Progress in mass and clonal selection of grapevine varieties in Portugal

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Summary: Since 1978 we have developed methods for mass and clonal selection of grapevine well adapted to the conditions of Portuguese viticulture. The most remarkable innovation of this methodology is the establishment of large experimental populations of clones in which good estimates of yield heritability and genetic gain can be obtained.

The aim of the present paper is to clarify some methodological aspects such as (1) the ideal composition and structure of the experimental populations of clones and (2) the application of this methodology to a large number of Portuguese varieties, in order to maximize the rate of yield improvement. At present we can point out the following results:

1. According to experimental data and computer simulations, we may conclude that the optimal number of clones to be included in each population is about 200-300, each one represented by 4 vines in 5 replications. By this way we can usually obtain heritability estimates between 30 and 80 % and genetic gain values higher than 15 %.

2. Until now, we have succeeded in applying this methodology to 30 of the most important Portuguese varieties, which represent more than 80 % of all varieties normally propagated in the country.

Key words: clone, selection, variety of vine, Portugal, yield, analysis, computer simulation, genetics.

Introduction

The methodology of selection followed in different countries usually comprises several cycles of selection from an initial cycle, with a high number of genotypes up to a final cycle in which a unique genotype is selected. Clonal selection may take from 20 to 25 years (Schöffling and Bender-Berland 1983).

However, the genotypes selected during the first cycle may already be used by vinegrowers as mass vegetative material, supplying high yield gains in a very short time. In countries that have recently started systematic selection, such as Portugal, there is urgent need to obtain better propagation materials, making this procedure particularly attractive.

In order to develop this mass selection methodology we are introducing statistical and genetical instruments of analysis – heritability (h^2) and genetic gain (R) – which have not yet been used in clonal grapevine selection of other countries. These techniques are applied to data analysis of clone populations yield, which corresponds to the second cycle of the integrated selection methodology that has been used since 1978 (Martins *et al.* 1987).

The possibility of obtaining useful results with this data analysis depends on the factors included in the expression for calculation of the genetic gain $R = i \cdot s \cdot h^2$. Owing to the lack of bibliographic information on this subject, the main purpose of this paper is to obtain, using computer simulations, the most favourable values of these factors. In the second part of this paper, the application of the described methods to the most important Portuguese grapevine varieties selection and the obtained data will be discussed.

Material and methods

The initial data consisted of the yield of individual plants out of a population of 189 clones of cv. Periquita, set up in two blocks and two replications of seven plants with the following statistical

indicators: mean of first block – 1.972; mean of second block – 1.472; general mean – 1.722; standard deviation – 0.556; heritability – 0.378. Using as standard values the yield of this population, BASIC programs (VAX BASIC V3.2) have been written to generate populations of random numbers (corresponding to total fruit weight per vine) with estimated variation values – medium per block, variance of media and heritabiity (h^2) – similar to those of the Periquita population.

The subroutine that generates the individual weights is, according to the classical model, $Y_{ij} = m + g_i + b_i + e_{ij}$ and it has the following values:

$$\text{vine} = 1.722 + r(\text{rep}) + f(c) + \text{rnd} \cdot m(\text{rep}) - m(\text{rep})/2$$

(vine = individual vine fruit weight; r(rep) = between block variation; f(c) = genotypic variation; m(rep) = modified factor of individual variance according to the block).

The intensity of selection (i) values in the calculation of the genetic gain (R), has been obtained from the SMITH's (1969) formula:

$$i = 0.8 + 0.4 \cdot \log(1/P-1).$$

Results and discussion

The selected number of clones in an experimental population has a high influence on the estimated gain (through i). However, this number has to be chosen regarding other aims, such as: a guarantee of a good stability for the observed gains, a high level of genetic variability and a maintenance of the initial enological quality level of the grapevine variety.

All the statements mentioned above suggest that a high number of clones is needed, while the value of gain and management of the propagation procedure recommends low numbers. In an empirical approach, we have adopted numbers around 40 clones.

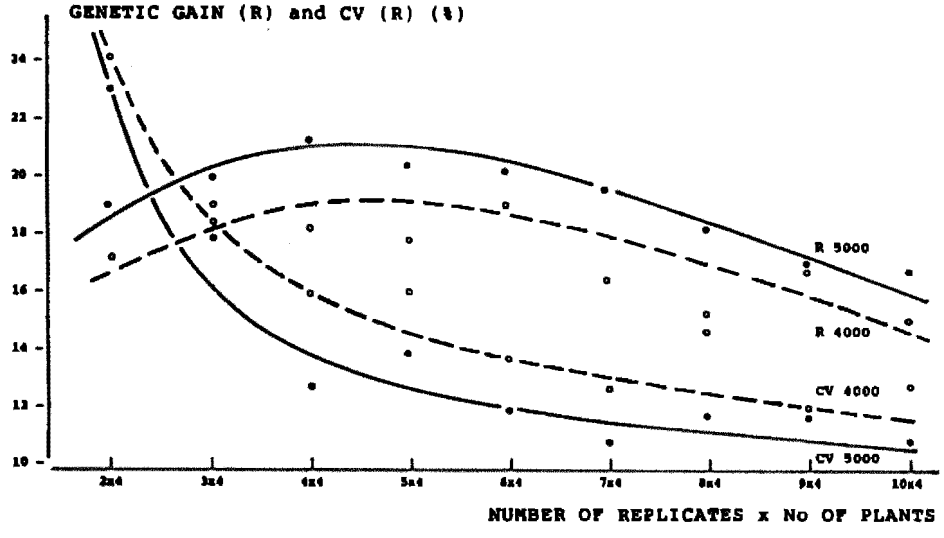

Fig. 1: Genetic gain (R) and coefficient of variation of the gain (CV(R)) when the number of replications varies from 2 to 10, the total numbers of plants in the experiment are 4000 or 5000 and the number of plants per replication is constant.

As far as the remaining gain factors are concerned, they depend on the number and the nature of the selected clones in the population and on the experimental design adopted. Moreover, some aspects of the experimental design adopted are due to some practical considerations that are independent from the methodology: (1) the size of plots (number of vines in a minor experimental unit) can hardly be less than 4, because this is a fairly small number and easy to limit in the field (half of the numbers of vines between two posts in our vineyard); (2) the total number of plants in an experiment which can hardly go beyond 4000-5000 (= 1.3-1.6 ha) otherwise it would drastically increase the environment variability and would make the completion of many experimental operations difficult.

According to what has been said, populations with areas of 1.3-1.6 ha (4000-5000 plants) having 4 vines per replicate, have been used in several computer simulations to better clarify the balance between the number of replications and the number of clones and to obtain the maximum rate of gain. We used the estimated gain (R) and the respective coefficient of variation among several experiments with the same structure as leading indicators of goodness of several types of experiments. The data corresponding to 1000 simulations are shown in Fig. 1.

The results show that the gain is maximum when experiments with 4-5 replications are considered, and the coefficient of variation decreases even more beyond this limit, although slowly. We may conclude that experiments with 5 replications of 4 vines each will give the highest and most accurate gain estimate (low coefficient of variation).

It may be verified that the genetic gains are higher and the coefficient of variation lower when the number of vines increases in the experiment. However, these data have to be carefully considered, as the environment variability increases when the area of the experiment increases, and this factor is not included in the simulated model which generated these data.

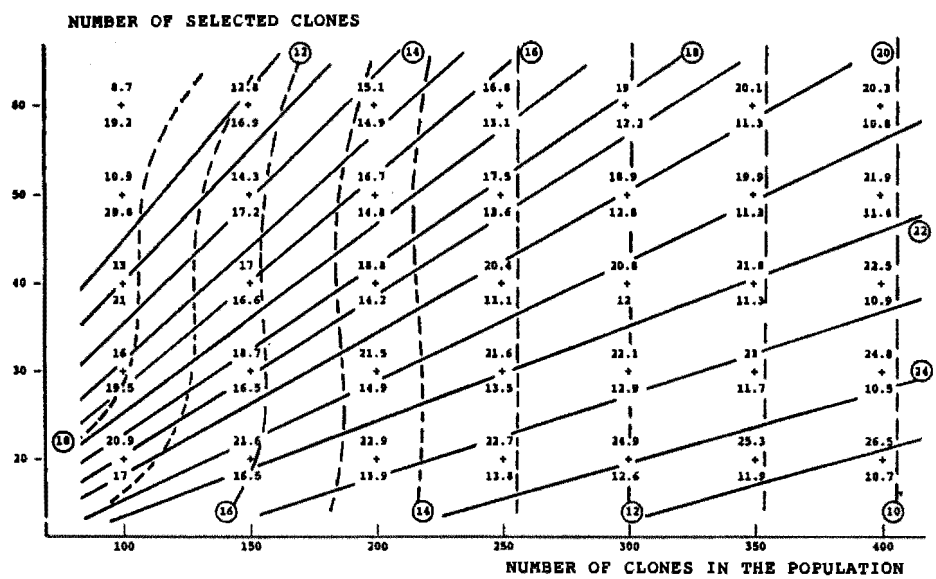

Fig. 2: Isolines of the gain (sloped lines, numbers above the chart) and of coefficient of variation of gain (vertical lines, numbers below the chart) as a function of the clones number in the experimental population and of the number of selected clones. Average data of 600 computer simulations regarding plots of 5 replications of 4 vines each (all values in the chart are in percent).

Estimate of heritability (h^2) and genetic gain of yield (R) in several experimental populations of clones

POPULATION-SITE, YEAR	No. CLONES POPULATION/SELECTED	MEAN	STAND. DEV.	HERIT. (h^2)	GENETIC GAIN (R)
Touriga-Douro,84	69 / 20	0.675	0.269	0.656	30.3 %
Periquita-Ribatejo,84	189 / 40	1.722	0.556	0.378	16.2 %
Touriga- Dão,85	49 / 20	1.777	0.712	0.780	29.6 %
Alvarelhão-Dão,86	32 / 20	2.429	0.658	0.738	11.9 %
Loureiro-V. Verdes,87	132 / 30	2.248	0.591	0.356	12.1 %
Touriga-Dão,87	52 / 20	3.354	1.047	0.805	24.8 %
Trajadura-V. Verdes,88	191 / 40	0.751	0.447	0.201	15.9 %
Vinhão-V. Verdes,88	211 / 40	0.697	0.351	0.386	26.8 %
Avesso-V. Verdes,88	164 / 40	1.553	0.427	0.228	8.8 %
Siria-Pinhel,88	239 / 40	2.282	0.799	0.777	39.2 %
Trincadeira-Ribatejo,88	272 / 40	0.413	0.136	0.587	29.1 %
Arinto-Oeste,88	266 / 40	0.522	0.268	0.650	49.8 %
Antão Vaz-Alentejo,88	210 / 40	1.465	0.322	0.423	12.8 %
Aragonêz-Alentejo,88	231 / 40	2.134	0.599	0.474	19.0 %

In a second set of computer simulations, the model structure was set to 5 replications of 4 plants each, and the number of population clones was varied as well as the number of selected clones.

The results are plotted in Fig. 2. We can observe that to the right hand of the chart the gain and the coefficient of variation of the gain isolines are progressively separating and the isolines of gain are also becoming flatter. In the population band of 250-300 clones we can visualize an area which divides the chart in two. On the left side the isolines are spaced closely with steep slopes, and on the right side they are separated by greater distances with shallow slopes. In reference to the numbers of clones, below 250-300 clones small increases of this number result in great increases of gain (R) and great decreases of coefficient of variation, above this limit the tendencies are reduced.

The data plotted in Figs. 1 and 2 suggest that populations of 250 clones and 5 replications of 4 plants each should be used. This design results in a suitable mass selection procedure when the 40 best clones are selected. Under these conditions and if the variation of genetic gain and environmental indicators are similar to those of the Periquita populations values (which have been taken as a standard value), the gains of estimated yield will be approximately 20 % and the coefficient of variation about 14 %.

According to these conclusions, we have been applying this methodology of mass selection (in the first cycle of clonal selection) to the most important Portuguese grapevine varieties and some of these results are shown in the table.

The field data, when compared with the results of computer simulations, revealed a satisfactory fit. Meanwhile, we are actually carrying out the first experiments to determine the gains of selected material and then we will be able to verify the consistency with the estimated gains.

Conclusions

The computer simulations allowed us to establish an adequate structure for experimental populations of grapevine clones – 250 clones, 5 replications and 4 plants each – which enable a 20 % gain in yield per cycle of mass selection, with each cycle lasting approximately 5 years.

The application of this methodology to Portuguese grapevine varieties supports the results foreseen in computer simulations and proves to be a valuable contribution to the improvement of Portuguese viticulture.

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Serological detection of closterovirus-like particles associated with grapevine leafroll disease - an improvement in clonal selection

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A b s t r a c t : Leafroll disease is one of the most widespread and damaging virus diseases of the grapevine. Up to now the diagnosis for sanitary selection was carried out by grafting an indicator cultivar (e. g. Blauer Spätburgunder) onto the scion to be tested. Very recently, closteroviruses have been detected in leafroll diseased grapevines. In all the cases, closteroviruses were associated with leafroll affected plants. In the meantime, antisera produced against two types of closterovirus associated with grapevine leafroll disease (GLRaV Type 1, Type 3) have become available.

The viral antigens could be detected serologically by ELISA in both diseased and symptom-free leaves, in dormant buds and in phloem extracts of infected plants.

A survey of leafroll disease was carried out in the viticultural regions of Baden-Württemberg. Samples based on visual observations made in commercial vineyards and in an indexing field were tested by ELISA using antisera against $GLRaV_1$ and $GLRaV_3$. $GLRaV_1$ could be detected by ELISA in all the plants from the commercial vineyards showing leafroll. $GLRaV_3$ only occurred in a few samples. Several samples were investigated by means of immunosorbent-electronmicroscopy (ISEM). In all the extracts from affected plants, closterovirus-like particles decorated with the specific antibodies could be detected. No decorated particles were found in extracts from healthy grapevines. All the plants from the indexing field showing symptoms over 3 years were infected with $GLRaV_1$ according to the ELISA test. Symptom-free indicator plants never showed a positive reaction either with $GLRaV_1$ or $GLRaV_3$.

It was thus possible to confirm the association between leafroll symptoms and serologically detectable closteroviruses. ELISA appears to represent a good tool for the diagnosis of grapevine leafroll disease and an improvement for the sanitary selection of clones.