of the musts were performed by standard methods: soluble solids by refractometry (probable alcohol by conversion), and acidity by titration.

2.4 Data analysis and identification of the most tolerant genotypes

For data analysis, linear mixed models were fitted following the experimental design of the field trial. Models included the genotypic effects, the resolvable replicate effects (which also include the effect of the day), the column effects within replicates (which also include the effect of the time of day), the row effects within replicates, and the random errors associated with observations.

For yield data, the mean yield of the plot was used. For physiological traits (NDVI, PRI, SPAD, and SLT) individual measurements on different leaves were made, as a result in the fitted models the effect of the plot was added. In all cases, model effects (with the exception of the overall mean) were assumed independent and identically distributed normal variables with zero mean and respective variances. All random effects were assumed mutually independent.

Residual maximum likelihood (REML) estimation method was used for covariance parameter estimation and Empirical Best Linear Unbiased Predictors (EBLUPs) of random effects were obtained from mixed model equations. The EBLUPs of the genotypic effects of SLT obtained were ranked and used to perform the selection. SLT was chosen as the primary abiotic stress tolerance indicator because it is the only of the physiological parameters measured that is quantified directly, without an associated model to obtain values. The most tolerant genotypes had the lowest EBLUP values of SLT. According to this criterion, the predicted genetic gains for all the other traits were obtained.

3 Results

3.1 Yield, quality, and abiotic stress tolerance

From the results obtained, it was possible to verify that there is significant genetic variability within the variety for all the traits analysed (P<0.001), and that the physiological indicators of abiotic stress showed values of broad sense heritability in the same range as those obtained for yield and quality traits of the berry (Table 1). SPAD had the highest value of broad sense heritability (0.674) of the abiotic stress indicators, while the other three traits were very similar to each other, with slightly lower values. The trait with the highest value of broad sense heritability was berry acidity, with 0.714, while berry weight had the lowest value, of 0.482.

The range of the predicted genotypic values obtained for the several evaluated traits is shown in (Table 2). Regarding SLT, there is a quantifiable genetic difference of 5.0 ºC between the coolest (more tolerant) and warmest (less tolerant) of the 165 clones measured. SPAD showed a 5.6 difference between the more tolerant (SPAD of 16.59) and the more sensitive clone (SPAD of 10.99). PRI was the parameter with the highest range of difference between the most tolerant and the most sensitive clone, while NDVI was a very conserved parameter, with very small differences between the most sensitive and most tolerant clones. Due to genetic causes yield ranges from 2.48 kg/plant in the lowest producing clone and 7.41 kg/plant in the highest producing clone, and is the parameter with the highest span between the extremes. Regarding quality parameters, pH is the one with the lowest range of variation, while berry acidity has the highest span of distribution.

Table 1. Overall mean, genotypic variance estimates and associated p-values, coefficient of genotypic variation (CV_G), and broad sense heritability (H^2) for all the traits analyzed.

Trait	Overall mean	Genotypic Variance estimate (p-value)	CV_G	H^2
NDVI	0.68	0.0001 (< 0.001)	1.08	0.565
PRI	0.019	0.0000 (< 0.001)	13.49	0.597
SPAD	14.28	1.742 (< 0.001)	9.25	0.674
SLT	31.16	1.488 (< 0.001)	3.92	0.583
Yield	4.59	0.995 (< 0.001)	21.75	0.636
Berry weight	1.09	0.006 (< 0.001)	7.31	0.482
Berry pH	3.59	0.007 (< 0.001)	2.25	0.648
Berry ºBrix	22.90	1.144 (< 0.001)	4.67	0.668
Berry Acidity	5.60	0.390 (< 0.001)	11.15	0.714

Table 2. Differences between the clones with the highest and the lowest Empirical Best Linear Unbiased Predictors (EBLUPs) of genotypic effects and predicted genotypic values for all the traits analyzed.

Trait	Predictor of the genotypic effect (EBLUP)		Predicted genotypic value for SLT	
	lowest	highest	lowest	highest
NVDI	-0.030	0.011	0.649	0.690
PRI	-0.012	0.003	0.006	0.022
SPAD	-3.29	2.32	10.99	16.59
SLT (ºC)	-2.60	2.40	28.5	33.6
Yield (kg/plant)	-2.07	2.86	2.48	7.41
Berry weight (g)	-0.124	0.230	0.968	1.322
Berry pH	-0.180	0.190	3.41	3.79
Berry ºBrix	-2.29	1.70	20.62	24.61
Berry Acidity	-0.88	2.74	4.72	8.34

3.2 Predicted genetic gains when selecting according to SLT

The predicted genetic gains (R) for all traits when the selection of the top ranking 15 genotypes is performed for SLT are presented in (Table 3). When selecting for SLT there is a genetically quantifiable 14% increase in yield of the selected clones, the highest % of gain resulting from this selection. Regarding the characteristics of the berry, selection for SLT will lead to a 2.2% increase in ºBrix and a 3.8% decrease in acidity. As for the other abiotic stress indicators, NDVI is not affected by the selection, while PRI and SPAD benefit from it, with increases of 3.6 and 4.5%, respectively.

Table 3. Predicted genetic gains (R) for each trait when the selection of the top ranking 15 genotypes is performed for SLT.

Trait	Overall mean	Predicted genetic Gain (*R*) Mean of the EBLUPs of the genotypic effects of the selected clones	Predicted genetic gain in percentage of the mean *R(%)*
NVDI	0.679	0.001	0.1
PRI	0.019	0.001	3.6
SPAD	14.275	0.648	4.5
SLT (ºC)	31.157	-1.680	-5.4
Yield (kg/plant)	4.586	0.640	14.0
Berry weight (g)	1.09	0.012	1.1
Berry pH	3.59	0.018	0.5
Berry ºBrix	22.29	0.497	2.2
Berry Acidity	5.60	-0.210	-3.8

4 Discussion

The Portuguese methodology of grapevine selection uses innovative methods that enable the increase of quality and yield in ancient varieties while simultaneously preserving genetic variability [8]. The availability of many different genotypes of a variety, together with a careful experimental design of field trials, enable the screening of large amounts of plant material for many different traits.

The analysis of SLT is based on the process of evaporative cooling, through which a plant can keep its leaf temperature lower than the atmosphere's when subjected to heat stress. For this process to be effective, especially under conditions of water shortage, the plant must achieve a good control of stomatal opening. This, in turn, will be related to a better modulation of gas exchange and thus of CO_2 uptake [7]. Therefore, when selecting for SLT, yield should also increase. This tendency was in fact verified, with a predicted genetic gain of 14% in yield when selecting the best clones for SLT.

Results showed that there was significant genetic variability within the variety for all the abiotic stress indicators, not just for SLT. However, the range of valuesobtained for the EBLUPs of genotypic effects for SLT between sensitive and tolerant genotypes was higher than that obtained for NDVI, probably as a result of NDVI's variety-specific characteristic [9], with very low range of values between genotypes. As for PRI and SPAD, the range of values obtained for the EBLUPs of genotypic effects for these traits were higher than for SLT, however, most genotypes presented values lower than the average of the variety, making selection based on them less effective than the one based on SLT. Furthermore, the selection for these traits did not result in increases of yield (data not shown).

The intra-varietal genetic variability for the abiotic stress indicators obtained in the Arinto clones can be used for the improvement of the ability of this variety to withstand stress conditions to better adapt to climate change. When selecting a superior group of genotypes for tolerance to abiotic stress using SLT, there is a significant increase in yield, while the quality characteristics of the must remain close to the mean of the variety.

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