Gonçalves E., Carrasquinho I., Martins A. 2020. A measure to evaluate the sensitivity to genotype-by-environment interaction in grapevine clones. *Australian Journal of Grape and Wine Research* 26(3): 259-270. https://doi.org/10.1111/ajgw.12432 <https://doi.org/10.1111/ajgw.12432>

A measure to evaluate the sensitivity to genotype-by-environment interaction in grapevine clones

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Short tittle: G×E interaction in grapevine clones

Abstract

Background and Aims: The development of an efficient clonal selection process requires the study of genotype-by-environment (G×E) interaction. This work aims to evaluate the variability of the G×E interaction among genotypes and to identify the less sensitive ones.

Methods and Results: The approach involves the fitting of mixed models to yield data taking into account the correlation induced by the repeated measurements of the same plot over the years. A measure for comparative evaluation of the G×E interaction among genotypes is proposed (Interaction Sensitivity, *IS*), based on the variance of the values of the empirical best linear unbiased predictors of G×E interaction effects across environments. In all cases studied significant G×E interaction variability was found, and the proposed measure to rank the sensitivity to G×E interaction varied widely among genotypes.

Conclusions: The existence of a common contribution shared by all observations made in the same plot was detected, independently of the lag between years. The proposed measure to rank the sensitivity to G×E interaction permitted identification of stable genotypes.

Significance of the Study: This work studied G×E interaction problem in the context of grapevine and proposes a measure for the comparative evaluation of the G×E interaction among genotypes.

Keywords: *clonal selection, G×E interaction, grapevine, multi-environmental trial, mixed models*

Introduction

Grapevine (*Vitis vinifera* L.) is one of the most important crops in the Mediterranean region and it is grown in many countries worldwide. As for any crop the genetic quality of the propagated materials is of utmost importance. For an ancient variety, the selection methodology currently used in Portugal by the Portuguese Association for Grapevine Diversity (PORVID) consists of three steps (Martins and Gonçalves 2015). The first is a random prospection of plants in old vineyards of the variety's main growing regions with the objective of obtaining a representative sample of the intravariety genetic diversity. To each single plant (genotype) prospected a code is given and it is vegetatively propagated originating a clone (a set of genetically identical plants). The second step is the planting of a large field trial according to an adequate experimental design using that sample (i.e. hundreds of genotypes/clones of the variety under selection) to quantify genetic diversity within the variety and perform selection of groups of superior genotypes (usually the selection of the top ranked 7–20 clones according to the target traits for the variety, polyclonal selection). This polyclonal selected material becomes available for new plantings. The third step of the methodology is implemented when the objective is to undertake clonal selection. It consists of the selection of a superior group of about 30–40 clones from the previous stage, in the establishment of several field trials in different locations, and in the evaluation during several years (usually two–four locations, if possible during 5–8 years). The main concern is to select individual clones which ideally present simultaneously good performance for the target traits and low sensitivity to genotype by environment (G×E) interaction. The development of an efficient clonal selection process requires knowledge about this type of interaction. Despite major advances concerning G×E analysis in plant breeding programs, such developments have not been routinely adopted in most of the grapevine clonal selection programs worldwide.

The phenotypic value of an individual for a given trait is controlled by its genotypic effect, the environmental effect and the G×E interaction effect. Such interaction exists when the comparative performance of genotypes vary according to the environment. Lynch and Walsh (1998) consider the G×E interaction can comprise two major types: (i) rank-change interaction, wherein genotypes are ranked in different orders in different environments (crossover interaction); and (ii) level-of-expression interaction, wherein the expression of genotypic differences varies across environments, but not necessarily with any change in the order of the genotype rankings. For selection purposes, rank-change interaction will generally be of greater interest to study (Li et al. 2017). The objective of the breeder is often to address G×E interaction either by selecting stable genotypes that are not sensitive to environmental changes, or by selecting genotypes for specific environments. According to Lynch and Walsh (1998) spatial aspects of the environment (such as location) tend to contain more predictable features than temporal aspects (such as yearly variation). Thus, breeders have to face two competing tasks. First, if there are different mega-environments (group of locations within which only modest G×E interaction occurs), genotypes that are widely adaptive within each mega-environment can often be found and selected. Second, if the environment has significant unpredictable components (such as year-to-year variation), G×E interaction cannot be exploited and, as an alternative, the breeder must try to mitigate its effects, for example, by selecting genotypes which are more stable over environments. As Lynch and Walsh (1998) stressed, G×E interaction is highly context-specific: is almost inevitable if genotypes are studied in a sufficiently large set of environments; if genotypes are examined within a small and appropriate chosen set of environments, G×E may largely disappear.

The subject of G×E interaction has been a research focus among biometricians and quantitative geneticists since the early 1900s (Yan and Tinker 2006). With the idea that it is an undesirable phenomenon which confounds genotype evaluation, much work has been devoted to developing new methods to study it. In fact, there are numerous methods for studying G×E interaction. Probably the simpler methods and the most intuitive ones use nonparametric statistics, which are based on the idea that a genotype is stable over environments if its ranks are similar over environments (Nassar and Hühn 1987). Historically, however, the most widely used techniques were focused on the regression analysis of the observations of the genotype on environmental indices (Finlay and Wilkinson 1963), on the genetic correlations between environments (Falconer and Mackay 1996), and on the use of biplots for the interpretation of G×E interactions (Kempton 1984, Yan and Tinker 2006). The additive main effects and multiplicative interaction (AMMI) and the genotype main effects and interaction effects (GGE) are the two main biplot analysis methods (Gauch, 2006, Yan et al. 2007, Gauch et al. 2008, Yang et al. 2009). Both are based on ANOVA (treat the main and interaction effects as fixed effects) and principal component analysis (PCA). The difference between them is that GGE biplot analysis is based on an environment-centred PCA, whereas AMMI analysis refers to a double-centred PCA. Other common approaches to assess the magnitude of genotype-by-environment interaction are based on the theory of mixed models (Smith et al. 2005, Yang 2007). According to Smith et al. (2005), the advantages of the linear mixed models include the ease to handle incomplete data, the ability to use more realistic within-trial models for error variance and the ability to assume some sets of effects (e.g. variety and/or environment effects) to be random rather than fixed. Thus linear mixed models have become popular for the analysis of multi-environmental trials (MET) data. Among these

113 approaches, one of the most mentioned is the factor analytic (FA) models (Piepho 1998,
114 Burgueño et al. 2008, Cullis et al. 2014, Smith et al. 2015). According to these authors, those
115 models can provide a reliable parsimonious and holistic approach for estimation of genetic
116 correlations between all pairs of trials and provide a natural framework for modelling G×E
117 patterns in complex multi-environment experiments. The use of FA models in multi-
118 environment trials is based on the use of eigenvectors from PCA and extended to
119 accommodate both additive and non-additive effects. In this approach predicted genotypic
120 effects for each environment are obtained (Smith et al. 2015).

121 As mentioned above, G×E interaction analysis in plant breeding programs have long
122 been implemented worldwide, mainly in annual crops. Commonly, potential new varietys are
123 evaluated in a large number of designed field experiments that cover a range of geographic
124 locations and years. For example, in Australia over 600 trials are conducted annually by
125 National Variety Trials (NVT) and cover a range of crops including wheat, barley, canola,
126 chick peas, faba beans, field peas, lentils, lupins, oats and triticale (Smith et al. 2015). Under
127 the grapevine clonal selection context, the evaluation of G×E interaction is also a key point in
128 the selection process. However, studies related to G × E interaction in grapevine clones are
129 scarce. In Germany, Laidig et al. (2009) studied the performance of Riesling clones at 16
130 locations but with a highly unbalanced data structure and some locations without replications.
131 In Portugal, some approaches have been conducted including graphical representation of
132 clones' ranking over environments, calculation of the coefficient of variation of phenotypic
133 values of one genotype in different environments, computation of non-parametric rank
134 measures (Martins et al. 1998, Martins and Gonçalves 2015). Another approach was the
135 quantification of G×E interaction from the genetic correlation between environments

(Gonçalves et al. 2016) from the fitting of a linear mixed model assuming different genetic variances and different genetic correlations among sites, and using an unstructured structure for genetic and error covariance matrices. These authors separated the two causes of G×E interaction as Cooper et al. (1996) proposed: the heterogeneity of genetic variance between environments (i.e. interaction due to scale) and heterogeneity of correlation between environments (i.e. interaction due to crossover) which affects the ranking of genotypes and hence selection. Concretely, in the work conducted by Gonçalves et al. (2016) the G×E interaction was studied for different traits (yield, and probable alcohol and acidity of the must) using different locations and years within each location. The results showed the presence of G×E interaction for all the studied traits. The effect of the year was also a remarkable result. Data from different years within a site were not genetically more strongly correlated than data from different sites.

Despite all these efforts, other approaches should be developed for a better understanding and interpretation of the G×E interaction in grapevine clones. In this context, there are some hurdles to overcome. One problem is related with the difficulty of field experimentation with this perennial crop, which is time consuming and implies high costs. As a consequence, few locations are used (frequently two to four) but the same genotypes are evaluated during several years in the same location. Under such conditions, some methods seeking specific adaptation, such as GGE and AMMI, are rarely applied in the grapevine context. Therefore, the objective should be to select genotypes that ideally show stability (low environmental sensitivity) over environments instead of attempting to select for locally-adaptive genotypes.

This work attempts to frame the G × E interaction problem in the context of grapevine. The main purpose of this work is to develop an expedient measure to easily rank genotypes according to their sensitivity to G×E interaction in the studied environments. The objective is to provide another criterion, besides those related to general performance of yield and quality traits of the must, to support selection decisions, and to inform grapegrowers about G×E interaction of selected clones. The theoretical approach involves the fitting of mixed models. The paper is arranged as follows: (i) analysis of yield data based on mixed models that combines the information across locations and across years within the location, and accommodates correlation induced by the repeated measurements of the same plot along the years in the same location; the specific objective is to know if there is significant G×E interaction variability among the clones of the same variety in the final stage of selection; and (ii) the development of a statistical measure for comparative evaluation of the G×E interaction among genotypes in order to identify the less sensitive ones.

Materials and methods

Plant material

To validate the methodology proposed in this study to analyse G×E interaction, multi-environmental trials of four grapevine varietys were considered: Alvarinho, Antão Vaz, Aragonez and Síria. The genotypes evaluated in these trials were selected from a previous stage of selection according to the yield in the varietys Antão Vaz, Aragonez and Síria. All plants were free of grapevine leafroll associated virus type 3 and grapevine fanleaf virus.

All information about the field trials can be found in Tables 1 and 2. For each variety, trials in two–three locations were available and the same genotypes were grown in all trials. They were planted in the main growing regions of the varietys in Portugal, and for each location, wine region, soil texture, altitude, and climate conditions are described in Table 1. In all trials, the training system was a vertical shoot position and the pruning system was a bilateral Royat cordon system, except for the variety Alvarinho, which was a double cordon system (high and low) alternated. The trials were laid out as a randomised complete block design, and the number of repetitions, number of plants per plot (experimental unit), rootstock, year of grafting, and planting density of each trial are presented in Table 2. Several traits were measured in these experiments, but the one under consideration to exemplify the methodology proposed in this paper is the yield. For each location, yield data from 2–11 years were collected. During these years, this trait was evaluated in all replicates of each field trial. That is, the mass of the grapes of all plants in each plot of each genotype was taken and in the statistical analysis the mean yield of each plot (kg/plant) was used for each repetition of each genotype. Additionally, previously to G×E interaction analysis, a preliminary data analysis for each year in each field trial was conducted to assess the broad sense heritability (the

proportion of phenotypic variance explained by genotypic causes), and thus, to evaluate genotypic variance component associated to yield data in those trials. The high values observed for this genetic parameter indicate the suitability of these field experiments to perform selection (Table 2). For each variety, the years evaluated in each location and the mean yield and the coefficient of variation of the mean yield phenotypic values in each environment are presented in Table 3.

In order to analyse G×E interaction, it is desirable to have a sample of the possible growing conditions to which those genotypes could be exposed to. Thus, the specific location-year combination was considered an 'environment'. It covers the specific conditions of the location, such as edapho-climatic conditions, rootstock and cultural practices, and the unique climatic features of the year.

Statistical methods

Mixed models for the analysis of G×E interaction in grapevine clones. A multi-environment single stage analysis was performed. In matrix formulation, the general model can be described as follows:

$$\boldsymbol{Y} = \boldsymbol{X\beta} + \boldsymbol{Zu} + \boldsymbol{e},$$

$\boldsymbol{Y}_{(n\times 1)}$ is the random vector of observations (mean yield of each plot), ordered by location, environment (combination location/year) and plot within each environment;

$\boldsymbol{\beta}_{(p\times 1)}$ is the vector of fixed effects (includes the overall mean and the main effects of the environments);

$\boldsymbol{X}_{(n\times p)}$ is the design matrix of fixed effects**;**

$\boldsymbol{u}_{(q\times 1)}$ is the vector of random effects (includes the effects of the blocks nested in environment, the genotypic main effects and the genotype by environment interaction effects); $q = \sum_{i=1}^{r} q_i$, where q_i is the number of levels of random effects factor i and r the number of random effects factors studied;

$\boldsymbol{Z}_{(n\times q)}$ is the design matrix of random effects**;**

$\boldsymbol{e}_{(n\times 1)}$ is the vector of random errors.

The vectors $\boldsymbol{u}$ and $\boldsymbol{e}$ are assumed mutually independent with multivariate normal distribution with vector of mean values $\mathbf{0}_{(n\times 1)}$ and covariance matrices $\boldsymbol{G}_{(q\times q)}$ and $\boldsymbol{R}_{(n\times n)}$, respectively:

$$\boldsymbol{Cov}[\boldsymbol{u}, \boldsymbol{e}] = \mathbf{0}, \boldsymbol{u} \cap \boldsymbol{\mathcal{N}_q}(\mathbf{0}, \boldsymbol{G}), \boldsymbol{e} \cap \boldsymbol{\mathcal{N}_n}(\mathbf{0}, \boldsymbol{R}).$$

Consequently, the distribution of $\boldsymbol{Y}$ is multivariate normal with mean value $\boldsymbol{X\beta}$ and covariance matrix $\boldsymbol{V} = \boldsymbol{ZGZ^T} + \boldsymbol{R}$, where $\boldsymbol{Z^T}$ is the transpose of $\boldsymbol{Z}$: $\boldsymbol{Y} \sim \boldsymbol{\mathcal{N}_n}(\boldsymbol{X\beta}, \boldsymbol{V})$.

Concerning the vector of random effects $\boldsymbol{u}_{(q\times 1)}$, it takes the form $\boldsymbol{u} = \left(\boldsymbol{u_1^T}, \boldsymbol{u_2^T}, \cdots \boldsymbol{u_r^T}\right)^{\boldsymbol{T}}$ where each sub-vector corresponds to the random effects of each factor. For the vector of random effects of factor i the covariance matrix is defined as $Var[\boldsymbol{u_i}] = \boldsymbol{G_i} = \sigma_i^2 \, \boldsymbol{I}_{q_i}, \ \forall i = 1, \ldots, r,$ where $\boldsymbol{I}_{q_i}$ is the identity matrix of order q_i; and $Cov[\boldsymbol{u_i}, \boldsymbol{u_{i'}}] = \mathbf{0}, \forall i \neq i'$. Therefore the covariance matrix of vector $\boldsymbol{u}$ is defined as $\boldsymbol{G} = \bigoplus_{i=1}^{r} \boldsymbol{G_i}.$, where $\oplus$ is the direct sum of matrices.

Concerning the vector of random errors $\boldsymbol{e}_{(n\times 1)}$, the simplest way to treat the problem is to assume that the elements of this vector are independent and identically distributed random variables, that is, $\boldsymbol{R} = \sigma_e^2 \boldsymbol{I_n}$ (hereafter named as model IND). It considers that random errors associated with observations made in different years in the same plot are independent random variables. With grapevine, however, this basic assumption is violated due to the sequential nature of the data on each plot over the years in the same location.

Let us consider vector $\boldsymbol{e}_{(n\times 1)}$, with $n = \sum_{j=1}^{l} n_j$, where n_j is the number of observations in location j, ordered by environment and plot within each location, takes the form $\boldsymbol{e} = \left(\boldsymbol{e_1^T}, \boldsymbol{e_2^T}, \cdots \boldsymbol{e_l^T}\right)^{\boldsymbol{T}}$, where each sub-vector corresponds to the random errors for each location. For location j the error covariance matrix, $Var\left[\boldsymbol{e_j}\right] = \boldsymbol{R_j}$, $\forall\, j = 1, \cdots, l$ and $Cov\left[\boldsymbol{e}_j, \boldsymbol{e}_{j\prime}\right] = \boldsymbol{0}$, $\forall j \neq j'$. Therefore the covariance matrix of vector $\boldsymbol{e}$ is defined as $\boldsymbol{R} = \bigoplus_{j=1}^{l} \boldsymbol{R}_j$.

The next step was to define the structure for the error covariance matrix in each location, that is, the structure of $\boldsymbol{R}_j$. In the following approaches, in the same location random errors associated to different experimental units were assumed to be independent; consequently covariance different from zero was only assumed for measurements on the same experimental unit (plot).

In location j with p plots matrix $\boldsymbol{R}_j$ takes the form $\boldsymbol{R_j} = \boldsymbol{I}_p \otimes \sum_{e_j}$, where $\boldsymbol{I}_p$ is the identity matrix of order p, $\otimes$ is the Kronecker product. There are several options to characterise this phenomenon with an appropriate covariance structure $\sum_{e_j}$. The most general

and complex form for $\sum_{e_j}$ is a so-called unstructured matrix that involves separate error variances for each year and separate correlations for all pairs of years. The objective, however, is to choose among those that make sense in this biological context and to find a structure that fits data adequately but is as simple as possible. From the specificity of the grapevine, emerges the following most probable covariance structures: the compound symmetry and the first order autoregressive model (when years are consecutive).

The compound symmetry (hereafter named as model CS): $\sum_{e_j}$ is a matrix with diagonal elements $\sigma^2_{e_j}$ (the error variance for location j) and non-diagonal elements defined as $\sigma^2_{e_j}\rho$ (ρ is the correlation between pairs of observations in the same plot of location j). This structure is a parsimonious covariance model which specifies that measures at all years have the same variance, and that all pairs of measures on the same plot have the same correlation. The implication is that the only aspect of the covariance between repeated measures is due to the plot contribution, independently of the lag between years.

The other matrix that makes sense to consider, when the evaluated years are consecutive, is the first order auto-regressive matrix (hereafter named as model AR1). In this case, matrix $\sum_{e_j}$ has diagonal elements $\sigma^2_{e_j}$ and non-diagonal elements defined as $\sigma^2_{e_j}\rho^{|k-k\prime|}$, where $|k-k'|$ is the lag between year k and k'. This model specifies that measures at all years have the same variance and considers that correlation between observations in the same plot is a function of their lag in time: nearby observations tend to be more highly correlated than observations farther apart in time.

The covariance model parameters were estimated by residual maximum likelihood method (REML) (Patterson and Thompson 1971), with average information algorithm. For

nested models, models IND and CS, and IND and AR1, model selection was performed by conducting a residual likelihood ratio test (REMLRT). These models were also compared using the Akaike information (AIC), defined as minus twice the residual log likelihood plus twice the number of variance parameters. Comparison of non-nested models (models CS and AR1) was based only using AIC criterion. Lower values of this criterion correspond to a best model fit.

Linear mixed models above described were fitted in R (R Core Team 2018), package ASReml-R (Butler et al. 2018).

A measure to select genotypes with low sensitivity to G×E interaction. The G×E interaction was assessed directly by testing the null hypothesis if the G×E variance component is zero ($H_0: \sigma^2_{G\times \mathrm{E}} = 0 \; vs \; H_1: \sigma^2_{G\times E} > 0$) by a REML ratio test (REMLRT), comparing minus twice the residual log-likelihood obtained with the fitting of two models, one with the interaction term (full model) and the other without it (reduced model, null hypothesis). The intravariety genetic variability among the tested genotypes ($H_0: \sigma^2_G = 0 \; vs \; H_1: \sigma^2_G > 0$) was also tested using a REMLRT. Under the null hypothesis that defines that a variance component is zero, the asymptotic distribution of the REMLRT statistic is a 50:50 mixture of chi-square distributions with zero and one degrees of freedom (Self and Liang 1987).

With the estimated covariance matrices, through the mixed model equations, the empirical best linear unbiased estimators (EBLUEs) of the fixed effects and the best linear unbiased predictors (EBLUPs) of the random effects were obtained as follows (Henderson 1975, Searle et al. 1992):

302 $$\widetilde{\boldsymbol{u}}_{\boldsymbol{EBLUP}} = \widehat{\boldsymbol{G}}\boldsymbol{Z}^{\boldsymbol{T}}\widehat{\boldsymbol{V}}\left(\boldsymbol{y} - \boldsymbol{X}\widehat{\boldsymbol{\beta}}_{\boldsymbol{EBLUE}}\right), \text{ with } \boldsymbol{X}\widehat{\boldsymbol{\beta}}_{\boldsymbol{EBLUE}} = \boldsymbol{X}\left(\boldsymbol{X}^{\boldsymbol{T}}\widehat{\boldsymbol{V}}^{\boldsymbol{-1}}\boldsymbol{X}\right)^{-}\boldsymbol{X}^{\boldsymbol{T}}\widehat{\boldsymbol{V}}^{\boldsymbol{-1}}\mathbf{Y}.$$

303 Ideally, a breeder would prefer to select for genotypes with both high mean performance (high
304 EBLUPs of genotypic effects) for the target traits and low sensitivity to G×E interaction (i.e.
305 increased stability in performance over environments, which means EBLUPs of G×E
306 interaction close to zero). Once rejected the null hypothesis for the G×E interaction, the study
307 was focused on the EBLUPs of G×E interaction effects ($EBLUP_{G\times E}$).
308 The EBLUPs of the G×E interaction effects depend on the variance components estimates.
309 When the estimated variance $\sigma^2_{G\times E}$ is zero, the EBLUPs of G×E are all zero; when the $\hat{\sigma}^2_{G\times E}$
310 is higher than zero, not all the EBLUPs of the interaction are zero. For each clone there are as
311 many EBLUPs of the interaction as the number of evaluated environments. Desiring that all
312 these EBLUPS are close to zero and knowing that the mean of the EBLUPs converges to zero
313 (Searle et al. 1992), then the variance of the EBLUPs of the effects of the interaction of a clone
314 will be a measure of its sensitivity to G × E interaction. But the meaning of the values of these
315 effects depends on the yield mean of the environment, therefore, it is desirable to define the
316 $EBLUP_{G\times E}$ for the genotype i in the environment k as the proportion of the yield mean of the
317 environment k ($EBLUP_{G_i\times E_k}(\%)$**)**:

318 $$EBLUP_{G_i\times E_k}(\%) = \left(EBLUP_{G_i\times E_k}/Overall\ mean\ environment\ k\right) \times 100.$$

319 The variance of the values $EBLUP_{G_i\times E_k}(\%)$ across the a environments studied is the
320 measure proposed to evaluate sensitivity of the genotype i to G × E interaction, hereafter
321 named as Interaction Sensitivity (*IS*):

322 $$IS = \frac{\sum_{k=1}^{a}\left(EBLUP_{G_i\times E_k}\% - \overline{EBLUP_{G\times E}(\%)}\right)^2}{a-1},$$

where $\overline{EBLUP_{G\times E}(\%)}$ is the mean of the values $EBLUP_{G_i\times E_k}(\%)$ across the a environments for the genotype i, which will be close to zero. The lower the value of IS, the lower the sensitivity of the genotype to the G×E interaction. Calculating the IS for each genotype it will be possible to select the less sensitive ones. In this analysis the inference to other environments will be weaker as the number and diversity of studied environments is lower.

Results

Models for the analysis of G×E interaction in grapevine clones

For the four varietys studied, the results to identify the best structure of the covariance matrix of the vector of random errors (matrix R) are shown in Tables 4, 5 and 6. In all studied varietys, models CS and AR1 were better than the model considering independent errors among observations of the same plot (IND). The latter always revealed higher values for AIC. This conclusion was also supported by the results obtained with the REML ratio test comparing models IND and CS and models IND and AR1. In either case, the result of the REMLRT was the rejection of the model IND for any usual significance level. Comparing models CS and AR1, lower values for AIC were observed for model CS in all the studied cases (Table 4), thus, CS model always revealed a better fit.

The estimates of the covariance parameters are illustrated in Table 5. Error variance heterogeneity among locations was observed from the fitting of models CS and AR1 (this can be seen through the values of the random errors variance component estimates for each location, $\hat{\sigma}^2_{eL.}$). It changed according to the varietys and was higher for Alvarinho and Antão Vaz. Additionally, depending on the varietys and location, low to moderate correlations

among observations of the same plot ($\hat{\rho}_{L.}$) were found. With a time lag of 5 or more years, the correlation between observations were approximately zero according to model AR1. The higher correlations among observations of the same plot were observed for Alvarinho in location L1, and Antão Vaz in location L2 (0.423 and 0.357, respectively). With regard to genotypic and block within environment variance components estimates, lower values were found for models CS and AR1 than for model IND. The opposite was observed for the G×E interaction variance component estimate, accomplished by an increase in its precision (higher value for the ratio $\hat{\sigma}^2_{G\times E}$/SE). With the fitting of models CS and AR1, significant G×E interaction variability was found (rejection of hypothesis $H_0: \sigma^2_{G\times \mathrm{E}} = 0$, P<0.05) for all studied cases (Table 6). The difference between residual log-likelihood of the models with and without interaction effects was higher in models CS and AR1, resulting in a high value for the REMLRT test statistic.

To sum up, variability concerning G×E interaction was detected for all the studied cases, genotypic variability was also significant (P<0.05), except for Antão Vaz (Table 6).

A measure to identify genotypes with low sensitivity to G×E interaction: interaction sensitivity (IS)

The EBLUPs of G×E interaction effects resulting from model CS (the best covariance structure for the matrix R for all varietys) were used to study the sensitivity to G×E interaction. The results obtained for the Interaction Sensitivity (IS) and for the predicted genotypic yield performance are provided in Table 7. The differences observed between the lowest and the highest values for *IS* demonstrate that this measure permits to differentiate the behaviour of

clones concerning their sensitivity to G×E interaction. For example, for Alvarinho, the highest value for IS was about 96 times superior to the lowest value (ranged from 52.45 to 5067.15). The genotypic predicted yield varied from 4.29 kg/plant to 7.64 kg/plant, but the three genotypes that revealed higher G×E sensitivity were the same that showed the lower yield predicted genotypic performance. The less sensitive genotypes revealed an average yield performance.

The differences between the maximum and minimum IS values for the clones of the other varietys were lower than the range observed for Alvarinho, however, the highest values for IS were about 10 times superior to the lowest values. The range for the yield predicted genotypic values for the other varietys studied was also lower. The predicted genotypic values among the tested genotypes ranged only from 3.48 kg/plant to 3.83kg/plant for Antão Vaz, from 2.89 kg/plant to 3.95 kg/plant for Aragonez, and from 2.51 kg/plant to 3.31 kg/plant for Síria. For these varietys, the less sensitive genotypes to G×E interaction usually revealed a mean yield performance (Table 7). The complete information about *IS*, predicted genotypic values, EBLUPs of the genotypic effects, and EBLUPs of the G×E interaction effects over the studied environments for all studied genotypes is provided in supporting information (Tables S1–S4).

For the four varietys studied, the EBLUPs of the effects of the G×E interaction as the proportion of the environment mean **(**$EBLUP_{G\times E}(\%)$**)** for the clones with the lowest and the highest IS values are represented in Figures 1– 4. In these figures the overall yield mean obtained for each environment is also presented. It should be noted that $EBLUP_{G\times E}(\%)$ of the clones with the lowest IS values were closer to zero. This means that those clones revealed

389 less sensitivity to G×E interaction. An opposite behaviour was observed for the clones that
390 showed the highest IS values. In this latter case, the oscillation around zero of $EBLUP_{G\times E}$%
391 increased.

392 In more detail, in variety Alvarinho (Figure 1), the genotype with the highest *IS* value
393 (AI35) showed high positive G×E interaction effects of the yield in several environments,
394 with EBLUPs of the G×E interaction effects higher than 30% of the yield mean of the
395 respective environments. A peak of 306% higher than the yield mean of the environment was
396 reached in L3-1994. This means that this genotype reacted better in these environments than
397 in the other ones, but this increasing in yield due G×E interaction does not mean that it had
398 the best yield among the genotypes studied. In fact, this genotype showed the lowest genotypic
399 mean yield performance (Table 7). In other environments, however, the same genotype
400 showed negative G×E interaction effects of the yield, less 30% of the mean of the
401 environments. Therefore, this means that it reacted worse in these environments than in the
402 others. Those variations resulted in a high value for *IS*. For the clone that showed the lowest
403 IS value (AI1), the variation of EBLUPs of the G×E interaction effects around zero was
404 smaller, ranging from -13.2% to +9.3% of the mean yield of the environments. The same
405 behaviour was also found in the genotypes of Antão Vaz variety (Figure 2). In this case, in
406 the clone that showed the highest value of *IS* (AN40), the variation of EBLUPs of the G×E
407 interaction effects ranged from -8.2 to +33.5% of the mean yield of the environments, whereas
408 for the clone with the lowest *IS* (AN1), it varied from -4.8 to +6.3%. For the genotypes of
409 Aragonez variety (Figure 3), the one that showed the highest value of *IS* (RZ40), the variation
410 of EBLUPs of the G×E interaction effects ranged from -19 to +10% of the mean yield of the

environments, whereas for the clone with the lowest *IS* (RZ1), it varied from -5.6 to +3.0%. Finally, for Síria variety (Figure 4), the genotype that showed the highest value of *IS* (CR40) presented a range of EBLUPs of the G×E interaction effects from -11% to +30.5% of the mean yield of the environments, whereas for the one with the lowest *IS* (CR1), it varied from -7.5% to +4.6%.

Importantly, for the four varietys studied, there was no relation between positive or negative effects of the G×E interaction and the overall yield mean of the environment (Figures 1–4). That is, it cannot be said that negative effects of G×E interaction always occur in 'poor' environments (with low overall mean yield) nor positive effects in 'good' environments (with high overall mean yield), or vice-versa. Similarly, it cannot be inferred that negative or positive effects of G×E interaction are dependent on the climate conditions. This latter finding is drawn from the results of Antão Vaz, Aragonez and Síria varietys (Table 1, Figures 2–4). In fact, for these field trials the temperature and precipitation varied according to location (Table 1 and Figures 2–4) and no pattern associated to the signal of G×E interaction effects among locations was detected. This type of variation is undesirable because it reveals the inconsistency of the genotype. Additionally, no systematic signal differences of G×E interaction effects and location were found. That is, over years in each location both genotypes under analysis reacted with positive and negative G×E interaction effects and, thus, no systematic behaviour was observed in each location (namely, all years with negative effects or positive effects). This finding reinforces the idea of unpredictable behaviour of a genotype. This is the most undesirable interaction, therefore genotypes revealing the highest values of *IS* should not be selected.

Discussion

Numerous methodologies are used worldwide to study G×E interaction in plant breeding. However, they are not currently and appropriately applied to grapevine clonal selection, particularly those techniques that search for specific adaptation, such as AMMI and GGE (Gauch 2006, Yan et al. 2007, Gauch et al. 2008, Yang et al. 2009). These practices are focused on performing mega-environment analysis, that is, to define a group of locations that consistently share the best set of genotypes across years. For this purpose the same set of genotypes is tested at the same set of test locations across multiple years (Yan et al. 2007). In viticulture, however, grapevine clones are studied only in a few locations and wine regions. Therefore, it is difficult to perform an analysis which recommends clones for a specific region. For example, in the practical examples handled in this work, which reflect what is usually done in grapevine clonal selection trials, the available number of locations and the number of trials in each one, do not permit to define specific adaptation. In fact, the genotypes of Alvarinho were studied in three different trials in one location (Monção); iwith Aragonez and Síria, the genotypes were evaluated in two locations, but only one trial in each one was planted; and, for Antão Vaz three locations were considered with only one trial in each one. But, as multiple years in each trial were evaluated, the most of the environmental contribution to G×E studied is unpredictable, such as year-to-year variation (for example, average temperature or rainfall during a growing season). As Lynch and Walsh (1998) mentioned, under such conditions, the best approach is to attempt to average performance of the genotype and to select for stability. The methodology proposed in this study responds precisely to this strategy: (i) permits the prediction of the genotypic effect for each clone at a global level of the environments; (ii) permits the prediction of the G×E interaction deviations for each clone

457 per environment; and (iii) takes into account that the random errors associated with
458 observations among different years in the same plot are correlated.

459 Considering the context of grapevine, the repeated measurements (yearly yield
460 observations) occur at a long enough interval so that a correlation close to zero relative to
461 other variation could be acceptable and, thus, the covariance structure IND could be
462 acceptable too. However, this study showed that, even with a low level of correlation among
463 repeated measurements, CS and AR1 models were always better than IND. Comparing CS
464 and AR1 models, an advantage of CS over AR1 was observed. Therefore, the existence of a
465 common contribution, such as the soil, radicular structure, shared by all observations made
466 in the same plot was detected, independently of the lag between years. Additionally, the
467 correlation among repeated measurements varied according to location, which can be
468 explained by the specific edapho-climatic conditions of each one. Importantly, the CS model
469 showed advantages for the study of G×E interaction, which is the key issue of the current
470 study. Indeed, regarding the estimates of the parameters obtained for the different fitted
471 models, in general the G×E variance component estimate increased with CS model and as
472 well as the ratio $\hat{\sigma}^2_{G\times E}$ /SE, which reveals an increase in the precision of this estimate.
473 Consequently, with the fitting of CS model, a higher precision in the prediction of the EBLUPs
474 of the effects of G×E interaction was also observed. On the other hand, the genotypic variance
475 component estimate obtained with the fitting of the latter model was lower, because the part
476 of this component resulting from scale differences was taken into account by the heterogeneity
477 variances assumed in R matrix. Considering other perennial crops, Piepho and Eckl (2014)
478 analysed ryegrass trials with 3 harvest years and found similar results for AR1 and CS models.

Still regarding the statistical methodology, a model that included the effects of the location (L) and the effects of the year nested in location (Y) could have been fitted in. In this way, the variability of G×E interaction could have been separated into G×L and G×Y interactions. This approach, however, was not followed. In previous studies conducted in grapevine clonal selection trials, differences between genotype-by-location and genotype-by-year within location interactions were not found (Gonçalves et al. 2016). On the other hand, in the context of grapevine clonal selection trials, the number of locations and years are few, and the estimation of G×L and G×Y variance components would be problematic. For this reason, the study was focused on a global level of the environments (each one including the effects of the local, year, cultural practices, and rootstock). As a result, a higher number of environments is achieved and a more accurate and precise estimate for the G×E variance component is obtained. This last issue is of the utmost importance in the context of this study because the measure proposed, Interaction Sensitivity (*IS*), is based on the EBLUPs of the G×E effects. In this case, the rankings of the predicted G×E interaction effects are required to be as close as possible to the rankings of the true effects. And, according to Searle et al. (1992), the estimates of the variance parameters have to be sufficiently precise to ensure that the optimality of BLUP is maintained with EBLUP. Additionally, if the effects of the location and the effects of the year nested in location are not separated in the analysis, the most correct approach is to select for stability, which is precisely the objective of the proposed measure. In this sense, *IS* is unbiased regarding these two components of interaction because it evaluates the overall genotype sensitivity to G×E interaction.

The measure proposed in this study, Interaction Sensitivity, to rank the sensitivity of clones to G×E interaction is expeditious and showed a wide range of variation among

genotypes, which reveals its ability to differentiate the genotypes concerning their sensitivity to G×E interaction. It should be noted, however, that there is no guarantee that genotypes with lower values for this measure could not exhibit an unexpected behaviour in a new environment. There is always the uncertainty linked to the cultivation of genetically homogeneous material. This type of behaviour was clearly observed through the analysis of Figures 1–4. Likewise, we will not be able to infer the results obtained in the studied environments to other climatic conditions. However, clones that show a more stable behaviour in the studied environments may tend to be more indifferent to new environmental conditions. The issue of the extrapolation of the results obtained from the environments studied to other environments is also dependent from the sample of the environments studied. As Lynch and Walsh (1998) mentioned, G×E interaction is almost inevitable if genotypes are studied in a sufficiently large set of environments; if genotypes are examined within a small and appropriate chosen set of environments, G×E may largely disappear.

Although the main objective at this stage of selection is to select for low sensitivity to G×E interaction, it is also important to match this information with the performance of the genotype to support the final selection decision. Ideally, a breeder wants to find genotypes which present simultaneously good performance for the target traits and low sensitivity to G×E interaction. Considering the data analysed in this study, what is desirable is to have genotypes with high EBLUPs of yield genotypic effects and EBLUPs of G×E interaction effects close to zero (which is reflected in a lower *IS*). The achievement of such objective will depend on the genetic diversity among the evaluated genotypes and the selection criteria used in the previous selection cycle. For example, if no significant yield genetic variability is found among the studied genotypes, the selection criterion should be based only on the lower

sensitivity to G×E interaction. In contrast, if significant genetic variability is found, genotypes with high EBLUPs of yield genotypic effects and low *IS* values, and genotypes with high EBLUPs of yield genotypic effects and high *IS* values can be found. In this latter case, it means that the genotype may be excellent in some environments and in others that might not happen (the genotype effect does not always overlap the negative effect of interaction).

The cases studied in this work exemplify the considerations previously made. For example, a narrow range of the yield predicted genotypic values was found among the genotypes studied in the varietys Antão Vaz, Aragonez and Síria, which is justified because those genotypes were selected from a previous stage according to the yield and its stability across years. In fact, in the Antão Vaz variety, the yield genetic variance found among the 40 studied genotypes was not significant. In these circumstances, the selection criterion should be based only on the sensitivity to G×E interaction. For Aragonez and Síria, although the null hypothesis $\sigma_G^2 = 0$ has been rejected, the main selection criterion should also be focused on the lower sensitivity to G×E interaction, given the narrow yield range observed among genotypes. For Aragonez, however, genotypes with high predicted genotypic values are among the genotypes with the least sensitivity (RZ3, RZ4, RZ9) (Table 7).

For Alvarinho the conditions were different. In Portugal, this variety has a high natural frequency of occurrence of grapevine leafroll associated virus type 3. Thus, the selection criterion from the previous stage was based on the condition to be free for this virus. As a result, the genotypic predicted yield differences found in the studied trials were higher. In this case, the three genotypes which are furthest from the mean yield (with the lowest EBLUPs of the genotypic effects) are those with higher sensitivity to G×E interaction. Several genotypes with the highest EBLUPs of the genotypic effects are ranked for IS from AI26 to AI32,

548 revealing sensitivity to G×E interaction (Tables 7, S1). As a consequence, their selection
549 should be viewed with caution and, above all, if selected, the information about their
550 sensitivity to G×E interaction should be provided to grapegrowers.

551 It should be highlighted that, in grapevine, G×E interaction is also found for other
552 important traits, for example, in compositional traits of the must, and the degree of G×E
553 interaction depends on the trait and variety (Gonçalves et al. 2016). For example, in the
554 aforementioned study, the highest G×E interaction was found for the yield in the case of
555 Fernão Pires variety, and for acidity in the case of the varietys Malvasia Fina and Rabo de
556 Ovelha. As a result, once detected G×E interaction, the EBLUPS of the effects of G×E
557 interaction for all the traits studied can be used to apply the proposed measure of interaction
558 sensitivity, and for each trait each genotype has an *IS* value. Hence, besides the criteria related
559 to general performances of yield and quality traits of the must, the *IS* for each trait should be
560 taken into account for final selection decisions. Usually, the final selection tries to prioritise
561 the most important traits of each variety, looking for genotypes that minimise the weaknesses
562 of the variety under selection. In practice, a table summarising the ranks of both *IS* and
563 EBLUPS of genotypic effects of the clones for the several traits evaluated should be the basis
564 for clonal selection. Alternatively, a selection index comprising all the previous information
565 could also be constructed. And, most importantly, the information about the sensitivity to G×E
566 interaction of the selected clones should be provided to grapegrowers.

567 In summary, the study of G×E interaction in grapevine clones should be strongly
568 implemented. In fact, it is inappropriate to study a clone in only one specific region taking
569 into account that it will be grown in other regions or even other countries. Finally, this new

approach for the study of G×E interaction in grapevine clones can also be applied to other perennial species.

Conclusions

Nowadays clonal materials are widely used worldwide to plant new vineyards. However, the clone is genetically homogenous, therefore it is likely to be sensitive to G×E interaction.

In order to implement a successful grapevine clonal selection, a multi-environmental trial should be conducted to provide information to grapegrowers about the sensitivity to G×E interaction of the available clones for planting new vineyards. The methodology proposed in this work to study G×E interaction is adapted to the context of grapevine and other perennial crops usually studied in few locations during several years. The existence of correlation among observations made in the same plot was detected, independently of the lag between years.

When using the proposed measure to evaluate the sensitivity to G×E interaction, differences among genotypes were found. This demonstrates the usefulness of this measure as an additional tool in grapevine clonal selection.

Acknowledgments

The authors would like to acknowledge the colleagues of National Network for Grapevine Selection for their help in management of field experiments and data collection. The research was funded by Fundação para a Ciência e Tecnologia (FCT) through LEAF funding (UID/AGR/04129/2013).

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Table 1. Description of the field trials of the four grapevine varietys studied.

Variety	Location	Portuguese wine region	Soil texture	Altitude (m)	Precipitation (mm)†	Tmax (°C)†	Tmin (°C)†
Alvarinho	Monção (A-L1)	VinhoVerde	Sandy loam	81	1465.7	27.5	4.1
	Monção - Pias (A-L2)	Vinho Verde	Sandy loam	78	1465.7	27.5	4.1
	Monção - Ceivães (A-L3)	Vinho Verde	Sandy	91	1465.7	27.5	4.1
Antão Vaz	Évora (AN-L1)	Alentejo	Slaty	259	609.4	30.2	5.8
	Palmela (AN-L2)	Península de Setúbal	Sandy	21	715.9	29.5	4.7
	Vidigueira (AN-L3)	Alentejo	Clayey	177	571.8	32.8	5.3
Aragonez	Estremoz (RZ-L1)	Alentejo	Clayey	506	609.4	30.2	5.8
	Tabuaço (RZ-L2)	Douro	Clayey	254	1073.7	28.7	2.1
Síria	Estremoz (CR-L1)	Alentejo	Clayey	506	609.4	30.2	5.8
	Pinhel (CR-L2)	Beira Interior	Sandy	590	882	24.6	1.2

†Source: http://www.ipma.pt/pt/oclima/normais.clima/ (accessed 1-12-2019). Tmax, average maximum air temperature of the hottest month (°C); Tmin, average minimum air temperature of the coldest month (°C) over a period of 30 years (1971–2000).

Table 2. Viticultural description of the field trials of the four grapevine varietys studied

Variety	Location	Rootstock†	Year of grafting	Planting density (m)	RCBD Ngen/Nrep/Nplant	H^2‡	Number of environments§
Alvarinho	Monção (A-L1)	1103P	1988	3.0 × 1.25	35 / 3 / 5	0.699	20
	Monção - Pias (A-L2)	SO4	1992	3.0 × 1.25	35 / 9 / 3	0.872	
	Monção - Ceivães (A-L3)	196/17	1993	3.0 × 1.25	35 / 9 / 4	0.856	
Antão Vaz	Évora (AN-L1)	99R	1986	3.0 × 1.20	40 / 5 / 5	0.688	14
	Palmela (AN-L2)	1103P	1991	2.80 × 1.20	40 / 8 / 7	0.432	
	Vidigueira (AN-L3)	140RU	1993	3.0 × 1.20	40 / 8 / 7	0.586	
Aragonez	Estremoz (RZ-L1)	99R	1990	3.0 × 1.10	40 /8 / 6	0.738	13
	Tabuaço (RZ-L2)	1103P	1991	2.50 × 1.10	40 /8 / 7	0.514	
Síria	Estremoz (CR-L1)	99R	1990	3.0 × 1.10	40 /8 / 6	0.899	10
	Pinhel (CR-L2)	99R	1986	2.50 × 1.10	40 /5 / 4	0.777	

†For each trial, a single clone for the rootstock; ‡ maximum value of broad sense heritability for the yield observed in each location; § total number of studied environments (combination location-year). Ngen, number of genotypes per variety; Nplant, number of plants per plot; Nrep, number of replicates; RCBD, randomised complete block design.

Table 3. Environmental code and the mean yield of the four varietys obtained in each environment.

Variety	Environmental code†	Mean yield (SE) (kg/plant)	CV (%)‡
Alvarinho	AI-L1-1990	2.433 (0.112)	27.34
	AI-L1-1991	4.380 (0.102)	13.80
	AI-L1-1992	3.373 (0.155)	27.12
	AI-L2-1995	1.662 (0.060)	21.47
	AI-L2-1996	11.372 (0.321)	16.69
	AI-L2-1997	7.406 (0.195)	15.54
	AI-L2-1998	6.230 (0.263)	24.93
	AI-L2-1999	15.565 (0.355)	13.48
	AI-L2-2001	14.780 (0.544)	21.77
	AI-L3-1994	0.587 (0.031)	31.32
	AI-L3-1995	3.035 (0.149)	29.06
	AI-L3-1996	3.595 (0.177)	29.17
	AI-L3-1997	4.592 (0.158)	20.39
	AI-L3-1998	1.991 (0.121)	36.01
	AI-L3-1999	7.485 (0.340)	26.88
	AI-L3-2000	3.853 (0.236)	35.51
	AI-L3-2001	8.766 (0.360)	24.07
	AI-L3-2002	7.156 (0.417)	34.09
	AI-L3-2003	6.804 (0.341)	29.35
	AI-L3-2004	13.085 (0.546)	24.53
Antão Vaz	AN-L1-1988	1.756 (0.048)	17.34
	AN-L1-1989	1.860 (0.057)	19.49
	AN-L1-1990	8.010 (0.114)	9.02
	AN-L2-1993	1.552 (0.050)	20.41
	AN-L2-1994	2.638 (0.068)	16.21
	AN-L2-1995	4.520 (0.103)	14.46
	AN-L2-1996	6.687 (0.167)	15.84
	AN-L2-1997	3.260 (0.086)	16.67
	AN-L2-1998	6.555 (0.135)	13.08
	AN-L3-1998	3.553 (0.078)	13.92
	AN-L3-1999	3.253 (0.070)	13.67
	AN-L3-2000	3.401 (0.080)	14.87
	AN-L3-2001	1.834 (0.072)	24.91
	AN-L3-2002	2.532 (0.096)	24.01
Aragonez	RZ-L1-1992	2.679 (0.039)	9.15
	RZ-L1-1993	4.088 (0.070)	10.80
	RZ-L1-1994	2.056 (0.058)	17.84
	RZ-L1-1995	4.720 (0.070)	9.40
	RZ-L1-1996	6.807 (0.080)	7.42
	RZ-L1-1997	5.819 (0.116)	12.60
	RZ-L1-1998	1.182 (0.036)	19.13
	RZ-L1-1999	5.559 (0.091)	10.36
	RZ-L2-1993	2.277 (0.046)	12.69
	RZ-L2-1994	2.378 (0.064)	17.04
	RZ-L2-1996	4.845 (0.090)	11.73
	RZ-L2-1997	2.027 (0.057)	17.91
	RZ-L2-1998	1.819 (0.050)	17.56
Síria	CR-L1-1992	2.674 (0.065)	15.25
	CR-L1-1993	1.958 (0.050)	16.11
	CR-L1-1994	1.584 (0.058)	23.20
	CR-L1-1995	4.630 (0.097)	13.31
	CR-L1-1996	5.844 (0.125)	13.50
	CR-L1-1997	3.471 (0.086)	15.76
	CR-L1-1998	1.308 (0.054)	25.90
	CR-L1-1999	2.444 (0.083)	21.39
	CR-L2-1988	3.224 (0.060)	11.69
	CR-L2-1989	2.037 (0.056)	17.45

† Combination of the location and year; ‡ coefficient of variation (CV) of the mean yield phenotypic values.

Table 4. Comparison of the three models fitted to yield data of the four grapevine varieties studied

Variety	Model	lr	npar	AIC	REMLRT (*P*-value)
Alvarinho	IND	-5509.5	4	11027.0	
	CS	-5202.5	9	10423.0	614.0 (<0.001)
	AR1	-5224.9	9	10467.8	569.2 (<0.001)
Antão Vaz	IND	-3506.1	4	7020.2	
	CS	-3155.9	9	6329.9	700.4 (<0.001)
	AR1	-3167.7	9	6353.3	676.9(<0.001)
Aragonez	IND	-1820.8	4	3649.6	
	CS	-1573.4	7	3160.9	494.7 (<0.001)
	AR1	-1711.0	7	3435.9	219.6 (<0.001)
Síria	IND	-1303.9	4	2615.9	
	CS	-1250.1	7	2514.2	107.7 (<0.001)
	AR1	-1285.7	7	2585.5	36.4 (<0.001)

Residual log-likelihood (lr), number of covariance parameters (npar), Akaike information criterion (AIC) obtained from the fitting of the models with matrix diagonal (IND), compound symmetry (CS) and first order autoregressive (AR1), and residual likelihood ratio test (REMLRT) for nested models IND and CS, and IND and AR1.

Table 5. Covariance parameters estimates (and respective standard errors (SE)) obtained from the fitting of the models IND, CS and AR1.:

Model	Covariance parameters estimates	Variety			
		Alvarinho	**Antão Vaz**	**Aragonez**	**Síria**
IND	$\hat{\sigma}_G^2$ (SE)	0.936 (0.246)	0.041 (0.015)	0.054 (0.015)	0.056 (0.017)
	$\hat{\sigma}_b^2$ (SE)	0.233 (0.046)	0.506 (0.083)	0.177 (0.029)	0.145 (0.029)
	$\hat{\sigma}_{G\times E}^2$ (SE)	0.883 (0.090)	0.072 (0.021)	0.046 (0.010)	0.084 (0.014)
	$\hat{\sigma}_e^2$ (SE)	3.684 (0.087)	1.830 (0.044)	0.775 (0.018)	0.757 (0.021)
CS	$\hat{\sigma}_G^2$ (SE)	0.797 (0.215)	0.013 (0.012)	0.037 (0.014)	0.040 (0.016)
	$\hat{\sigma}_b^2$ (SE)	0.161 (0.037)	0.468 (0.078)	0.164 (0.027)	0.138 (0.028)
	$\hat{\sigma}_{G\times E}^2$ (SE)	0.808 (0.077)	0.100 (0.018)	0.065 (0.009)	0.093(0.014)
	$\hat{\sigma}_{eL1}^2$ (SE)	1.251 (0.139)	1.842 (0.112)	0.819 (0.030)	0.741 (0.024)
	$\hat{\rho}_{L1}$ (SE)	0.423 (0.072)	0.089 (0.046)	0.273 (0.024)	0.142 (0.020)
	$\hat{\sigma}_{eL2}^2$ (SE)	5.862 (0.223)	2.496 (0.108)	0.702 (0.030)	0.875 (0.067)
	$\hat{\rho}_{L2}$ (SE)	0.087 (0.023)	0.357 (0.027)	0.284 (0.029)	0.110 (0.075)
	$\hat{\sigma}_{eL3}^2$ (SE)	2.571 (0.092)	1.026 (0.042)		
	$\hat{\rho}_{L3}$ (SE)	0.189 (0.023)	0.190 (0.027)		
AR1	$\hat{\sigma}_G^2$ (SE)	0.842 (0.224)	0.019 (0.012)	0.048 (0.014)	0.054 (0.017)
	$\hat{\sigma}_b^2$ (SE)	0.168 (0.038)	0.464 (0.077)	0.170 (0.028)	0.144 (0.029)
	$\hat{\sigma}_{G\times E}^2$ (SE)	0.805 (0.078)	0.091 (0.017)	0.058 (0.009)	0.090 (0.014)
	$\hat{\sigma}_{eL1}^2$ (SE)	1.247 (0.130)	1.850 (0.113)	0.813 (0.025)	0.735 (0.022)
	$\hat{\rho}_{L1}$ (SE)	0.394 (0.066)	0.106 (0.055)	0.232 (0.019)	0.120 (0.021)
	$\hat{\sigma}_{eL2}^2$ (SE)	5.840 (0.219)	2.479 (0.096)	0.697 (0.027)	0.882 (0.067)
	$\hat{\rho}_{L2}$ (SE)	0.056 (0.028)	0.438 (0.020)	0.271 (0.024)	0.116 (0.075)
	$\hat{\sigma}_{eL3}^2$ (SE)	2.607 (0.089)	1.029 (0.041)		
	$\hat{\rho}_{L3}$ (SE)	0.305 (0.021)	0.258 (0.029)		

$\hat{\sigma}_G^2$ – genotypic variance component estimate; $\hat{\sigma}_b^2$ – block nested in environment variance component estimate; $\hat{\sigma}_{G\times E}^2$ - genotype by environment interaction variance component estimate; $\hat{\sigma}_e^2$ - random errors variance component estimate for model IND; $\hat{\sigma}_{eL.}^2$ - random errors variance component estimates for each location for models CS and AR1; $\hat{\rho}_{L.}$ – correlation estimates between observations in the same plot across years for each location (in AR1 model in two consecutive years)

Table 6. Residual likelihood ratio tests for genotype×enviroment (G×E) interaction and genotypic (G) variance components.

Variety	Modelo	$REMLRT_{G\times E}$ (*P*-value)	$REMLRT_{G}$ (*P*-value)
Alvarinho	IND	238.5 (<0.001)	228.5 (<0.001)
	CS	253.0 (<0.001)	176.7 (<0.001)
	AR1	257.2 (<0.001)	212.2 (<0.001)
Antão Vaz	IND	15.9 (<0.001)	24.3 (<0.001)
	CS	57.1 (<0.001)	1.8
	AR1	51.4 (<0.001)	4.3
Aragonez	IND	35.0 (<0.001)	93.3 (<0.001)
	CS	97.7 (<0.001)	21.4 (<0.001)
	AR1	71.0 (<0.001)	56.3 (<0.001)
Síria	IND	64.6 (<0.001)	46.1 (<0.001)
	CS	95.9 (<0.001)	17.0 (<0.001)
	AR1	81.2 (<0.001)	37.6 (<0.001)

Residual maximum log-likelihood ratio test (REMLRT) for the G×E variance component ($REMLRT_{G\times E}$) ($H_0: \sigma^2_{G\times \mathrm{E}} = 0$ vs $H_1: \sigma^2_{G\times \mathrm{E}} > 0$) and for the intravariety genetic variability among the tested genotypes ($REMLRT_G$) ($H_0: \sigma^2_G = 0$ vs $H_1: \sigma^2_G > 0$) according to the fitted models with matrices diagonal (IND), compound symmetry (CS) and first order autoregressive (AR1)

Table 7. Interaction sensitivity (*IS*) for each genotype and variety, listed from the lowest (AI1, AN1, RZ1, CR1) to the highest (AI35, AN40, RZ40, CR40) sensitivity to G×E interaction, and predicted genotypic value (PGV) of the yield (kg/plant) for each genotype and their respective ranking number (rank) and prediction standard error (PSE[§])

Alvarinho		**Antão Vaz**		**Aragonez**		**Síria**	
***IS* (rank)**	***PGV*(rank)** (PSE=0.220)	***IS* (rank)**	***PGV* (rank)/** (PSE=0.115)	***IS* (rank)**	***PGV* (rank)** (PSE=0.115)	***IS* (rank)**	***PGV* (rank)** (PSE=0.118)
52.45 (AI1)	6.49 (18)	9.81 (AN1)	3.63 (28)	7.80 (RZ1)	3.58 (19)	16.95 (CR1)	2.76 (31)
60.15 (AI2)	6.27 (23)	12.43 (AN2)	3.73 (12)	9.29 (RZ2)	3.45 (35)	22.47 (CR2)	2.91 (21)
75.39 (AI3)	6.12 (24)	12.75 (AN3)	3.62 (32)	13.78 (RZ3)	3.68 (7)	23.35 (CR3)	2.85 (23)
79.28 (AI4)	6.66 (16)	12.93 (AN4)	3.69 (22)	14.68 (RZ4)	3.62 (11)	23.46 (CR4)	2.57 (38)
89.97 (AI5)	6.33 (21)	13.86 (AN5)	3.62 (31)	14.72 (RZ5)	3.56 (22)	23.57 (CR5)	2.75 (34)
105.44 (AI6)	6.35 (20)	16.06 (AN6)	3.63 (29)	15.41 (RZ6)	3.48 (28)	23.68 (CR6)	2.82 (29)
134.44 (AI7)	6.04 (25)	17.95 (AN7)	3.83 (2)	15.52 (RZ7)	3.57 (20)	25.08 (CR7)	3.01 (15)
135.00 (AI8)	6.50 (17)	19.63 (AN8)	3.64 (25)	16.03 (RZ8)	3.59 (16)	25.22 (CR8)	2.93 (20)
147.13 (AI9)	6.31 (22)	20.21 (AN9)	3.71 (17)	17.22 (RZ9)	3.84 (2)	30.03 (CR9)	2.71 (37)
173.69 (AI10)	6.86 (14)	21.56 (AN10)	3.79 (3)	18.28 (RZ10)	3.34 (39)	30.32 (CR10)	3.02 (14)
204.71 (AI11)	5.89 (27)	22.59 (AN11)	3.70 (19)	18.39 (RZ11)	3.60 (13)	30.50 (CR11)	2.85 (24)
211.86 (AI12)	6.88 (13)	22.75 (AN12)	3.69 (20)	18.84 (RZ12)	3.58 (18)	31.98 (CR12)	2.51 (39)
212.67 (AI13)	5.77 (28)	26.16 (AN13)	3.55 (35)	19.05 (RZ13)	3.66 (9)	33.98 (CR13)	2.75 (32)
257.12 (AI14)	6.47 (19)	27.53 (AN14)	3.66 (23)	20.18 (RZ14)	3.45 (36)	37.34 (CR14)	3.15 (5)
276.94 (AI15)	6.97 (12)	28.30 (AN15)	3.77 (5)	21.15 (RZ15)	3.34 (37)	40.68 (CR15)	2.85 (27)
323.48 (AI16)	5.96 (26)	28.58 (AN16)	3.48 (40)	21.32 (RZ16)	3.48 (29)	41.03 (CR16)	2.74 (36)
330.85 (AI17)	7.06 (10)	28.60 (AN17)	3.62 (30)	22.68 (RZ17)	3.46 (32)	41.95 (CR17)	2.74 (35)
468.93 (AI18)	6.84 (15)	30.14 (AN18)	3.51 (38)	23.46 (RZ18)	3.52 (27)	42.41 (CR18)	2.76 (30)
503.38 (AI19)	7.08 (9)	31.09 (AN19)	3.71 (16)	23.78 (RZ19)	3.46 (31)	42.94 (CR19)	2.94 (18)
517.98 (AI20)	6.99 (11)	32.34 (AN20)	3.59 (34)	26.06 (RZ20)	3.95 (1)	46.38 (CR20)	2.75 (33)
531.68 (AI21)	5.52 (30)	32.58 (AN21)	3.53 (36)	26.53 (RZ21)	3.55 (24)	49.10 (CR21)	3.04 (12)
543.30 (AI22)	7.13 (7)	34.08 (AN22)	3.72 (14)	27.64 (RZ22)	3.54 (25)	49.22 (CR22)	2.85 (25)
587.17 (AI23)	5.68 (29)	34.66 (AN23)	3.75 (10)	29.78 (RZ23)	3.80 (3)	53.95 (CR23)	2.51 (40)
625.74 (AI24)	5.35 (31)	34.88 (AN24)	3.52 (37)	30.39 (RZ24)	3.53 (26)	54.60 (CR24)	2.85 (26)
627.82 (AI25)	5.28 (32)	35.09 (AN25)	3.61 (33)	30.90 (RZ25)	3.48 (30)	56.69 (CR25)	3.03 (13)
644.16 (AI26)	7.13 (8)	35.39 (AN26)	3.72 (13)	31.01 (RZ26)	3.65 (10)	62.76 (CR26)	2.89 (22)
700.57 (AI27)	7.55 (3)	40.34 (AN27)	3.75 (7)	31.22 (RZ27)	3.61 (12)	65.47 (CR27)	3.10 (7)
819.01 (AI28)	7.46 (5)	43.02 (AN28)	3.77 (4)	31.26 (RZ28)	3.45 (33)	66.30 (CR28)	3.04 (11)
829.26 (AI29)	7.31 (6)	44.42 (AN29)	3.50 (39)	35.06 (RZ29)	3.56 (23)	70.79 (CR29)	3.23 (2)
914.74 (AI30)	7.62 (2)	45.86 (AN30)	3.74 (11)	36.82 (RZ30)	3.59 (14)	71.13 (CR30)	2.97 (17)
937.57 (AI31)	7.64 (1)	46.17 (AN31)	3.64 (26)	40.29 (RZ31)	3.56 (21)	72.65 (CR31)	3.19 (3)
982.68 (AI32)	7.47 (4)	50.52 (AN32)	3.76 (6)	41.46 (RZ32)	3.71 (6)	73.93 (CR32)	3.07 (9)
2459.84 (AI33)	4.69 (33)	51.45 (AN33)	3.69 (21)	44.17 (RZ33)	3.77 (5)	79.56 (CR33)	3.05 (10)
4204.49 (AI34)	4.41 (34)	51.51 (AN34)	3.65 (24)	45.19 (RZ34)	3.34 (38)	80.51 (CR34)	3.00 (16)
5067.15 (AI35)	4.29 (35)	58.64 (AN35)	3.70 (18)	53.21 (RZ35)	3.59 (15)	81.57 (CR35)	3.18 (4)
		64.37 (AN36)	3.75 (9)	53.23 (RZ36)	3.58 (17)	95.28 (CR36)	2.94 (19)
		73.11 (AN37)	3.71 (15)	53.85 (RZ37)	3.45 (34)	95.90 (CR37)	3.10 (8)
		80.04 (AN38)	3.83 (1)	72.11 (RZ38)	3.67 (8)	112.21 (CR38)	3.14 (6)
		80.91 (AN39)	3.75 (8)	74.00 (RZ39)	2.89 (40)	114.03 (CR39)	3.31 (1)
		106.74 (AN40)	3.63 (27)	89.34 (RZ40)	3.77 (4)	176.34 (CR40)	2.84 (28)
	Overall mean PGV=6.41		Overall mean PGV=3.67		Overall mean PGV =3.56		Overall mean PGV =2.92

[§] For each variety, PSE is the same for all genotypes because the design is balanced - all genotypes were evaluated in the same number of environments and repetitions.

Figure 1. EBLUPs of the effects of the G×E interaction as the proportion of the environment mean [$EBLUP_{G\times E}$ (%)**]** over the studied environments [after the environment code, in brackets, is presented the overall mean yield of the environment, kg/plant (pt)], for the genotypes with the highest (AI35) (●) and the lowest (AI1) (●)sensitivity to G×E interaction in Alvarinho variety.

Figure 2. EBLUPs of the effects of the G×E interaction as the proportion of the environment mean **(**$EBLUP_{G\times E}$%**)** over the studied environments [after the environment code, in brackets, is presented the overall mean yield of the environment, kg/plant (pt)], for the genotypes with the highest (●) and the lowest (●) sensitivity to G×E interaction in Antão Vaz variety.

Figure 3. EBLUPs of the effects of the G×E interaction as the proportion of the environment mean **(**$EBLUP_{G\times E}$%**)** over the studied environments [after the environment code, in brackets, is presented the overall mean yield of the environment, kg/plant (pt)], for the genotypes with the highest (●) and the lowest (●) sensitivity to G×E interaction in Aragonez variety.

Figure 4. EBLUPs of the effects of the G×E interaction as the proportion of the environment mean **(**$EBLUP_{G\times E}$%**)** over the studied environments [after the environment code, in brackets, is presented the overall mean yield of the environment, kg/plant pt)], for the genotypes with the highest (●) and the lowest (●) sensitivity to G×E interaction in Síria variety.

Supporting information

Table S1. List of Interaction Sensitivity (*IS*) ranked from the lowest to the highest, predicted genotypic values (PGV) of the yield (kg/plant), EBLUPs of the genotypic effects as the proportion of the yield overall mean (*EBLUP(G)* (%)) and EBLUPs of the G×E interaction effects as the proportion of the environment mean (*EBLUP(G×E) (%)*) over the studied environments for each genotype in the Alvarinho variety.

Table S2. List of Interaction Sensitivity (*IS*) ranked from the lowest to the highest, predicted genotypic values (PGV) of the yield (kg/plant), EBLUPs of the genotypic effects as the proportion of the yield overall mean (*EBLUP(G)* (%)) and EBLUPs of the G×E interaction effects as the proportion of the environment mean (*EBLUP(G×E)* (%)) over the studied environments for each genotype in Antão Vaz variety.

Table S3. List of Interaction Sensitivity (*IS*) ranked from the lowest to the highest, predicted genotypic values (PGV) of the yield (kg/plant), EBLUPs of the genotypic effects as the proportion of the yield overall mean (*EBLUP(G)* (%)) and EBLUPs of the G×E interaction effects as the proportion of the environment mean (*EBLUP(G×E)* (%)) over the studied environments for each genotype in Aragonez variety.

Table S4. List of Interaction Sensitivity (*IS*) ranked from the lowest to the highest, predicted genotypic values (PGV) of the yield (kg/plant), EBLUPs of the genotypic effects as the proportion of the yield overall mean (*EBLUP(G)* (%)) and EBLUPs of the G×E interaction effects as the proportion of the environment mean (*EBLUP(G×E)* (%)) over the studied environments for each genotype in Síria variety.