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Research Article

Fully and Partially Replicated Experimental Designs for Evaluating Intravarietal Variability in Grapevine

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Background and Aims. In ancient grapevine varieties, the experimental design of field trials is crucial to providing a reliable evaluation of quantitative traits. The main purposes of this study are to demonstrate the benefits of the resolvable row-column design (RCD) for quantifying intravarietal variability and performing polyclonal selection and to compare the efficiency of fully and partially replicated designs for quantifying intravarietal variability to implement the latter designs for a preliminary analysis of that variability. *Methods and Results*. Linear mixed models were fitted to yield data obtained in field trials with fully and partially replicated designs. The results pointed out the importance of the RCD in controlling the spatial variability present in large field trials. Although less precise, a partially replicated design proved to be useful in evaluating intravarietal variability when the average of years was used. *Conclusions*. The results reinforced the importance of the RCD in increasing the efficiency of intravarietal variability quantification and polyclonal selection. The partially replicated design proved to be useful when the only objective was to perform a preliminary analysis of intravarietal variability. *Significance of the Study*. Understanding the role of experimental design in grapevine selection field trials will help grapevine breeders enhance their knowledge about variability within ancient varieties and implement more successful polyclonal selection.

1. Introduction

Plant breeding plays an important role in agriculture by providing plant materials with superior genetic quality to be used by farmers.

Ancient grapevine varieties have a high level of intravarietal variability with respect to the most important traits (yield, soluble solids content, and acidity) [1, 2]. This variability enables the best genotypes within a variety to be selected. The traditional method used for grapevine improvement is clonal selection (selection of individual clones). As grapevine is perennial, the well-conducted clonal selection is very time-consuming. It requires establishing multienvironmental trials in the main regions where the variety is cultivated to study the genotype by environment (G × E) interaction [3]. Typically, in each location, a field trial with 20–40 clones is established with an experimental design preferentially of the family of incomplete block designs, with 6–10 repetitions and 5–8 plants per experimental unit [4]. Even with a sound study of G × E interactions, clonal selection (homogeneous genetic material) always suffers from high sensitivity to G × E interactions and helps accelerate the erosion of intravarietal variability. However, Europe has thousands of varieties [5], and implementing a clonal selection programme for each variety is unfeasible. Conserving and exploring the intravarietal variability of ancient varieties is essential to fostering their use in the future and thus preserving the traditions and history of ancient growing regions and their wines. Therefore, to accomplish this latter goal, two approaches can be adopted: studying intravarietal variability and performing polyclonal selection for widely used varieties and studying the intravarietal variability of rarely grown and little-known varieties.

For widely used varieties, a field trial is established with a representative sample of the intravarietal variability of the variety [1, 6]. In such field trials, many genotypes are evaluated over several years (usually 3–6 years) in terms of the most important economic traits, and then, polyclonal selection is performed. Polyclonal selection is the selection of a superior group of genotypes to predict the genetic gains for important economic traits, that is, a varietal subpart, usually 20 genotypes, is selected according to its superiority in yield and other important traits and lesser sensitivity to genotype × year interaction and distributed to producers and planted as a balanced and indivisible mixture. This circumstance favours stability in different environments. Using a mixture of genotypes buffers the effect of the possible negative behaviour of an individual clone in a particular environment. Therefore, field trials for polyclonal selection are usually implemented at one site. Additionally, polyclonal selection enables the intravarietal variability of the variety in the field to be conserved for at least 30 years and several types of polyclonal material to be selected according to different criteria over time; these benefits of polyclonal selection respond quickly to the demands of producers, consumers, and climate change.

For rarely grown and little-known varieties, the cheapest and less space-consuming strategies are desirable to evaluate intravarietal variability in terms of important traits (to decide if there is potential to move on to another stage of selection) and to conserve this diversity in the field for at least 30 years.

This work focused on the experimental designs to be applied in the polyclonal selection methodology and in the preliminary study of the intravarietal variability of rarely grown and little-known varieties.

In plant breeding, the experimental design of field trials is crucial to provide a reliable evaluation of the genotypes under study. Grapevines are an outstanding example of a successful crop in Portugal, where the results of selection have fostered important economic gains [1, 4]. Therefore, to increase the efficiency of intravarietal variability evaluation and the genetic gains of selection, studies related to experimental designs implemented in the field are continuously conducted.

To evaluate the most important economic traits, which are quantitative traits, well-designed experiments, which rely on the well-known principles of randomization, replication, and blocking, are needed [7, 8]. In the plant breeding context, fully replicated designs and partially replicated designs are used.

In fully replicated designs, all the genotypes are replicated, and in large field trials, the efficiency of those designs depends essentially on the randomization process used to control environmental variation. Blocking plays a key role in controlling spatial variability, water regimes, and farming operations. The need to control all these sources of variation led to establishing a two-dimensional layout of field trials; two-dimensional field trials have a strong tradition in agricultural experiments. After Fisher [9] introduced a randomized complete block design, Yates [10, 11] was the first to describe balanced incomplete block designs, including balanced square lattice designs. There are many variants of these designs, but the most relevant for working with many treatments are alpha designs [12], which constitute a particular class of generalized lattice designs; row-column designs [13] impose blocks in both row and column directions; these designs correspond to groups of more complex Latin square designs [14] and resolvable spatial row-column designs [15]. Generating efficient row-column designs is a very important topic in experimental design research [15–19]. The overall objective is optimizing neighbour balance, ensuring that the number of pairwise adjacencies is as equal as possible across pairs of treatments over the field layout.

Row-column designs have been strongly implemented for annual and perennial crops [20, 21] and forestry [22–24], in which breeding field trials typically occupy large areas. Concerning grapevines, fully replicated designs are useful for evaluating intravarietal genetic variability and for performing genetic selection [4]. Simulation studies with this species revealed that alpha and row-column designs are the most efficient when many genotypes are used [25]. Additionally, according to Gonçalves et al. [25], the higher efficiency of the row-column design relative to the alpha design was observed when the number of plots per incomplete block was greater than or equal to 10. This type of experimental design is also recommended in the OIV resolution [6] to perform polyclonal selection.

Partially replicated designs (also known as unreplicated trials) are frequently used in plant breeding early generation field trials. The objective is to make a preliminary assessment of the available germplasm and to select a subgroup (normally approximately 1/3) of genotypes to enter a more advanced stage of selection [26–28]. Numerous variants of partially replicated trials are frequently used in plant breeding. Two types of treatments are used: checks (which correspond to replicated treatments) and tests (which correspond to nonreplicated treatments). The checks can be planted according to several experimental designs, among which are the augmented randomized complete block design, augmented randomized incomplete block design, and augmented row-column designs [29–32]. Other more complex experimental designs have also been proposed, such as the augmented lattice square design [33] and alpha-alpha design [34]. When spatial correlation is considered, the approximations of Martin et al. [27] and the p-rep designs of Cullis et al. [28] were also proposed. To improve the evenness of replicated treatment plot distribution, Vo-Thanh and Piepho [35] proposed the augmented quasi-sudoku designs.

For grapevine, partially replicated designs were proposed to quantify the intravarietal variability in rarely grown varieties [36]. Simulation studies performed by these authors showed a greater precision in collections with an alpha-alpha design, over 250 genotypes, and a minimum of 33% of plots containing check genotypes.

Field trials to evaluate the intravarietal genetic variability of grapevines and to perform genetic selection were traditionally planted according to a randomized complete block design with a row-column arrangement. However, over the past decade, with methodological advances in experimental

designs, designs of the family of incomplete blocks have begun to be implemented, particularly resolvable row-column designs. Methodological studies with resolvable row-column designs and partially replicated designs were also implemented in the field.

The main purposes of this paper are to demonstrate the importance and benefits of the resolvable row-column design to quantify intravarietal genetic variability and to perform polyclonal selection and to compare the efficiency of fully and partially replicated designs for intravarietal variability quantification by using yield data obtained in field trials constructed accordingly.

2. Materials and Methods

2.1. Description of Field Trials. To evaluate the importance of the experimental design for quantifying intravarietal variability and for performing polyclonal selection methodology, yield data from the field trials of several autochthonous Portuguese grapevine varieties were considered. Six varieties (Antão Vaz, Arinto, Bastardo, Gouveio, Rufete, and Tinta Caiada) were planted according to a fully replicated design (resolvable row-column design), and one (Bastardo) was also planted according to a partially replicated design ($\alpha - \alpha$ design). In the latter case, the proportion of check and test genotypes followed the guidelines provided by Gonçalves et al. [36]. For each variety, the field trial contains a representative sample of the intravarietal variability prospected in the main ancient growing regions: Antão Vaz in Alentejo region (Portugal); Arinto in Vinhos Verdes, Bairrada, Lafões, and Lisboa wine demarked regions (Portugal); Gouveio in Dão, Douro, and Trás-os-Montes regions (Portugal); Rufete in Beira Interior, Dão, and Douro regions (Portugal); Tinta Caiada in Alentejo, Douro (Portugal), and Somontano (Spain); Bastardo in Dão, Beira Interior, Douro, Trás-os-Montes (Portugal), and Jura (France). The genotypes of the Bastardo variety present in the partially replicated design were also present in the fully replicated design. The two types of field trials were planted contiguously in the field.

All information about the field trials and respective experimental designs can be found in Table 1. The experimental designs were generated by using CycDesigN 4.0 software (http//www.vsni.co.uk/software/cycdesign/), a software package for generating several efficient experimental designs, the foundations of which are described by John and Williams [16]. For row-column designs, the spatial option was used, and the separation of different genotypes in rows and columns was ensured according to a modified exponential variance weight function, with a value of 0.9 for the decay factor; this value is consistent with values from real grapevine selection trials [37].

Field trials are planted at the Experimental Centre for the Conservation of Grapevine Diversity (Pegões, Southern Portugal) of the Portuguese Association for Grapevine Diversity (PORVID), which is a farm dedicated to conserving the intravarietal variability of all autochthonous Portuguese varieties. In all trials, the training system was a vertical shoot position, the pruning system was a bilateral Royat cordon system, and the planting density was 2.50 m × 1.20 m. In each trial, all the plants were grafted onto a single clone of the 1103 Paulsen rootstock.

2.2. Models for Yield Data Analysis. The model for a resolvable row-column design can be described as follows:

$$y_{\text{ijlm}} = \mu + u_{g_i} + u_{r_j} + u_{\text{col}(r)_{jl}} + u_{\text{row}(r)_{jm}} + e_{\text{ijlm}}, \quad (1)$$

for $i = 1, \cdots, v$; $j = 1, \cdots, r$; $l = 1, \cdots, s$; and $m = 1, \cdots, k$ (v, number of genotypes; r, number of resolvable replicates; s, number of columns; k, number of rows). In this model, y_{ijlm} is the response (the mean yield of each plot (kg/plant)) of the ith genotype in the jth replicate, $l - th$th column, and mth row; μ is the general intercept, u_{g_i} is the ith genotype effect, u_{r_j} is the jth replicate effect, $u_{\text{col}(r)_{jl}}$ is the lth column effect within the j replicate, $u_{\text{row}(r)_{jm}}$ is the mth row effect within the jth replicate, and e_{ijlm} is the random error corresponding to the observation y_{ijlm}.

Model effects (with the exception of μ) were assumed to be independent and identically distributed normal variables with zero mean and variances σ^2_g, σ^2_r, $\sigma^2_{\text{col}(r)}$, $\sigma^2_{\text{row}(r)}$, and σ^2_e. All random effects were assumed to be mutually independent.

The model for a partially replicated design ($\alpha - \alpha$ design) is similar to the previously described model for a resolvable row-column design, including the genotypic effects of the replicated (check) and nonreplicated (test) genotypes.

For covariance parameters estimation, the residual maximum likelihood (REML) estimation method was used. Given the estimates of these parameters, the empirical best linear unbiased predictors (EBLUPs) of random effects were obtained through the mixed model equations, and the respective prediction error variances (PEVs) of these predictors were obtained by using the inverse elements of the mixed model equations.

Linear mixed models were fitted using the ASReml-R package [38] in R software [39].

2.3. Evaluation Criteria to Compare Analyses in Fully Replicated Design. The analysis performed was focused on the study of the effects of experimental design and how they control spatial variability in each year; therefore, an individual year analysis was performed.

When a resolvable row-column design (RCD) is adopted, the effects of the experimental design should be included in the model to respect the randomization process. However, when these effects are removed from the analysis, their importance can be assessed by comparing the model without the design effects (hereafter named M0) with the RCD model. The Akaike information criterion, $\text{AIC} = -2l_R + 2n_{\text{par}}$ [40], was used to compare the relative goodness-of-fit between these two models (where l_R is the residual log-likelihood obtained for the fitted model and n_{par} is the number of estimated covariance parameters). This criterion penalizes more complex models, and smaller AIC values indicate a better fit. The simplest model (model M0) is a reduced form of the RCD model. Therefore, the models are

Table 1: Description of the fully replicated design (resolvable row-column design, RCD) adopted for the field trials of six grapevine varieties and the partially replicated design (PRD) adopted for the field trial of Bastardo variety.

Variety	Year of grafting	Experimental design	Data
Antão Vaz (AN)	2013	RCD, $v = 110$, $k = 11$, $s = 10$, $r = 6$ 3 plants per plot	Yield from 1 year (2019)
Arinto (AR)	2013	RCD, $v = 165$, $k = 11$, $s = 15$, $r = 6$ 3 plants per plot	Yield from 3 years (2019, 2020, 2021)
Gouveio (GV)	2016	RCD, $v = 154$, $k = 14$, $s = 11$, $r = 5$ 3 plants per plot	Yield from 2 years (2019, 2020)
Rufete (RU)	2016	RCD, $v = 242$, $k = 11$, $s = 22$, $r = 6$ 3 plants per plot	Yield from 1 year (2020)
Tinta Caiada (CA)	2013	RCD, $v = 220$, $k = 11$, $s = 20$, $r = 6$ 3 plants per plot	Yield from 2 years (2020, 2019)
Bastardo (BT)	2013	RCD, $v = 374$, $k = 17$, $s = 22$, $r = 4$ 3 plants per plot 341 genotypes: 77 check and 264 test For check genotypes, $\alpha - \alpha$ design: $k = 11$, $s = 11$, $r = 6$, 3 plants per plot	Yield from 4 years (2018, 2019, 2020, 2021)

v, number of genotypes; k, number of rows; s, number of columns; r, number of replicates, plot, experimental unit) and the partially replicated designs adopted for the field trial of Bastardo variety. All field trials are located at Experimental Centre for the Conservation of Grapevine Diversity (Pegões, Portugal).

nested and can be compared using the residual likelihood ratio test (REMLRT), comparing minus twice the residual log-likelihood obtained with the fitting of the two models, one without the design effects (reduced model, M0; null hypothesis H_0: $\sigma_r^2 = 0$, $\sigma_{\text{col}(r)}^2 = 0$, $\sigma_{\text{row}(r)}^2 = 0$) and the other with the design effects (full model, RCD; alternative hypothesis H_1: at least one of these variance components is not zero). The distribution of the residual likelihood ratio test statistic consists of mixtures of Chi-square distributions because the tested parameters are in the boundary of parameter space. Determining the correct asymptotic null distribution for the likelihood test statistic requires simulation studies. Thus, a conservative solution was used, the naive approach of using a Chi-squared distribution with the number of degrees of freedom equal to the increase in the number of parameters between the two models; in this case, it corresponds to a Chi-squared distribution with three degrees of freedom.

The variability associated with each design effect (that is, with the resolvable replicate (H_0: $\sigma_r^2 = 0$ vs. H_1: $\sigma_r^2 > 0$), with the column within the replicate (H_0: $\sigma_{\text{col}(r)}^2 = 0$ *vs* H_1: $\sigma_{\text{col}(r)}^2 > 0$), and with the row within the replicate (H_0: $\sigma_{\text{row}(r)}^2 = 0$ vs. H_1: $\sigma_{\text{row}(r)}^2 > 0$)) and the intravarietal genetic variability among the tested genotypes (H_0: $\sigma_g^2 = 0$ vs. H_1: $\sigma_g^2 > 0$) were also tested using REMLRT. Under the null hypothesis, the asymptotic distribution of the REMLRT statistic is a 50 : 50 mixture of Chi-square distributions with zero and one degree of freedom [41]. This p value of the test was half of the reported p value from the distribution with one degree of freedom [41, 42].

With the experimental designs used in this study, the prediction error variances may differ for different genotypes, as genotypes with more information have smaller prediction errors. As a consequence, a generalized measure of broad-sense heritability was used to evaluate the efficiency of selection. A generalized measure of broad-sense heritability appropriate for complex experimental designs was introduced by Cullis et al. [28] and discussed by Piepho and Möhring [43] and later by other authors. In the context of grapevines, the generalized measure of broad-sense heritability was studied by Gonçalves et al. [44]. In this study, the generalized measure of broad-sense heritability applied was as follows:

$$H^2 = 1 - \frac{\overline{\text{PEV}}}{\widehat{\sigma}_g^2}, \tag{2}$$

where $\overline{\text{PEV}}$ is the average of the prediction error variance of genotypic effects and $\widehat{\sigma}_g^2$ is the estimate of the genotypic variance. This definition of heritability is related to prediction error variances. The closer the predictions are to the true values, the closer the heritability is to 1, and the smaller the prediction error variance is. Consequently, a more efficient selection will be performed.

Therefore, the increase in efficiency of selection (EF) when comparing the RCD model with the M0 model was based on the H^2 values obtained in the two models as follows:

$$\text{EF}(\%) = \frac{H_{\text{RCD}}^2 - H_{M0}^2}{H_{M0}^2} \times 100. \tag{3}$$

Additionally, plots of the sample semivariograms of the residuals from the RCD model were used as a tool for diagnosing spatial correlation. The packages gstat [45] and sp [46] in R software [39] were used.

2.4. Evaluation Criteria to Compare Designs. Fully and partially replicated designs were compared to provide a reference about the deviation of the result obtained for quantifying intravarietal variability with a partially replicated design in relation to a design that would be more efficient (fully replicated). For this comparison, different yield datasets were used. As a consequence, the results of these two designs could not be formally compared by using a residual likelihood ratio test or the AIC.

The comparison between fully replicated and partially replicated designs was based on the results obtained by each design individually for the following: genotypic variance component estimate, generalized broad-sense heritability, and genotypic coefficient of variation (CV_G, the ratio between the estimate for the genotypic standard deviation and the overall mean). In this study, the yield data were analysed for individual years and for the average of years, since a more precise quantification of intravarietal variability is achieved with an average of years [4].

3. Results

3.1. Fully Replicated Design: The Row-Column Design (RCD). The results reporting the importance of including all the experimental design effects (resolvable replicate effects, column effects within replicates, and row effects within replicates) in the analysis are shown in Table 2 and Figure 1. For all the cases studied, the RCD analysis showed a better fit than the analysis without the effects of the experimental design (M0). Lower AIC values were always obtained with the RCD model. When testing variance components associated with the effects of the experimental design, the null hypothesis (H_0: $\sigma_r^2 = 0$, $\sigma_{\text{col}(r)}^2 = 0$, $\sigma_{\text{row}(r)}^2 = 0$) was always rejected for any usual level of significance (p value <0.001). Analysing the variance components of the experimental design individually, for all the cases being studied, the column within replicate variance was always significant (rejection of H_0: $\sigma_{\text{col}(r)}^2 = 0$, p value <0.05). However, the replicate and row within replicate variances were not always significant. The replicate variance was not significant (nonrejection of H_0: $\sigma_r^2 = 0$, p value >0.05) in Bastardo /2018, Gouveio/2021, and Rufete/2020; the row within replicate variance was not significant (nonrejection of H_0: $\sigma_{\text{row}(r)}^2 = 0$, p value >0.05) in Antão Vaz/2019, Arinto/2020, Arinto/2021, Bastardo/2020, and Rufete/2020. Therefore, in the RCD model, the importance of each experimental design variance component estimate ($\widehat{\sigma}_r^2$, $\widehat{\sigma}_{\text{col}(r)}^2$, and $\widehat{\sigma}_{\text{row}(r)}^2$) depended on the field trial and on the year (Table 2). Figure 1 shows the proportion of each design effect variance. A higher proportion of variability among

Table 2: Variance components estimates, REML log-likelihood ratio test for σ^2_r, $\sigma^2_{\mathrm{col}(r)}$, and $\sigma^2_{\mathrm{row}(r)}$ (to test the variance components associated to the effects of the experimental design), and the increase in efficiency (EF) of RCD analysis compared to the analysis without the effects of the experimental design (based on H^2values).

Field trial[1]/year	Model	$\widehat{\sigma}^2_g$ (SE)	$\widehat{\sigma}^2_r$ (SE)	$\widehat{\sigma}^2_{\mathrm{col}(r)}$ (SE)	$\widehat{\sigma}^2_{\mathrm{row}(r)}$ (SE)	$\widehat{\sigma}^2_e$ (SE)	AIC	REMLRT (p value)	H^2	EF (%)
AN/2019	M0	1.697 (0.460)				9.728 (0.587)	2246.82		0.507	
	RCD	1.797 (0.451)	0.834 (0.611)	0.344 (0.233)	0.228 (0.219)	8.344 (0.557)	2208.63	44.18 (<0.001)	0.549	+8.27
AR/2019	M0	0.992 (0.179)				3.590 (0.177)	2409.55		0.622	
	RCD	1.074 (0.173)	0.462 (0.327)	0.398 (0.103)	0.140 (0.063)	2.587 (0.140)	2263.92	151.62 (<0.001)	0.695	+11.63
AR/2020	M0	0.917 (0.192)				4.702 (0.232)	2657.87		0.537	
	RCD	1.095 (0.193)	0.869 (0.573)	0.180 (0.086)	0.039 (0.058)	3.601 (0.194)	2503.58	160.29 (<0.001)	0.636	+18.41
AR/2021	M0	2.064 (0.327)				5.249 (0.258)	2838.40		0.702	
	RCD	2.176 (0.325)	0.510 (0.361)	0.437 (0.136)	0.072 (0.074)	4.220 (0.228)	2746.68	97.72 (<0.001)	0.744	+6.02
BT/2018	M0	0.777 (0.082)				1.311 (0.055)	2358.09		0.703	
	RCD	0.768 (0.080)	0.010 (0.017)	0.103 (0.029)	0.035 (0.018)	1.166 (0.053)	2317.88	46.21 (<0.001)	0.712	+1.31
BT/2019	M0	0.822 (0.135)				3.747 (0.158)	3706.29		0.466	
	RCD	0.877 (0.133)	0.083 (0.091)	0.249 (0.073)	0.149 (0.057)	3.249 (0.146)	3649.09	63.21 (<0.001)	0.501	+7.54
BT/2020	M0	0.577 (0.101)				2.936 (0.124)	3324.10		0.438	
	RCD	0.637 (0.099)	0.198 (0.174)	0.170 (0.053)	0.009 (0.026)	2.555 (0.114)	3236.39	93.70 (<0.001)	0.488	+11.29
BT/2021	M0	1.385 (0.192)				4.592 (0.194)	4069.52		0.546	
	RCD	1.406 (0.180)	0.283 (0.265)	0.526 (0.121)	0.130 (0.058)	3.682 (0.166)	3933.34	142.18 (<0.001)	0.585	+7.24
GV/2020	M0	0.468 (0.078)				0.969 (0.056)	910.86		0.704	
	RCD	0.498 (0.077)	0.126 (0.100)	0.075 (0.028)	0.052 (0.024)	0.720 (0.046)	820.88	95.98 (<0.001)	0.757	+7.51
GV/2021	M0	1.008 (0.156)				1.625 (0.094)	1321.77		0.754	
	RCD	1.050 (0.158)	0.015 (0.029)	0.094 (0.044)	0.111 (0.048)	1.395 (0.090)	1305.76	22.01 (<0.001)	0.773	+2.60
RU/2020	M0	0.223 (0.036)				0.940 (0.039)	1509.98		0.572	
	RCD	0.224 (0.034)	0.007 (0.011)	0.147 (0.029)	0.000	0.784 (0.035)	1426.68	89.31 (<0.001)	0.600	+5.06
CA/2020	M0	2.263 (0.315)				6.034 (0.257)	3959.06		0.692	
	RCD	2.349 (0.312)	0.388 (0.286)	0.456 (0.131)	0.198 (0.089)	4.984 (0.231)	3867.44	97.63 (<0.001)	0.725	+4.83
CA/2021	M0	3.543 (0.436)				5.982 (0.255)	4018.68		0.780	
	RCD	3.694 (0.435)	0.460 (0.337)	0.249 (0.096)	0.554 (0.141)	4.704 (0.219)	3897.67	127.01 (<0.001)	0.812	+4.13

$\widehat{\sigma}^2_g$, genotypic variance estimate; $\widehat{\sigma}^2_r$, resolvable replicate variance estimate; $\widehat{\sigma}^2_{\mathrm{col}(r)}$, columns within replicate variance estimate; $\widehat{\sigma}^2_{\mathrm{row}(r)}$, rows within replicate variance estimate; $\widehat{\sigma}^2_e$, random error variance estimate; SE, respective standard error; AIC, Akaike information criterion. Broad-sense heritability (H^2) obtained with the fitting of two models (model without the effects of the experimental design-M0 and model considering a resolvable row-column design-RCD). [1]The abbreviations of the varieties are referred in Table 1.

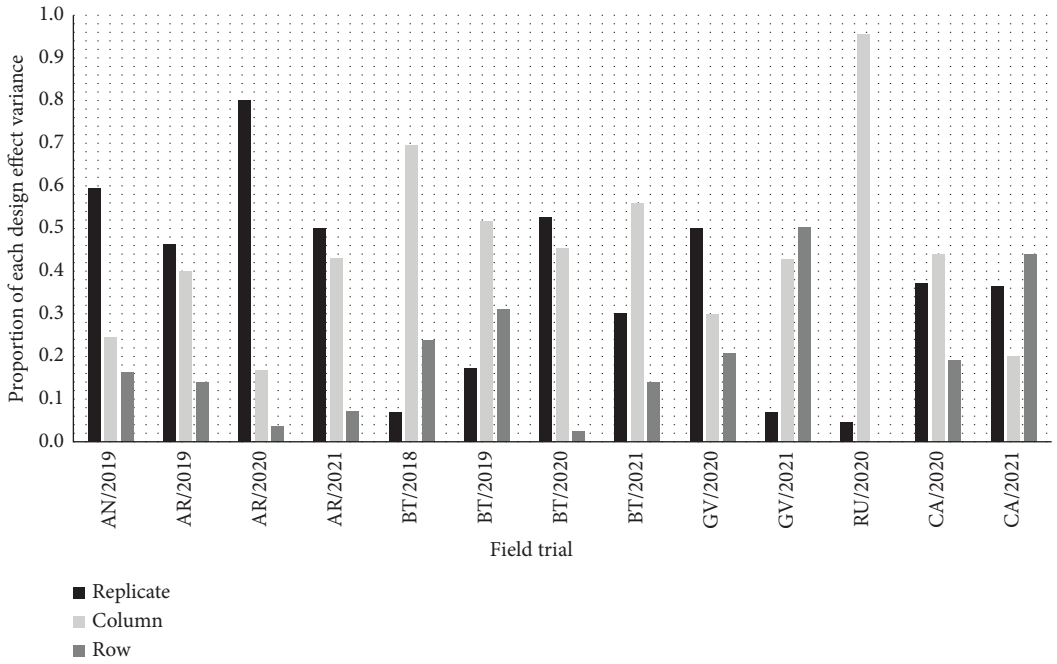

Figure 1: Proportion of each design effects variance (resolvable replicates, columns within replicate, and rows within replicate) in the total design effects variance for all the studied field trials and years. The abbreviations of the varieties are referred to in Table 1.

resolvable replicates was found in Antão Vaz/2019, Arinto/2020, Arinto/2021, Arinto/2019, Bastardo/2020, and Gouveio/2020. For the column within replicate variance, a higher proportion was obtained in Bastardo/2018, Bastardo2019, Bastardo/2021, Rufete2020, and Tinta Caiada/2020. The highest proportions for the row within replicate variance were found in Gouveio/2021 and Tinta Caiada/2021.

When the effects of the experimental design were not included in the analysis (model M0), the results obtained with the estimates of the variance components and their related standard errors showed a higher error variance estimate. Additionally, underestimated and less precise genotypic variance estimates were obtained with the M0 model. For all the cases studied, the genotypic variance component was significant (rejection of H_0: $\sigma_g^2 = 0$, p value <0.05) with RCD analysis.

The results for comparing the efficiency of the experimental design to perform selection within a variety are illustrated through generalized broad-sense heritability (H^2). For all field trials and evaluated years, the values for H^2 are presented to assess the information provided by genotypic causes associated with yield data in these trials. Depending on the field trial and year, the values obtained with the RCD ranged between 0.501 and 0.812. The generally high values observed in this genetic parameter indicate the suitability of these field experiments to perform the selection. The H^2 values were always higher for the RCD analysis, indicating a higher precision in genetic selection. The greater efficiency of the RCD analysis depended on the field trial (the efficiency was greater in Arinto) and on the year. For the Arinto variety, the effects of the experimental design were more effective in 2020 and 2019; for Bastardo in 2019, 2020, and 2021; for Gouveio in 2020; and for Tinta Caiada in 2021. These results are supported by the values of the increase in the efficiency (EF) of the RCD analysis compared with the M0 analysis (Table 2).

3.2. Comparing Fully and Partially Replicated Designs. Two types of experimental designs were compared for the Bastardo variety. Table 3 shows the results obtained for yield data in 4 years with the RCD (fully replicated design) and $\alpha - \alpha$ design (partially replicated). The quantification of genetic variability and, particularly, the detection of intravarietal variability were observed for both experimental designs. However, the precision associated with the genotypic variance component estimate was always higher for the RCD, as shown by the lower standard error associated with this estimate and the higher values for the REML likelihood test statistic. The values of H^2 were also always higher for the RCD, varying between 0.488 and 0.712, whereas for the $\alpha - \alpha$ design, these values ranged from 0.219 to 0.453.

When the average of the 4 years was used, the values of the coefficient of genotypic variation (CV_G) obtained for both designs were similar, although a higher ratio $\widehat{\sigma}_g^2/SE$ for the RCD was obtained; this finding reveals a higher precision in the quantification of intravarietal variability with fully replicated design. When analysing the precision of the selection, a higher value of H^2 was obtained with the RCD (the variation between the two designs was 0.24). Consequently, a more efficient selection will be performed with RCD.

4. Discussion

Controlling error in large field trials represents a key issue in agricultural experiments, and classical randomized complete block designs are not the best option. Hence, two types of

Table 3: Genotypic variance estimate and respective standard error ($\widehat{\sigma}_g^2$ (SE)), broad-sense heritability (H^2), genotypic coefficient of variation (CV_G), and REML log-likelihood ratio test for genotypic variance (σ_g^2) obtained for fully replicated design (resolvable row-column design, RCD) and partially replicated design ($\alpha - \alpha$ design) in Bastardo (BT) variety, for individual years and the average of years.

Field trial/year	Design	$\widehat{\sigma}_g^2$ (SE)	H^2	CV_G (%)	REMLRT (p value)
BT/2018	RCD	0.7684 (0.0800)	0.712	28.47	255.76 (<0.001)
	$\alpha - \alpha$	0.7102 (0.1271)	0.429	22.60	81.12 (<0.001)
BT/2019	RCD	0.8775 (0.1327)	0.501	17.76	75.36 (<0.001)
	$\alpha - \alpha$	0.8228 (0.1897)	0.321	18.10	40.51 (<0.001)
BT/2020	RCD	0.6370 (0.0992)	0.488	16.75	70.06 (<0.001)
	$\alpha - \alpha$	0.5255 (0.1575)	0.219	14.51	20.87 (<0.001)
BT/2021	RCD	1.4065 (0.1800)	0.585	22.34	124.65 (<0.001)
	$\alpha - \alpha$	1.4323 (0.2558)	0.453	25.09	77.34 (<0.001)
BT/mean	RCD	0.6038 (0.0689)	0.653	16.87	182.396 (<0.001)
	$\alpha - \alpha$	0.6664 (0.1243)	0.412	17.82	74.7874 (<0.001)

practices have been proposed to overcome this issue. One type is to use spatial models for the correlation between neighbouring plots, and the other is the adoption of efficient experimental designs. The first uses models that assume that neighbouring plots will share a similar environment [47, 48]. In large grapevine selection field trials, this approach has been shown to be effective [37]. These authors compared the efficiency of mixed spatial models with that of a classical randomized complete block model (with independent and identically distributed errors). Comparisons were based on yield data from large selection field trials of the Arinto, Aragonez, and Viosinho grapevine varieties. The results showed that fitting spatial mixed models to yield data was significantly better than the classical approach, resulting in a positive impact on selection decisions and increasing the accuracy in predicting genetic gain. The other approach, and the most advisable one, is to be ambitious in implementing the experimental design in a field trial. The approach described in this work was found to be particularly useful for illustrating the importance of applying resolvable row-column designs in grapevine field trials. In fact, for all the examples studied, the RCD analysis was shown to outperform the one without the effects of the experimental design. Therefore, better control of spatial and other sources of environmental variation and background noise was achieved using RCD analysis. The model described in equation (1) assumed random effects for the experimental design factors. This finding implies covariance between observations that are made in the same resolvable replicate (complete block), covariance between observations in the same row within a replicate, and covariance between observations in the same column within a replicate. Therefore, observations in the same row and column within a replicate have a covariance of $\sigma_r^2 + \sigma_{\mathrm{col}(r)}^2 + \sigma_{\mathrm{row}(r)}^2$ because they share not only the same replicate effect but also the same row and column effects. Therefore, RCD analysis allows us to model spatial covariance among observations in the field trial. The effectiveness of RCD analysis can also be reinforced by the observation of directional empirical semivariograms of the residuals (Figure 2). For all the field trials studied, the semivariance between residuals is similar as the lag distance increases, showing no pattern of spatial correlation. Hence, these results support the ability of the RCD to control spatial variability. As a complement, it is also important to note that when performing an analysis keeping the design effects and considering a separable first-order autoregressive process to the variance structure of the plot errors, the spatial structure was found to be more appropriate for grapevine selection field trials according to [37], the effectiveness of the RCD analysis was also proved. In fact, convergence problems with spatial models were observed in the field trials of the Bastardo, Gouveio, and Rufete varieties (resulting in very small and poorly estimated variance components associated with the experimental design effects). For this reason, these results are not included in Table 2. Additionally, the higher complexity of the spatial model was penalized in the Antão Vaz and Arinto field trials (compared with the AIC values described in Table 2, higher or similar AIC values were obtained by the spatial model). When compared with the results of the RCD analysis (Table 2), with the complement of the spatial analysis in the Tinta Caiada variety, slightly lower values for AIC and similar values for H^2 were obtained.

In sum, the correct strategy for field experiments should start with a well-planned, fully replicated design, followed by the fitting of a model with all design effects, in accordance with the randomization scheme. Then, in the diagnosis of the residuals, spatial correlation did not accounted for among the design effects should be checked. Spatial models should be used as an alternative when the experimental design is unsuccessful and, when necessary, as a complement to the experimental design.

The high efficiency of the RCD analysis depended on the field trial and, for each field trial, on the year (Table 2, Figure 1). In fact, each field trial has its own unique features in terms of soil, slope, and cultural techniques, which define the efficiency of the effects of the experimental design. In addition, the conditions in each field trial change from year to year, resulting from the effects of several cultural techniques, pests and diseases, rainfall, and other specific conditions. This justifies the different results obtained according to the year for the proportion of the variability of the different design effects (Figure 1), mainly in the cases of the field trials of Gouveio and Tinta Caiada varieties, as well as

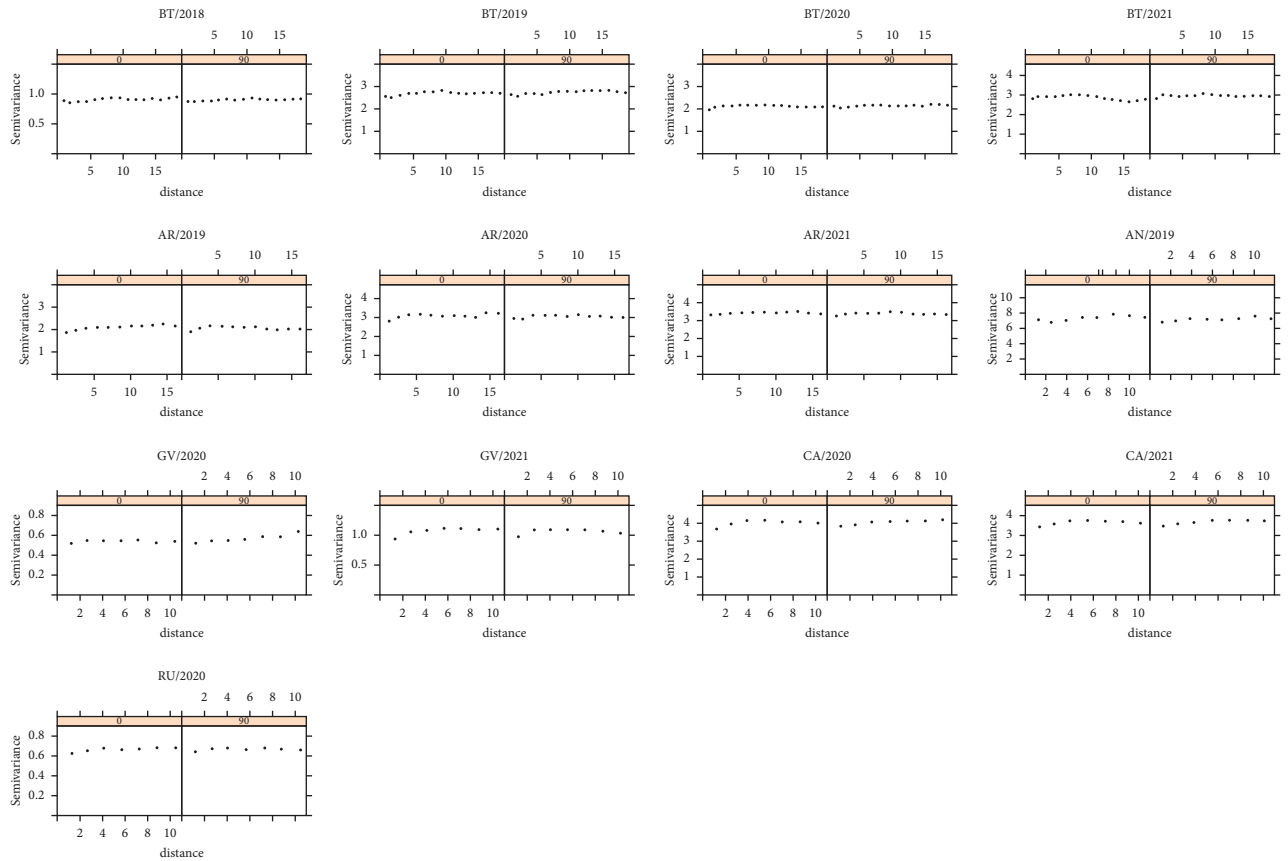

Figure 2: Empirical semivariograms of the residuals resulting from the fitting of the RCD model for row (0) and column (90) directions for all field trials studied. The abbreviations of the varieties are referred to in Table 1.

for the low value of efficiency of RCD (EF, Table 2) for Bastardo variety in 2018. As a consequence, in some years, the effectiveness of the design effects was higher. Therefore, as the conditions in the field are unpredictable, establishing the experimental design should be ambitious, and all possible sources of variation that can affect an experiment should be considered.

The high values of H^2 obtained are the results of not only the randomization process of the experimental design (resolvable row-column design), as previously discussed but also other reasons. One reason is related to the genotypic variability within each variety; genotypic variability obviously influences the value of heritability (the higher the genotypic variability within a variety, the higher the value of H^2). The other reason is related to the overall planning of the field trial. It is essential to be demanding when choosing the location of the field trial and to try to ensure that the conditions are as homogeneous as possible. Another factor in the success of an experiment is the number of replications. As is well known, the number of replicates is essential to allow a valid estimation of the error variance and to reduce its estimate. In this study, most of the field trials were established with 6 replicates, helping to reduce prediction error variances and therefore increasing H^2. In fact, the number of replicates (no less than 4 replicates for yield data of grapevine) proved to be a very important issue in the accuracy and precision of the performed selection in a methodological study conducted by [49]. The last factor relevant to be mentioned is related to the number of plants per plot. Plots with 3 plants and their average yield were used for the analysis. Using an average of 3 plants instead of using a plot with a single plant is also another strategy to reduce the error variance (the variance of a mean is lower).

Another important question related to a randomized design is neighbour balance, meaning that each treatment (in this case, each clone) has varying neighbours across replications, and the number of direct adjacencies is balanced between pairs of clones. Neighbour balance is ensured by imposing spatial restrictions during the design search. The field trials studied in this work were installed for several years and were generated using the package CycDesigN 4.0 with the "spatial" option, which assumed an exponential covariance model. However, the generation of row-column designs with higher efficiency in good neighbour balance properties has been continuously studied and discussed [17, 18], and more recent approaches have been recently implemented in experimental design packages [19].

The results obtained with a partially replicated design using real yield field data were presented for the first time in grapevine conservation. This type of experimental design showed a lower value of broad-sense heritability, therefore, higher prediction error variances of genotypic effects and less efficiency in selection. On the other hand, this type of experimental design proved to be useful for quantifying the

intravarietal variability; the results accord with those obtained by simulation in grapevine field trials [36]. Concretely, a partially replicated design was more useful when the average of years was used, although with lower precision than a fully replicated design. In fact, in a field trial, the quantification of the intravarietal variability of the yield will differ among years because several sources of error variation (such as evaluation errors and other environmental deviations among years) are present and because the range of genetic differences among genotypes differs with the year due to G × E interaction. Therefore, an overall view of the genetic variability within a variety requires more than one year of evaluation, and usually, the average of years is used for quantifying intravarietal variability [4].

As previously mentioned, for other species, the topic of partially replicated designs is currently addressed, but in a different context from the one proposed in this work for grapevine varieties. In fact, the use of these designs has been restricted to early-generation testing in plant breeding, where the seeds of new candidate lines are usually limited and multienvironment trials have been established. In early-generation testing, replication usually occurs at a higher level because trials are replicated across sites, meaning that for the multilocation design, there will be replication for all entries [50]. The approach presented in this work implies no replication for some genotypes and thus reinforces the strategy of using this type of experimental design only for quantifying intravarietal variability but not for performing the selection. In sum, to perform polyclonal selection, a field trial with a fully replicated design must be installed. Nevertheless, for the study of intravarietal variability in a rarely grown and little-known variety, a partially replicated design can be an interesting option. Based on simulation studies in the context of grapevines, the utilization of partially replicated designs has already been indicated for research on rarely grown varieties [36]. In fact, in Mediterranean countries, there is a large richness of ancient varieties, and for many of these varieties, there is a lack of knowledge about their intravarietal variability and usefulness for selection. With yield data, this work proved that a preliminary analysis of the potential of these varieties would be possible in a smaller experimental area with lower costs and efforts.

5. Conclusions

Useful grapevine selection experiments require efficient experimental designs. The results obtained with real field data reinforce the importance of resolvable row-column designs to improve the control of spatial variation and background noise and the efficiency of grapevine selection. The results obtained with a partially replicated design have proven their usefulness in evaluating the intravarietal variability when the average of years is used.

Data Availability

The data used to support the findings of this study cannot be made available due to its proprietary nature.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors have contributed significantly to this work and all are in agreement with the manuscript.

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