Grapevine clonal selection in Portugal: A different approach

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Abstract. The methodology for selection of an ancient grapevine variety conducted by PORVID (Portuguese Association for Grapevine Diversity) and the Portuguese Network for Grapevine Selection, focuses on quantitative genetic tools resulting in an integrated strategy that comprises two types of selected material – clonal and polyclonal – both carrying high genetic and economic gains for relevant agronomic and oenological traits. This work focused on the clonal selection methodology, particularly on the model for communication with users of selected materials (grape growers and winemakers). The approach is innovative, since detailed information of the whole experimental process leading to certified clones is provided, including predicted genetic gains for several traits and information about the clone's environmental stability (G × E interaction). This last analysis, combined with the utilisation of several certified clones (approximately 7), contributes to a better adaptation to environmental changes.

1. Introduction

In Portugal, grapevine genetic selection began in 1978. This work has been conducted by the National Network for Grapevine Selection, later joined by PORVID (Portuguese Association for Grapevine Diversity). In total, about 120 researchers and technicians are partially involved in this work at a national level. After 40 years of methodological development [1,2], the methodology of grapevine selection currently used includes a global strategy of conservation and evaluation of intra-varietal genetic variability, and selection. It is an integrated approach comprising two types of selected material – polyclonal and clonal – both carrying high genetic and economic gains for important agronomic and oenological traits. At present, 30000 genotypes of over 200 ancient varieties are conserved, 61 varieties undergo selection and 178 selection field trials have been planted. The first polyclonal selected materials started to be distributed to grape growers in 1984, and since 2005, 150 clones from 24 varieties were obtained.

The polyclonal material is a group of superior genotypes selected from the initial large field trial of selection, which was planted with a representative sample (100–400 genotypes) of the genetic variability within the variety. The clonal material corresponds to the individual clone, selected after a third stage of selection based on multi-environmental trials to evaluate the genotype × environment (G × E) interaction of the clones. The method ends with the selection of a plural number of different clones (usually 7), allowing the grower to avoid monoclonal vineyards and minimize G × E interaction.

The main difference of this methodology compared to the standard clonal selection methodology [3], is related to the evaluation of intravarietal genetic variability in a field trial and the application of quantitative genetics theory. In fact, an ancient grapevine variety is a heterogeneous population concerning important quantitative traits (yield, sugar, acidity, anthocyanins and many others), therefore the application of quantitative genetics theory to quantify the efficiency of selection, that is, the genetic gain of selection, is a key issue for both polyclonal and clonal selection.

This work is focused on the clonal selection methodology, particularly, on the model for communication with users of selected materials (grape growers and winemakers). To inform users about clonal selected materials in an objective, useful and practical way, the first catalogue of PORVID clones was published in 2018 (available at `https://tinyurl.com/y7498ppt`). The approach is innovative, since detailed information of the whole experimental process leading to certified clones is provided, including: (1) the selection procedure, describing locations and experimental design of field trials, studied traits and years of evaluation; (2) the predicted genetic gains of the clone for several traits and (3) the clone's environmental stability (G × E interaction).

2. Methodological bases

The theory of linear mixed models [4,5] is the support for quantitative genetic analysis. Some key concepts are summarized below to clarify the type of information provided about selected materials.

The success of selecting a quantitative trait within a genetically heterogeneous population is measured by the genetic gain. Therefore, this genetic indicator should be always provided to users of selected materials. Genetic gain (R) is the part of the difference between the mean of the phenotypic values (observed) of the selected genotypes and the mean of the whole population (selection

differential, S), which is genetically determined and transmissible (heritability, h^2) [6]:

$$R = S \times h^2. \quad (1)$$

When vegetative propagation is used, as is the case of an ancient grapevine variety, h^2 in expression (1) is the broad-sense heritability (usually denoted as H^2). In classical models of quantitative genetics (i.e., balanced data with no random effects other than those associated with genotypes and error and diagonal variance-covariance matrices), the proportion of total variance (phenotypic variance) that is genetic is called heritability. In the classical context, the broad-sense heritability (at the level of genotype mean) of a quantitative trait in a given population and environment is computed as:

$$H^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}_e^2/r}, \quad (2)$$

where $\hat{\sigma}_g^2$, $\hat{\sigma}_e^2$ and r are the genotypic variance estimate, the error variance estimate and the number of replicates of the trial, respectively. This genetic parameter varies from 0 to 1. It is important to remark that when more complex models are used, the above referred expression is not applicable. Several studies have considered the problem of defining the heritability for more complex models [7–9], including in the context of genetic analysis of ancient grapevine varieties [10]. However, independently of its computation, the general meaning of heritability (according to [6], the squared correlation between the predicted and the true genetic effects), is a key concept to evaluate data quality and the success of selection, as well as to predict genetic gain.

Another key concept in genetic selection terminology is the empirical best linear unbiased predictor (EBLUP) [11] of the genotypic effect of a trait (obtained from fitting a linear mixed model). Briefly, the EBLUP of the genotypic effect is the part of the difference between the genotype mean and the population mean, which is genetically determined and transmissible [4,11]. When an individual clone is selected, this concept represents the genetic gain associated to its selection. When a group of clones is selected (polyclonal selection), the associated predicted genetic gain corresponds to the average of the EBLUPs of the genotypic effects of those selected clones (in all contexts, using classic or more complex models). Obviously, the precision associated to the predicted genetic gain for a clone is lower than the precision of the predicted genetic gain for the polyclonal selection (the standard error of a mean is always lower), which consequently constitutes a weakness of clonal selection when compared to polyclonal selection.

As previously mentioned, this work is focused on clonal selection. The clonal material corresponds to a clone. In the process of clonal selection, the second stage (Fig. 1) is essential to predict the genetic gains of selection (as the selected material is compared to the mean of a representative sample of the intra-varietal variability of the variety). But, in the context of clonal selection, one must be well aware that the phenotypic value of an individual for a given trait is controlled by its genotypic effect, the effect of the environment and the G × E interaction effect. To assess the latter, a third stage of selection must be executed, consisting of several trials in the variety's usual growing regions, with 20–40 clones selected in the second stage (Fig. 1). This is a crucial stage for studying G × E interaction.

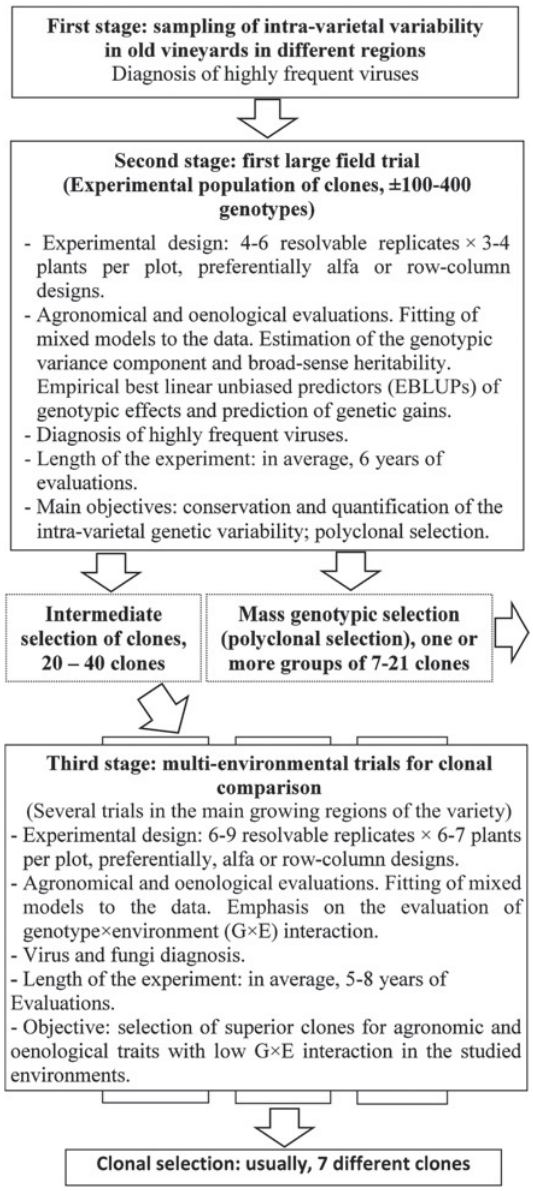

Figure 1. Methodology of grapevine selection in Portugal (adapted from [2]).

G × E interaction is a complex phenomenon, and it exists when the comparative performances of genotypes vary according to the environment. It is, thus, understandable that a rigorous study of this phenomenon requires a large number of different environments. As mentioned by [12], G × E is highly context-specific: it 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.

A precise study of G × E interaction in grapevine clones has some hurdles. One is related with the difficulty of field experimentation with this perennial crop, which is time consuming and has high cost. Therefore, few locations are usually available (commonly 2 to 5), but the genotypes are evaluated during several years in each of

the locations. When a limited number of environments are evaluated, ideal results will hardly be achieved. Besides, there will always be the unpredictable behaviour of a clone in an unknown condition. Hence, the complementary strategy to overcome G × E interaction problems is to select several different clones. For this reason, the methodology (Fig. 1) ends with the selection of a plural number of different clones (usually 7).

There are numerous methods for studying G × E interaction. Some of the most important ones used worldwide to study this phenomenon were reviewed by [12]. In Portugal, some approaches were conducted including graphical representation of clones' ranking over environments, calculation of the coefficient of variation of phenotypic values of one genotype in different environments, computation of non-parametric rank measures [2,14], and quantification of G × E interaction from the genetic correlation between environments [15]. However, for practical reasons, the information provided to grape growers will not into such detail. Instead, it provides a simple and intuitive way of visualizing the phenomenon.

As previously mentioned, the phenotype value of an individual clone for a given trait and environment is due to its genotypic effect, the effect of the environment, the effect of the G × E interaction, and a random error associated to the observation. After fitting an appropriate mixed model to multi-environmental data, the EBLUP of the G × E interaction effect of the clone for each studied environment is obtained. One way to understand the sensitivity of any given clone to G × E interaction is to comparatively observe, in a graph, the values of those EBLUPs across different environments. Being obviously desirable that all EBLUPs of the G × E interaction effects on the clone will be zero (no interaction effects in all environments), this method was chosen to provide grape growers with a visual perception of each clone's sensitivity to the interaction.

3. Case study

The following case-study is related to the clonal selection procedure applied to the Portuguese ancient variety Vinhão. This variety is mostly grown in the Northwest of Portugal, in the "Vinho Verde" region [16]. The information provided to grape growers about the selection procedure is described below.

3.1. Information about field trials, including experimental design, evaluated traits and years

The large field trial (second stage of selection) was planted in Arcos de Valdevez, with a representative sample of the intra-varietal variability of the variety (211 clones). All clones were grafted on 196/17 rootstock, the experimental design was a randomized complete block design (with 5 resolvable replicates), with a row-column arrangement, and two plants per plot. Yield was evaluated in years 1988–1991, 1993 and 1997. Potential alcohol, total acidity, pH, anthocyanins and total phenol index were assessed in 1993 and 1997, and berry weight, malic and tartaric acids in 1997.

For the third stage of selection, 3 clonal comparison field trials were installed in 3 different sites (located in the main areas where the variety is grown, Fig. 2), comprising 34 clones selected from the large field trial used for the second stage. Those clones carried on a predicted genetic gain for yield of +17%.

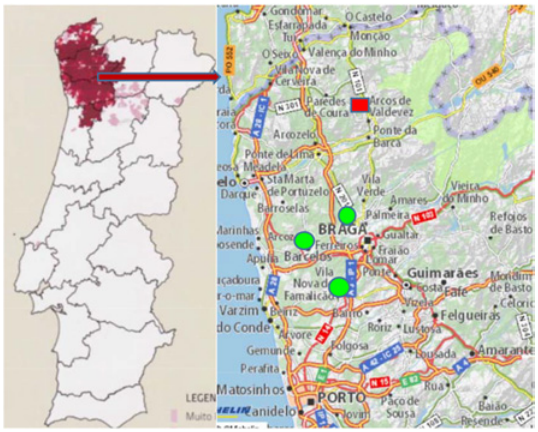

Figure 2. Vinhão is mostly grown in NW Portugal (rouge region highlighted on the map [16]). The selection procedure comprised the installation of 4 field trials in this region (■, the first large field trial; ●, the field trials for clonal comparison).

The information about clonal comparison field trials is following described.

- Location 1: Barcelos (S. Miguel da Carreira). Plants were grafted on 196/17 rootstock, the experimental design was a randomized complete block design (6 resolvable replicates), with a row-column arrangement, and 4 plants per plot. Yield was evaluated in 1993, 1994, 1995, 1996 and 1998. Potential alcohol, total acidity and pH were assessed in 1994, 1995 and 1996.
- Location 2: Braga (S. Paio de Pousada). Plants were grafted on 1103P rootstock, the experimental design was a randomized complete block design (5 resolvable replicates), with a row-column arrangement, and 4 plants per plot. Yield was evaluated from 1994 to 1999. Potential alcohol, total acidity and pH were evaluated from 1995 to 1999.
- Location 3: Vila Nova de Famalicão (Seide). Plants were grafted on 161/49 rootstock and the experimental design was a randomized complete block design (9 resolvable replicates), with a row-column arrangement, and 3 plants per plot. Yield was evaluated in 1997, 1999, 2000, 2001, 2003 and 2004. Potential alcohol, total acidity and pH, were assessed in 1999, 2000, 2001, 2002, 2003 and 2005, berry weight in 2000, 2001, 2003 and 2005, anthocyanins and total phenol index in 2003 and 2005.

Additionally, vigour and rootstock affinity with 2 rootstocks (SO4, 99R) were evaluated. Microvinifications and diagnosis of virus (GFLV, ArMV, GLRaV1, 2 and 3) by enzyme linked immunosorbent assay (ELISA) were also performed.

Mixed models were fitted to data, variance components were estimated and several genetic indicators were computed.

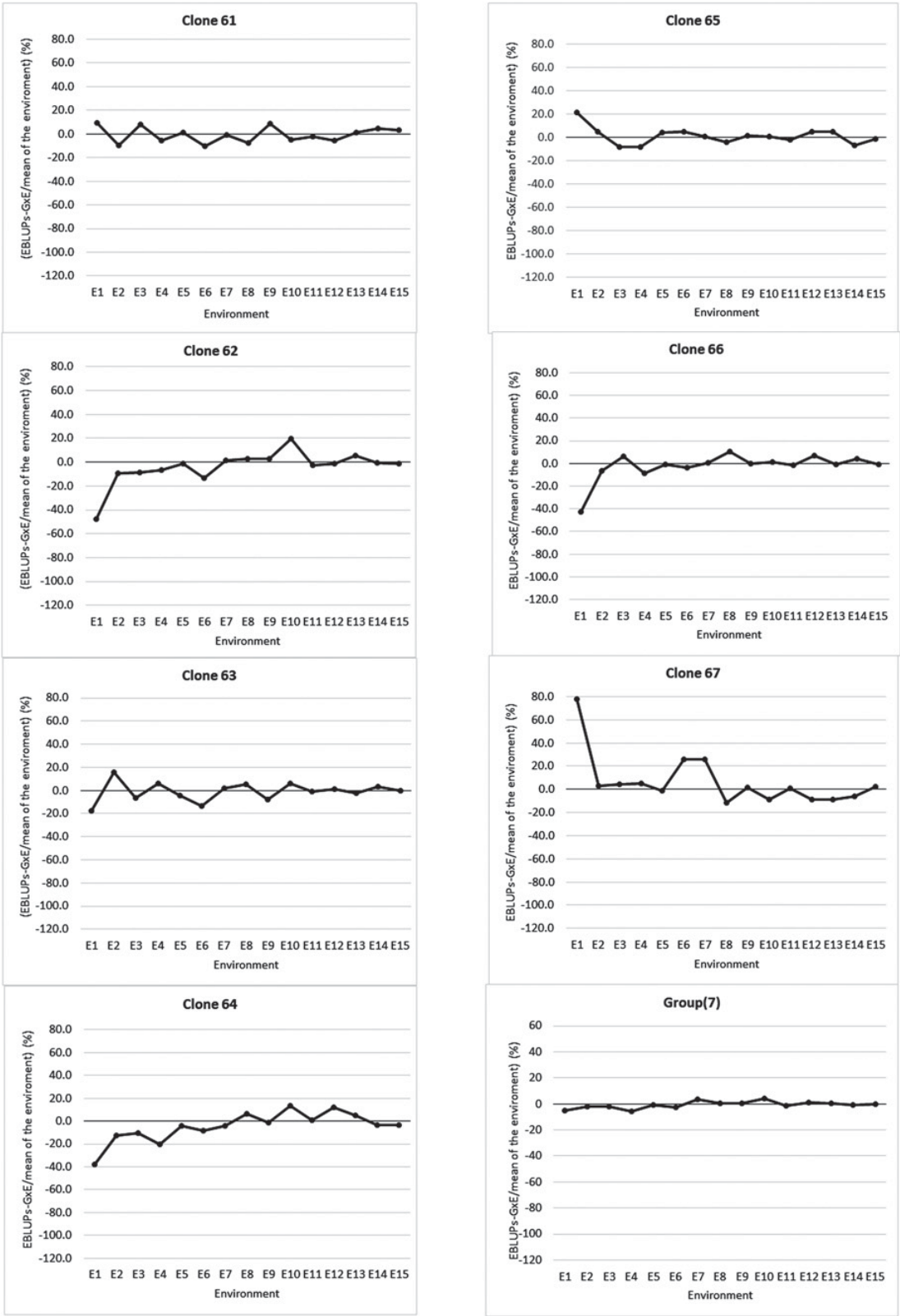

Figure 3. Charts detailing, for each Vinhão selected clone and for the group of the 7 clones, values for the EBLUPs of G × E interaction effects across all 15 tested environments.

3.2. Information about predicted genetic gains for each clone for the main traits evaluated

The clonal selection procedure ended with the selection of 7 clones, designated by numbers 61 to 67.

Information about predicted genetic gains for yield and potential alcohol for each clone are shown in Table 1.

The individual clone gains ranged from +7.5% to +27.6% for yield and from −5.6% to +6.6% for potential alcohol. Five clones revealed gains for both traits. The group of the 7 clones also showed gains for both traits.

The gains for other traits, such as total acidity, berry weight and anthocyanins, are detailed in Table 2. Predicted genetic gains ranged from −4.3% to +2.0% for total

Table 1. Predicted genetic gains for yield and potential alcohol for each clone and for the group of the 7 clones (in percentage relative to the population mean of the second stage trial: yield = 5.3 kg/plant, $H^2 = 0.652$; probable alcohol = 8.4%V/V, $H^2 = 0.613$).

Clone	Yield	Potential alcohol
Clone 61	+18.2%	+5.7%
Clone 62	+9.6%	−5.6%
Clone 63	+11.9%	+6.6%
Clone 64	+10.3%	+3.9%
Clone 65	+7.5%	−1.6%
Clone 66	+10.4%	+5.5%
Clone 67	+27.6%	+3.1%
Group (7)	+13.6%	+2.5%

Table 2. Predicted genetic gains for total acidity, berry weight and anthocyanins for each clone and for the group of the 7 clones (in percentage relative to the mean of clonal comparison trials: total acidity = 10.7 g/dm^3; berry weight = 1.9 g; anthocyanins = 1454.4 mg/l).

Clone	Total acidity	Berry weight	Anthocyanins
Clone 61	−2.2%	+3.1%	−0.1%
Clone 62	+2.0%	+2.2%	−2.2%
Clone 63	−0.8%	+1.9%	+3.8%
Clone 64	+1.3%	+0.9%	+1.1%
Clone 65	−0.8%	−1.2%	+0.1%
Clone 66	−4.3%	−2.1%	+1.9%
Clone 67	−0.8%	−4.9%	−2.7%
Group (7)	−0.8%	0.0%	+0.3%

Table 3. Environments (E) used for the study of G × E interaction.

E1	Famalicao 1997 (0.760 kg/plant, $H^2 = 31.2$)
E2	Braga 1998 (1.831 kg/plant, $H^2 = 49.7$)
E3	Barcelos 1993 (1.960 kg/plant, $H^2 = 40.9$)
E4	Famalicao 1999 (2.170 kg/plant, $H^2 = 32.4$)
E5	Arcos 1997 (2.938 kg/plant, $H^2 = 49.7$)
E6	Barcelos 1998 (3.265 kg/plant, $H^2 = 42.1$)
E7	Braga 1997 (3.821 kg/plant, $H^2 = 0.447$)
E8	Famalicao 2000 (5.198 kg/plant, $H^2 = 0.638$)
E9	Braga 1996 (5.905 kg/plant, $H^2 = 0.347$)
E10	Famalicao 2001 (7.736 kg/plant, $H^2 = 0.622$)
E11	Arcos 1993 (9.621 kg/plant, $H^2 = 0.608$)
E12	Barcelos 1996 (10.323 kg/plant, $H^2 = 0.365$)
E13	Famalicao 2003 (10.738 kg/plant, $H^2 = 0.825$)
E14	Famalicao 2004 (12.271 kg/plant, $H^2 = 0.704$)
E15	Arcos 1991 (12.861 kg/plant, $H^2 = 0.598$)

acidity, between −4.9% and +3.1% for berry weight, and from −2.7% to +3.8% for anthocyanins. Interestingly, clone 64 revealed genetic gains for all evaluated traits.

3.3. Information about yield sensitivity to G × E interaction

Analysis of G × E interaction was conducted using yield data from each site/year with broad sense heritability higher than 0.30. By environment (E) was meant the combination site/year. In total, the 34 clones selected for the third stage of selection were evaluated in 15 environments (Table 3). After fitting an appropriate mixed model to multi-environmental data, G × E interaction was significant for yield (rejection of hypothesis $H_0 : \sigma^2_{G\times E} = 0$, $p < 0.05$).

For each clone, a graphic detailing the EBLUP of the G × E interaction effect for each environment is included in Fig. 3. Differences among clones for the sensitivity to G × E interaction were found. For example, the EBLUPs of the G × E interaction effects for the several environments studied are closer to zero in clones 61, 65 and 63 than in the other clones. This means that, in the studied environments, those three clones revealed less sensitivity to G × E interaction. In the lowest yielding environment (E1), some clones revealed a positive effect of interaction (clones 67, 65 and 61), whereas others revealed a negative effect. There is a tendency for lower sensitivity to G × E interaction in environments presenting higher yields.

Analysing the behaviour of the group of the 7 clones, it can be observed that in all environments their EBLUPs of the G × E interaction effects are close to zero. This last option permitted to minimize the G × E interaction across all tested environments. To sum up, the utilisation of several certified clones (approximately 7), contributed to a better control of effects due to G × E interaction.

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References

[1] A. Martins, L. Carneiro, R. Castro, Vitis, special issue, 485–489 (1990)
[2] A. Martins, E. Gonçalves, In *Grapevine Breeding Programs for the Wine Industry: Traditional and Molecular Techniques*, edited by A.G. Reynolds (Woodhead Publishing, Elsevier, UK, 2015), p. 159
[3] Resolution OIV-VITI 564A-2017. *Standard protocol for the clonal selection of grapevine varieties* (2017)
[4] S. Searle, G. Casella, C. McCulloch, *Variance Components* (John Wiley & Sons Inc., Hoboken, New Jersey, 1992)
[5] C. McCulloch, S. Searle, J. Neuhaus, *Generalized Linear and Mixed Models*, 2nd edn. (John Wiley & Sons, New York, 2008)
[6] D. Falconer, T. Mackay, *An Introduction to Quantitative Genetics*, 4th edn. (Prentice Hall, London, 1996)
[7] B. Cullis, A. Smith, N. Coombes, J. Agr. Biol. Envir. St. **11**, 381 (2006)
[8] H. Oakey, A. Verbyla, W. Pitchford, B. Cullis, H. Kuchel, Theor. Appl. Genet. **113**, 809 (2006)
[9] H.P. Piepho, J. Möhring, Genetics **177**, 1881 (2007)
[10] E. Gonçalves, I. Carrasquinho, A. St. Aubyn, A. Martins, Euphytica **189**, 379 (2013)

[11] C. Henderson, Biometrics **31**, 423 (1975)
[12] M. Lynch, B. Walsh, *Genetics and Analysis of Quantitative Traits* (Sinauer Associates, Inc., Sunderland, 1998)
[13] Y. Li, M. Suontama, R. Burdon, H. Dungey, Tree Genet Genomes **13**, 60 (2017)
[14] A. Martins, L. Carneiro, S. Mestre, E. Gonçalves, J. Neves-Martins, C. Almeida, I. Ramadas, J. Eiras-Dias, D. Madeira, N. Magalhães, Proc. XXIII Congrès Mondial de la Vigne et du Vin **1**, 169 (1998)
[15] E. Gonçalves, E.I. Carrasquinho, I.R. Almeida, V. Pedroso, A. Martins, Aust. J. Grape Wine R. **22**, 52 (2016)
[16] IVV, *Catálogo das Castas Para Vinho Cultivadas em Portugal* (2011)