PRESERVATION OF INTRAVARIETAL DIVERSITY IN CLONAL AND SANITARY PRE-SELECTION FOR A MINORITY VARIETY IN DANGER OF EXTINCTION: MATURANA BLANCA

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Abstract

Aim: An objective methodology to preserve intravarietal diversity during the stage of clonal and sanitary pre-selection is exposed. The case of the minority variety in danger of extinction called Maturana Blanca is analyzed.

Methods and results: The cluster analysis of the initial population's data (OIV descriptors) allows leading the pre-selection with the objective of preserving the intravarietal diversity. The pre-selection process, where one accession from each cluster is selected and its sanitary status evaluated, is carried out for three consecutive years. It is verified that the intravarietal diversity is kept after the pre-selection by comparing the distribution of the selected vines to the ones of the initial population for each variable and the principal component analysis of both populations.

Significance and impact of the study: The advantage of this method consists of grouping the individuals by a statistical procedure of cluster analysis, enabling a randomized selection.

Conclusion: The methodology that has been established to carry out the clonal and sanitary pre-selection of the variety Maturana Blanca allows the preservation of the existing intravarietal diversity.

Key words: pre-selection, cluster analysis, grapevine, weak type selection

Résumé

Objectifs: Afin de préserver la diversité intra-variétale pendant la phase de présélection clonale et sanitaire, une méthodologie objective a été effectuée. Le cas de la variété minoritaire en danger d'extinction appelée Maturana Blanca a été analysée.

Méthodes et resultats: L'analyse des données de la population initiale (descripteurs OIV) permet de diriger la présélection avec l'objectif de préserver la diversité intra-variétale. Le processus de présélection, où une accession de chaque groupe est sélectionnée et son statut sanitaire évalué, est effectué pendant trois années consécutives. Il est vérifié que la diversité intravariétale est conservée après la présélection en comparant la répartition des vignes sélectionnées à celles de la population initiale pour chaque variable et l'analyse en composantes principales des deux populations.

Conclusion: La méthodologie qui a été établie pour effectuer la présélection clonale et sanitaire de la variété Maturana Blanca permet la préservation de la diversité intra-variétale existante.

Importance et impact de l'étude: L'avantage de cette méthode consiste à regrouper les individus par une méthode statistique d'analyse de regroupement, permettant une sélection aléatoire.

Mots clés: présélection, analyse de regroupement, vigne, sélection de type faible

manuscript received 30th October 2010 - revised manuscript received 3th May 2012

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J. Int. Sci. Vigne Vin, 2012, **46**, n°2, 123-130

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INTRODUCTION

In general, the objective of clonal selection is focused on getting individuals that are more resistant to certain circumstances (e.g., environmental factors such as soil salinity or drought, biotic stresses such as viruses and pests, or sensory and physico-chemical features), while preserving the genetic characteristics of the plant with regard to the variety. In fact, the preservation of the varietal heritage, which generates wines with personality over the years in a grape growing region, is the main objective when working with grape varieties.

All the varieties have two possible origins : one seed resulting in an individual that has been propagated vegetatively and has incorporated somatic mutations over the years, or more than one seed (polyclonal) with morphological uniformity derived from isolated and closely related wild grape populations (Rives, 1961, 1981 ; Roselli and Scaramuzzi, 1974 ; Mullins and Meredith, 1989). The polyclonal genetic origin of grape varieties and the progressive accumulation of genetic mutations may explain the great genetic variability (Rives, 1961 ; Ulanovsky *et al*., 2002 ; Sefc *et al*., 2009).

It is important to take into account that, in viticulture, most varieties consist of a population of clones or individuals, with very similar genetic profile ; hence, they constitute a variety, with very similar features and phenotypical characteristics. However, they also show morphological differences in several aspects of the typical characteristics of the variety, such as the time of bud burst, the length of vegetative cycle, vigour, yield, bunch compactness, or phenols and soluble solids accumulation (Yuste *et al*., 1998).

These differences are sometimes due to the influence of ambient factors such as viruses, climate, soil, etc. The current works of selection take into consideration not only the varietal aptitude and the adaptation to the environment but also the search for plants that are free of the most common viruses (Walter and Martelli, 1996).

Nowadays, the process of grapevine selection is divided in three parts : clonal pre-selection (exploration), sanitary selection and clonal selection. The pre-selection implies a methodical monitoring of the accessions, in order to select those that represent the general characteristics of the variety. In this respect, and with regard to the objectives of the selection, it is important to emphasize the importance of selecting the greater intravarietal diversity rather than restricting the selection to a few genotypes that might have interesting characteristics but do not represent the whole potential of the variety (Lacombe *et al*., 2004).

The second part of grapevine selection, the sanitary selection, implies the performance of legal regulations set up by two different organisms :

- The Technical Regulations of Control and Certification of the Vine Plants "Reglamento Técnico de Control y Certificación de Plantas de Vid", Spanish Official Gazette, 1st July 1986.

- The European Guidelines 68/193, 74/649 and 77/129, where it is stated that the vine-growing plant material, which is a marketing object, must fulfil a series of requirements concerning varietal and sanitary quality, taking into account the Grapevine Fanleaf, Leafroll, and Fleck viruses.

Clonal selection has provided high quality propagation material with undoubted advantages, especially as regards to phytosanitary standards. However, it has also been detrimental to the preservation of the grapevine biodiversity. For the local grape varieties in particular, it is proposed a non-intensive and objective selection (Osler *et al*., 2008).

Recently, Martínez de Toda and Sancha (1995) started a project in order to recover, preserve and study the endangered grapevine germplasms in Rioja (Spain). One of the most interesting cultivars studied in this project, initially known as Ribadavia, has been proved to be a synonym of Maturana Blanca. This variety is almost extinct in the Rioja appellation (Cervera *et al*., 1998 ; Martínez de Toda and Sancha, 1997 ; Ulanovsky *et al*., 2002). Initially, there exists only one old vineyard in Rioja and in all the northern region of Spain where Maturana Blanca is cultivated widely. It is located in the municipality of Sotés. This work aims to tackle the clonal and sanitary pre-selection of Maturana Blanca in this vineyard, preserving as much intravarietal diversity as possible.

MATERIALS AND METHODS

1. Plant material

The plant material under study was located in the only old vineyard planted with Maturana Blanca of the Rioja appellation (north of Spain). This commercial vineyard was located in Sotés (42°24'1" N, 2°37'35" W, 672 m above sea level). The vines were grown as bush vines and the parcel was in a flat area on homogeneous clay soil. The vineyard was rain fed and subjected to the common agricultural practices of the region. There was a total of 524 Maturana Blanca individuals in the vineyard ; of which 287 showed morphological anomalies, anomalies in their vegetative cycle, signs of virus and/or susceptibility to pests and diseases. The 287 individuals that showed lack of varietal identity were removed from the study, and the

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remaining 237 accessions were used for the pre-selection of the Maturana Blanca vineyard.

2. Collection of data

This work studies several parameters associated to different phenological stages of the accessions during the vegetative cycle. In order to assess these parameters, the following data were collected:

- Bud burst: number of spurs per vine, number of buds left at pruning season, number of sprouted buds and number of secondary buds per vine.

- Berry set: number of bunches and number of shoots per vine.

- Ripening: bunch size (characters OIV 202 and 203), bunch compactness (character OIV 204) and berry size (characters OIV 220 and 221), estimated according to the OIV descriptor list for grapevine varieties and Vitis species (OIV, 1983). Furthermore, the average bunch weight and the average yield per vine were assessed for each accession.

These data were used to graphically represent the distribution of the accessions according to each variable: percentage of bud burst, number of bunches per shoot, mean bunch weight, bunch compactness, berry size and average yield per accession.

3. Clonal and sanitary pre-selection

The criterion followed in order to do the pre-selection consisted of two parts. In the first part, a cluster analysis was carried out using the SPSS statistical package (SPSS for Windows 11.0, Chicago; SPSS Inc., 2001). This analysis was done by means of the option 'Hierarchical cluster analysis', applying the method of Pearson's correlation and standardizing the data according to the 'Zscore' method (mean = 0 and standard deviation = 1). Unweighted pair-group method using arithmetic averages (UPGMA) was used in order to group the accessions together and to obtain the dendrogram. From this dendrogram, several groups of accessions were determined for a dissimilarity level. The limit was established on each dendrogram according to the distribution. The level depended on the accessions that were analyzed and the rigour of the researcher's criteria: the more permissive the criterion, the higher the level established and the more accessions considered as similar within each group.

Table 1. Objective varietal characteristics for the selection in each group.

Characteristic		*Optimum value*
Bud burst		100 %
Bunches per shoot		2
Mean bunch weight		100-200 g
Bunch density		medium-low
Berry size		medium-low
Average yield per vine		2 kg
Virus:	Grapevine Fanleaf	free
	Grapevine LeafRoll	free
	Grapevine Fleck	free

In the second part of the methodology, one accession was selected from each group established in the first part. The grouping methodology developed in the first part was defined according to the statistical analysis criteria, thereby ensuring that the work of pre-selection was carried out over all the intravarietal diversity. The selection of one accession in each group could be done at random, but here it was based on the varietal characteristics that were established as a selection objective (Table 1). As a consequence, one accession whose characteristics were most similar to the defined ones was selected from each group of accessions (all along the intravarietal diversity). It was a way of preserving the intravarietal diversity, since all the groups were represented. At the same time, the presence of those interesting characters in the selected accessions was guaranteed.

These pre-selected accessions were analyzed by ELISA in order to determine their sanitary status and the grapevine viruses: Grapevine Fanleaf Virus (GFLV), Grapevine LeafRoll associated Virus (GLRaV), and Grapevine Fleck Virus (GFV). After that, the virus-positive accessions were eliminated from further analysis.

The "Clonal and Sanitary Pre-selection" process, where one accession of each group is selected and evaluated for grapevine viruses (sanitary status), was carried out every year for three consecutive years. After three years, the absence of viruses is guaranteed because the selected individuals have been analyzed more than one year. Furthermore, its agricultural characteristics are consistent, avoiding the influence of one year. Both factors are the main advantages of a three-year pre-selection over a one-year pre-selection.

4. Checking the Preservation of Intravarietal Diversity after Pre-selection

In order to prove that the intravarietal diversity of the initial population was preserved among the selected individuals every studied year, the distribution of the selected accessions was represented graphically among

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the original population for the considered variables. The data considered each year for each individual were the average values of that year and the previous ones. The curves of the distribution of the three populations were compared: similar kind of curves would indicate that the intravarietal diversity has been preserved, according to the original population.

The principal components analysis (PCA) is also a tool for this checking. This analysis is included in the "Factor analysis" option of the statistical program SPSS 11.0. The correlation coefficients are used in order to find the 'Correlation Matrix' and two factors have been extracted. The obtained factor scores constitute the X and Y axis in the graphic representation of the studied accessions. PCAs were carried out over the initial population of 237 accessions and over the selected populations for three years. The results of the four populations were compared according to two criteria: variables with the most weight in the variability of the population and distribution of the accessions according to the components I and II. The intravarietal diversity was preserved if, according to the observed parameters of the study, there were no differences among these results.

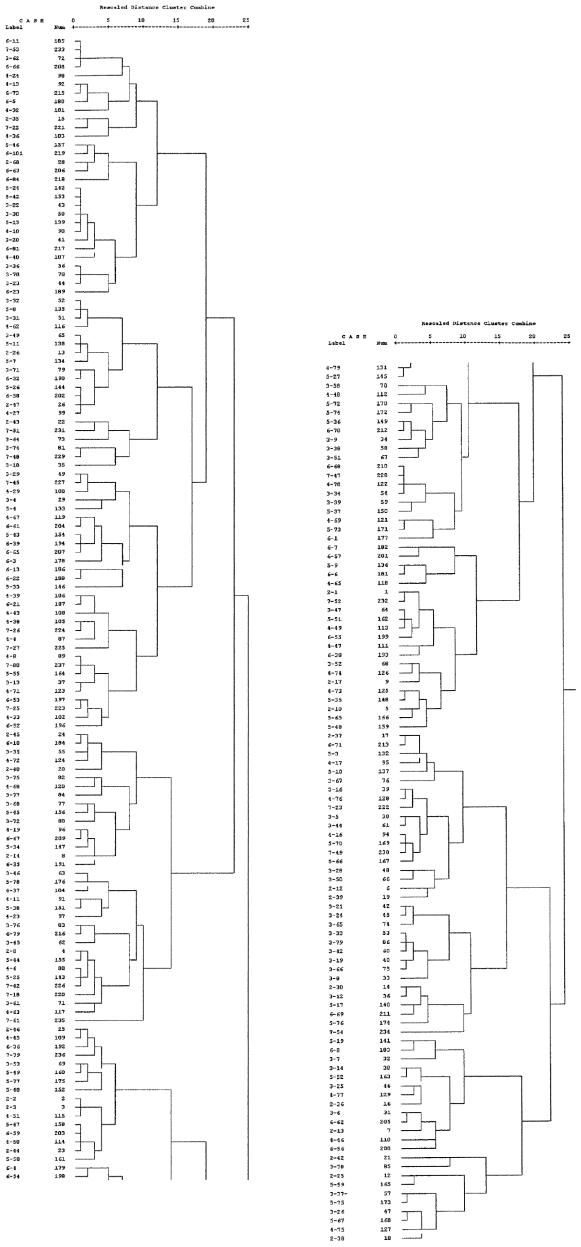


Figure 1. Dendrogram of all the variables, obtained in the first year, among 237 accessions. The dendrogram was obtained by Pearson coefficient and UPGMA analysis using SPSS 11.0 software.

In the last two years of the study, a more complete analysis of the berry was developed. For each accession, 100 berries were weighed and a must analysis was carried out. The acidity ($g \cdot L^{-1}$ tartaric acid) and the probable alcohol were analyzed. The results of these analyses were not used as selection variables, but they provided an idea of the selected population diversity (6 accessions). The chemical parameters were not included as pre-selection criteria because the vegetative and productive indexes provided enough information to study the intravarietal heterogeneity. The chemical characteristics were measured at the end of the study, in order to corroborate that the six selected accessions had a suitable grape composition and there were not any deviance.

RESULTS AND DISCUSSION

The 237 accessions were submitted to the first cluster analysis, according to the studied variables. The result among all the accessions is represented in figure 1. This dendrogram was the basis for the pre-selection. All the accessions were included in several groups, according to their similarity for the variables considered in the study. A level of distance was selected. The more restrictive is the level, the higher is the number of groups, and the more accessions are selected for the following year. For a three-year pre-selection program, approximately one out of five accessions is selected every year. Depending on the selection intensity, the number of accessions left is more restrictive. Considering the mentioned criteria, for a medium restrictive level, the accessions were considered in the same group for a level of distance of 6.

The first year, 53 different groups were established and one accession from each group was selected ("First selection"). The criterion based on the characteristics that have been established as objective of the selection was used, as indicated in Materials and Methods (Table 1). That way, it is assumed that the selected accessions represent the best intravarietal diversity, as there is one accession from each group. After that, the sanitary status

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(grapevine viruses) was checked in the 53 different accessions, resulting in 0 virus-positive cases.

The same method was followed the second year of the study. The new dendrogram allowed us to identify 15 different groups and again one accession from each group was selected ("Second selection"). In the last year, the dendrogram allowed the selection of 6 accessions ("Third selection", underlined in Figure 2). It was assumed that these selected accessions represent the intravarietal diversity of the initial population.

The interest of this method over traditional methods is that the plant selection is performed on the groups obtained by statistical analysis (cluster analysis), implying greater objectivity and ensuring the maintenance of population diversity.

In order to check that this was achieved, the data obtained from the initial population (237 accessions) were represented graphically and contrasted against the distribution of the first, second, and third round of selected accessions (Figure 3). In this figure, the accessions were distributed according to the values of each one of the variables that have been considered.

In relation to the initial population, we can see that, for the percentage of bud burst, most of the accessions (more than 200 of 237) vary from 90 to 100 % of bud burst. As regards to the ratio of bunches per shoot, the greater group of accessions ranges between 1.75 and 2, although the range of the variable is wide, including accessions between 0.75 and 2.25 bunches. The mean bunch weight has two noteworthy groups of accessions: from 70 to 150 g and from 150 to 280 g. According to

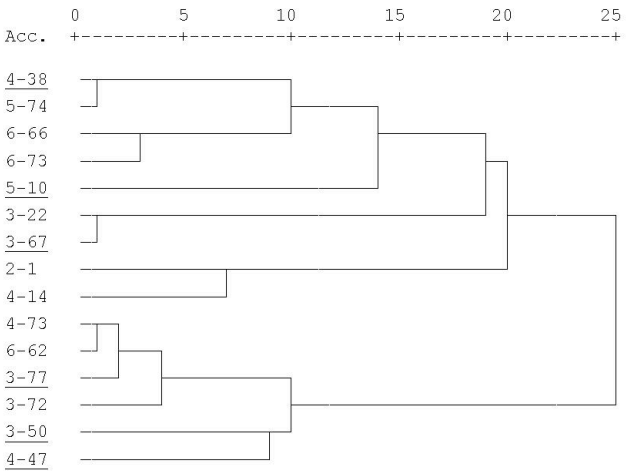


Figure 2. Dendrogram of all the variables, obtained in the last year of the study, among 15 accessions. The dendrogram was obtained by Pearson coefficient and UPGMA analysis using SPSS 11.0 software. Accessions underlined are the pre-selected ones.

bunch density and berry size, most of the accessions have average values. Finally, the average yield per vine shows that the main group ranges between 0.5-2,5 kg. In all cases, except for the percentage of bud burst, the distribution of the accessions shows a normal distribution curve, which suggests that the intravarietal diversity has been preserved.

If we focus on the selected accessions - first, second, and third year - in relation to the percentage of bud burst, the 90-100 % group is still the majority one after the selection. The program has been favourable to these accessions, to the detriment of those that have a bud burst between 80 and 90 %, which are now less numerous. In the ratio of bunches per shoot, the groups of accessions with 1.25-2 bunches continue being the majority group during the three years. The same applies for the representations of berry size, mean bunch weight, and average yield per vine: the majority groups in the initial population continue being the majority groups in the selected populations, and the rest of the groups decrease in a proportional way. In the case of bunch density, the selection seems to have been favourable to the accessions with more compact bunches, to the detriment of those with looser bunches (this fact could have been influenced by the selected individuals considering other criterion such as bunch weight and yield per vine).

In summary, it is possible to see that in most cases the same tendency -a normal distribution curve- has been observed. This fact shows that the pre-selection method exposed is not favourable to the preservation of the most productive accessions or the accessions with particular characteristics. According to the hypothesis described by Scienza and Valenti (1999), this method of clonal selection is a weak type selection.

According to these authors, the traditional model of clonal selection is considered a stabilizer, insofar as it is favourable to the medium biotype at the expense of those that are in the two asymptotes of the normal distribution curve, decreasing the population variability. Meanwhile, the directed selection rewards the extreme phenotype, both right and left side of the distribution. On the other hand, the destructive selection favours both extremes of the distribution at the expense of the medium area (double selection), thus creating two new different populations.

The adoption of a weak type clonal selection method makes the maximum genetic progress possible for the variety: it turns the germplasm collection into a secondary centre of variability, where a great part of the biotypes that are representative of the population variability are present. However, it requires a longer period of time than the strong selection approach.

On the other hand, the comparison of the PCAs of the initial population and the accessions selected during the

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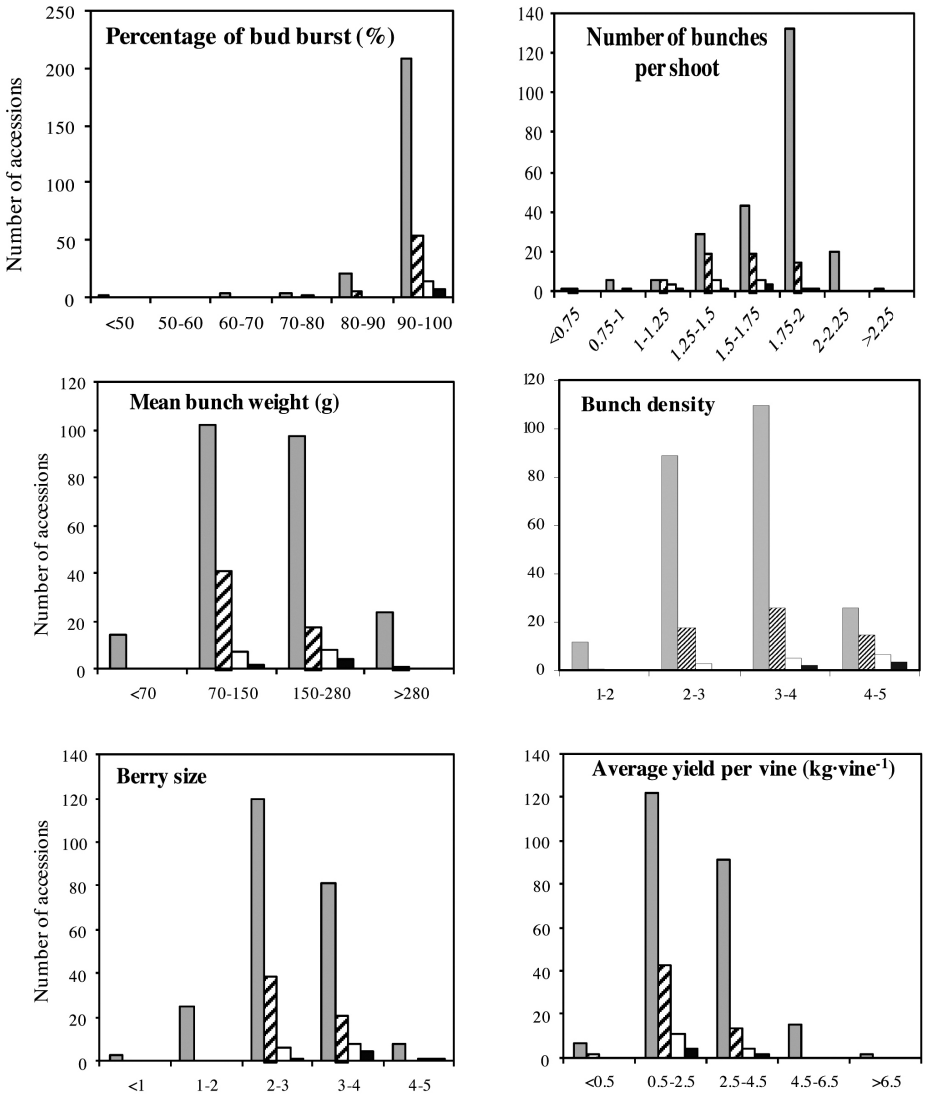


Figure 3. Distribution of the accessions according to the considered variables. The initial population is in grey, the "first selection" population in black with diagonal white stripes, the "second selection" in white and the "third selection" in black.

first, second, and third year (Table 2), allowed us to confirm the maintenance of the intravarietal diversity after the pre-selection process. The data analysis converted the variables into two components that explain the percentages of the variances that appear in the table. The most important variables that define the first component were the average yield per vine and the size of the bunch, for the four populations. The second component was defined mainly by the bud burst in the initial population and in the first selection and, to a lesser extent, by the bunch density and the secondary bud burst. In the second and the third selection, the bunch density acquired more importance than bud burst.

The representation of the accessions according to these principal components led to the graphs represented in Figures 4 and 5. In these graphs, we can observe that,

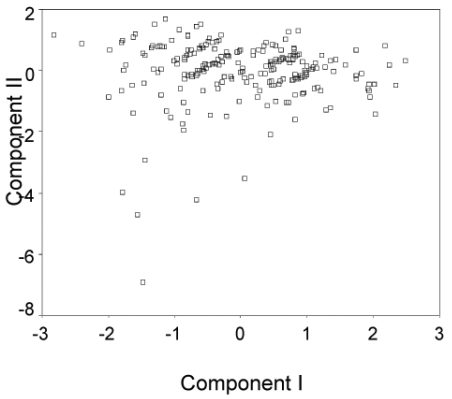


Figure 4. Graphic representation of the initial population according to the principal components.

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Table 2. Correlation coefficients between the variables and two principal components. Percentage of the total variation expressed by the eigenvalue for two extracted components.

	Original population		First selection		Second selection		Third selection	
	Component		Component		Component		Component	
	I	II	I	II	I	II	I	II
Yield	0.937	0.042	0.927	0.080	0.868	-0.106	0.987	-0.014
Bunch size	0.915	-0.104	0.889	0.016	0.878	0.278	0.878	-0.288
Bunches per shoot	0.651	0.434	0.500	0.022	0.757	-0.387	0.894	0.239
Berry size	0.580	-0.413	0.487	-0.334	0.561	-0.122	0.916	-0.119
Secondary bud burst	0.282	0.195	0.215	0.651	0.440	0.508	0.074	0.823
Bud burst	0.170	0.789	-0.006	0.798	-0.501	0.182	-0.396	0.362
Bunch density	0.365	-0.483	0.522	-0.138	0.090	0.854	-0.266	-0.919
% of the variance	56.891		52.201		59.122		77.452	

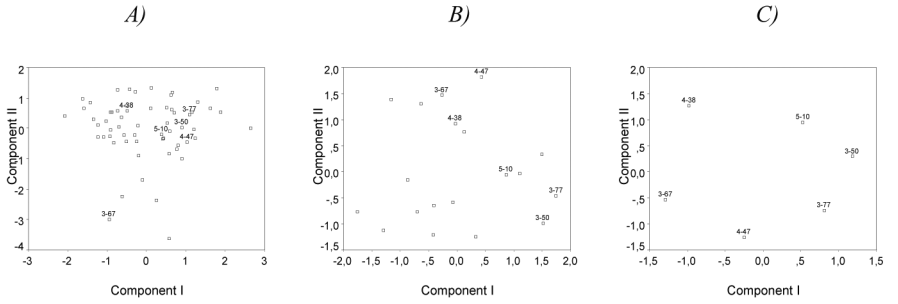


Figure 5. Graphic representation of the first year pre-selected population (A), the second year pre-selection (B), and the last year pre-selection (C) according to the principal components. The accessions that were selected in the last year of the study are noted in each plot.

at the beginning, the component I allows for a better distinction among individuals, because these individuals are dispersed in a wide range of values. The component II did not divide many accessions; however, the differences among the rest were not so evident as they were gathered together in a narrow range. On the other hand, the weights of the different variables in both components for the differentiation of individuals were on similar levels. In view of these graphs, it can be concluded that the intravarietal diversity was maintained in the new populations of selected accessions in relation to the initial population.

Regarding the must analysis of the selected accessions, the total acidity ($g \cdot L^{-1}$ tartaric acid) and probable alcohol are shown in Figure 6. The average acidity was 6.13 $g \cdot L^{-1}$, ranging from 4.9 $g \cdot L^{-1}$ (accession 4-47) to 9.4 $g \cdot L^{-1}$ (3-67). The average probable alcohol was around 12.5º, and any significant variations among the different accessions have been found. The accessions 3-50 and 5-10 (12.5º) showed the highest probable alcohol, while the accession 3-67 showed the lowest one (12.2º). These data allowed us to deduce that the selected accessions form a heterogeneous population and that the pre-selection did not follow a specific direction.

CONCLUSIONS

The methodology that has been established to carry out the clonal and sanitary pre-selection of the variety Maturana Blanca allows the preservation of the existing intravarietal diversity. The interest of this method over traditional methods is that the plant selection is performed on the groups obtained by statistical analysis (cluster analysis) of the plants studied, implying greater objectivity and ensuring the maintenance of the population diversity.

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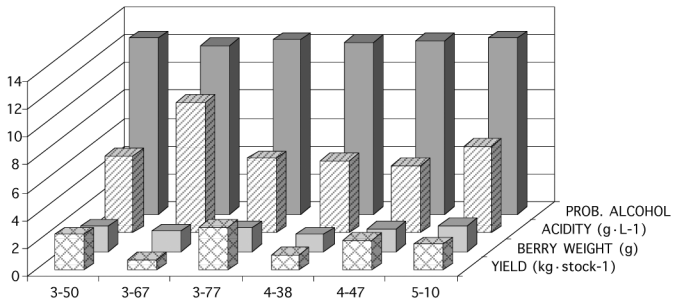


Figure 6. Graphic representation of the values shown by each selected accession according to several considered parameters.

The comparison between the original population and the group of accessions obtained after the pre-selection process - through the distribution of the accessions for the considered variables and the principal component analysis - showed that the intravarietal diversity is preserved following the process mentioned above.

This weak type clonal selection is not favourable to the accessions that have specific characteristics, but it allows to a great part of the biotypes that are representative of the original population variability to be selected for further studies.

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