Clonal selection of Aragonez variety (syn. Tinta Roriz, Tempranillo) in the Douro Wine Region focused on quality improvement

Sélection clonale de la variété Aragonez (syn. Tinta Roriz, Tempranillo) dans la Région Délimitée du Douro ciblée vers l'amélioration de la qualité

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Abstract

Two field trials with 30 genotypes of the grapevine variety Aragonez (Portuguese official list code PRT52603, syn. Tinta Roriz, Tempranillo), coming from a first selection cycle, were established in 2012 in the Douro Wine Region (DWR), North Portugal: one in a steep slope area located at 194 m altitude and the other in a flat area at 471 m altitude. The objective is to select genotypes with below-average yield, higher berry quality and stable behaviour in different environments. In 2014 several traits were evaluated: fertility indexes, yield, number and weight of bunches per plant, berry weight, volume of grape must, soluble solids, titratable acidity and pH.

Results showed that genetic variability found within the group of 30 clones evaluated in the two trials was significant (for a probability level of 0.05) for almost all traits. Values of broad sense heritability varied from 0.36 to 0.97. Obtained results in both field trials are compared and the genotype by site interaction is studied. A summarized characterization of genotypes considering all evaluated traits in both trials is also done and genotypes with superior performance were identified for possible selection.

***Keywords**: Vitis vinifera, Aragonez, clonal selection, Douro Wine Region.*

Résumé

Deux essais de champ avec 30 génotypes du cépage Aragonez (code PRT52603 dans la liste officielle portugaise, syn. Tinta Roriz, Tempranillo), venus d'un premier cycle de sélection, ont été greffés en 2012 dans la Région Délimitée du Douro (RDD), au nord du Portugal: le premier dans une pente très forte située à 194 m d'altitude et l'autre dans une zone plane à 471 m d'altitude. L'objectif est de sélectionner des génotypes avec des rendements inférieurs à la moyenne, une meilleure qualité des baies et une bonne stabilité des paramétres évalués dans différents environnements. En 2014, plusieurs caractères ont été évalués: les indices de fertilité, le rendement, le nombre et le poids des grappes par pied, le poids des baies, le volume de moût de raisins, la teneur en sucre, acidité totale et pH.

Les résultats ont montré que la variabilité génétique dans le groupe des 30 génotypes évalués dans les deux essais était significative (pour un niveau de signification de 0,05) pour presque toutes les caractéristiques. Les valeurs de l'héritabilité au sens large ont varié de 0,36 à 0,97. Les résultats obtenus dans les deux essais sont comparés et l'interaction génotype X environnement est étudiée. Une caractérisation sommaire des génotypes concernant tous les caractères évalués dans les deux essais se fait aussi et différents génotypes sont identifiés comme possible candidats à sélectionner.

***Mots-clés:** Vitis vinifera, Aragonez, sélection clonale, Région Délimitée du Douro*

1. Introduction

Tinta Roriz (code PRT52603 in Portuguese official list, syn. Aragonez, Tempranillo) is the most widely grown grapevine variety in Portugal (13 877 ha), representing 6% of the total vineyard area in the country (IVV, 2013), and around 15% of the total vineyard area in Douro Wine Region (DWR) and is used as a main recommended variety in DWR appellations.

This variety underwent clonal selection during the 1980's – 1990's meant to increase its productivity which resulted in several certified, commercially available clones, which have been recently reported as providing medium to below-average quality wines, namely low colour, low polyphenols and medium to low sensory quality, associated with high yields (Tente, 2010). These observations are confirmed by technical staff (viticulturists, winemakers) in several private companies using this variety and seems to have specific incidence in warmer, drier regions, leading to the assumption of a nexus between these environmental conditions and observed loss of quality (ADVID, 2011).

The objectives of the work developed by ADVID, with the collaboration of two wine companies (Sogrape Vinhos, S.A. and Sogevinus Fine Wines, S.A.), under the supervision of PORVID, are: (1) to make a preliminary evaluation of the genotypic variability available within a group of 30 clones of 'Aragonez' variety coming from a previous first cycle of selection carried out before; (2) to understand the adequacy of the trials for performing, in the future, an efficient selection of clones with below-average yield, higher quality of the berry and good stability in different environments, expecting that the decrease in yield will further accentuate the gain in berry concentration, regulating the ripening process.

2. Material and methods

A selection of 30 genotypes was made from the original field trial, of the first experimental cycle of selection (located at Alentejo region, South of Portugal), focusing quality and using EBLUPS of genotypic effects of different traits. This selection was made according to the following criteria: the 21 genotypes with the lowest yield and the 16 genotypes with the highest anthocyanins content (some of them satisfying simultaneously both criteria).

Two trials were setup in 2012 in commercial vineyards supplying grapes on the DWR, one located in the Baixo Corgo sub-region (Vila Real, 471 m altitude), and one in Cima Corgo sub-region (Tabuaço, 194 m altitude). Each trial was established according to a resolvable row-column experimental design, latinized by column, with 6 replicates (5-6 plants). In order to understand the effect of higher temperatures and water deficit on this variety, two other clonal trial fields will be installed in 2015 in the Douro Superior sub-region.

In 2014 several traits were evaluated: two fertility indexes, determined by the following ratios, number of bunches/number of buds (IFPR) and number of bunches/number of shoots (IFPO); yield (kg/plant), bunch weight (kg/bunch), volume of grape must (mL/60 berries), weight of berries (g/60 berries), soluble solids (ºBrix), titratable acidity (tartaric acid, g/l) and pH. The yield, weight of bunches, and the fertility indexes were evaluated in all trial replicates. For the evaluation of quality traits of must, a sample of 60 berries per plot was collected in three replicates. The analyses of musts were performed by standard methods: soluble solids by refractometry, acidity by titration, pH by potenciometry.

For each site, a linear mixed model was fitted to the data of each trait. In this model, the effects of replicates were assumed as fixed and the effects of genotypes, rows within the replicate and columns within the replicate were assumed as random. All random effects and errors were assumed to be independent and identically distributed normal random variables. Data analysis was performed using Proc Mixed procedure of SAS version 9.2 (SAS Institute 2008). The estimation of the model parameters

was conducted using the residual maximum likelihood method. A residual likelihood ratio test was used to test the null hypothesis that the genotypic variance component was equal to zero.

To evaluate the efficiency of selection, the broad-sense heritability was computed (Gonçalves et al., 2013). To assess the performance of the several genotypes under study, the empirical best linear unbiased predictors (EBLUPs) of genotypic effects of each trait were obtained through the mixed model equations (Henderson, 1975; Searle et al., 1992). Moreover, an analysis of genotype by site interaction was conducted. In this case, a mixed model was fitted for each trait, considering the effects of sites and replicates within a site as fixed, and the effects of genotypes, genotype by site interactions, rows within replicate within a site and columns within replicate within a site as random. All random effects and errors were assumed mutually independent and identically distributed. A residual likelihood ratio test was used to test the null hypothesis that the genotype by site variance component was equal to zero.

3. Results and discussion

The results obtained for each field trial corresponding to all the evaluated traits are shown in Table 1. For a significance level of 0.05, the genetic variability found within the group of 30 evaluated genotypes in the two trials was significant for all traits (except for titratable acidity at Tabuaço). This means that, although these trials belong to a second cycle of selection, there is still quite raw material to perform selection.

In the trial located at Vila Real, the overall mean of the yield was lower but the range of variation of the mean phenotypic values was higher. This tendency was observed for almost all traits under analysis. It seems that, under the conditions of this trial, the genetic differences among genotypes were more expressed. In fact, in general, in the trial located at Vila Real, the results were better than those obtained in the trial located at Tabuaço. In the former, the values of broad-sense heritability for the evaluated traits were higher, ranging from 0.40 to 0.966. In the field trial located at Tabuaço, these values varied from 0.36 to 0.89. Among traits, higher values for this genetic parameter were obtained for yield and bunch weight for both trials. In those cases, the control of environmental variation was successful and the proportion of the phenotypic variation explained by genetic causes was high (above 90% in the trial of Vila Real). Also, for the trial located at Vila Real, the values of heritability were superior to 0.70 for weight of berries, volume of the grape must and soluble solids. Therefore, for these traits a successful selection is expected. For other traits, such as IFPR and IFPO, moderate values for the heritability were obtained, thus the efficiency of selection will be moderate. However, for the first time the genotypes under study were evaluated concerning these traits and, although moderate, the results are promising.

For the traits with higher values of broad-sense heritability, a summarized characterization of the genotypes in both trials is illustrated in Table 2. Through the observation of the ranks of the EBLUPs for the several traits under analysis, genotypes with different performances are identified. In general, those with higher values of yield and bunch weight, revealed lower values for soluble solids content (for example, RZ0633, RZ0717, RZ1702 and RZ4005). However, there are also other that showed a balanced behaviour for several traits, including yield and soluble solids, but revealing a higher change of rank between field sites (such as RZ3902, RZ4009, RZ6303 and RZ8207). The changing of ranking for the same trait in the two trials for some genotypes is an indicator of genotype by site interaction. In fact, the results of Table 3 showed the existence of genotype by site interaction for some traits. For a significance level of 0.05, significant genotype by site interaction was found for yield, bunch weight, weight of berries, volume of grape must and soluble solids.

4. Conclusions

The results concerning genotypes and trials are preliminary. However, the genotypic variation found within the group of 30 genotypes and the high values of heritability obtained for some traits are promising to perform, in the future, a successful selection in order to meet the objectives of the grape growers of the Douro Wine Region. In the next year, data collection will be continued, with special importance for the evaluation of the quality traits of berries, namely anthocyanins, polyphenols and tannins.

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Table 1 – Results of individual analysis for trials located at Tabuaço and Vila Real. Overall mean, minimum and maximum mean phenotypic values, σ_g^2 - genotypic variance component, h_g^2 - broad-sense heritability estimate

Site	Trait	Mean	Min.-Max.	p-value of the test $H_o: \sigma_g^2 = 0 \quad vs \quad H_1: \sigma_g^2 > 0$	h_g^2
Tabuaço	Yield (kg/plant)	3.854	1.947-5.421	< 0.001	0.779
	Bunch weight (kg/bunch)	0.321	0.189-0.479	< 0.001	0.894
	IFPR	2.254	1.708-2.898	0.0012	0.553
	IFPO	1.538	1.172-1.952	0.0015	0.542
	Volume of grape must	80.756	64.667-94.667	< 0.001	0.666
	Weight of berries (g/60 berries)	126.767	105.0-152.233	< 0.001	0.684
	Soluble solids (ºBrix)	21.080	16.833-23.5	0.0241	0.474
	Titratable Acidity (tartaric acid, g/L)	3.260	2.675-4.180	0.1103	0.363
	pH	3.892	3.703-4.05	0.0256	0.490
Vila Real	Yield (kg/plant)	2.770	0.696-6.138	< 0.001	0.907
	Bunch weight (kg/bunch)	0.263	0.069-0.508	< 0.001	0.966
	IFPR	2.238	1.142-3.022	0.0289	0.404
	IFPO	1.520	0.740-2.167	0.0160	0.444
	Volume of grape must (mL/60	95.567	67.666-112.333	< 0.001	0.730
	Weight of berries (g/60 berries)	146.581	106.300-168.767	< 0.001	0.759
	Soluble solids (ºBrix)	21.557	19.167-23.367	< 0.001	0.713
	Titratable Acidity (tartaric acid, g/L)	4.068	3.469-5.025	0.0017	0.623
	pH	4.076	3.823-4.223	0.0012	0.650

Table 2 - EBLUPs of genotypic effects of 30 genotypes in both sites for traits showing higher heritability

Genotype	Rank of the EBLUPs									
	Tabuaço yield	**Vila Real yield**	**Tabuaço Bunch weight**	**Vila Real Bunch weight**	**Tabuaço Soluble solids**	**Vila Real Soluble solids**	**Tabuaço Volume of grape must**	**Vila Real Volume of grape must**	**Tabuaço weight of berries**	**Vila Real weight of berries**
RZ0107	22	19	22	19	26	17	27	19	27	19
RZ0129	21	8	26	18	6	5	23	25	24	25
RZ0633	3	2	4	4	23	21	18	20	16	13
RZ0717	1	1	7	1	21	28	16	9	20	9
RZ1513	12	18	15	20	11	15	26	24	25	26
RZ1702	4	7	3	6	25	22	22	13	22	6
RZ3306	18	22	17	24	8	1	17	18	11	22
RZ3311	15	11	21	15	19	30	9	14	12	15
RZ3408	29	26	30	26	16	8	28	29	29	28
RZ3507	14	13	5	9	24	29	5	15	5	18
RZ3705	23	23	18	23	12	3	3	23	4	24
RZ3801	24	24	23	22	28	20	6	4	8	5
RZ3803	28	21	27	21	1	7	25	27	26	27
RZ3807	13	9	13	14	15	25	1	10	1	4
RZ3902	7	15	10	17	7	11	2	17	3	17
RZ3911	26	17	16	11	17	16	13	12	13	11
RZ3915	11	20	2	7	5	6	15	5	10	16
RZ4005	5	5	11	8	20	26	8	7	7	8
RZ4009	17	6	12	10	2	12	4	16	2	12
RZ4010	6	16	8	13	27	13	10	2	14	3
RZ4101	30	29	29	27	29	9	20	3	19	2
RZ4105	10	12	14	12	9	14	7	6	6	10
RZ4110	27	28	25	29	30	24	30	28	30	29
RZ4305	20	27	24	28	14	18	19	26	18	20
RZ4307	25	30	28	30	13	2	24	30	21	30
RZ4505	2	4	1	5	10	23	14	22	23	23
RZ6303	8	3	9	2	3	27	11	1	17	1
RZ8020	16	25	20	25	4	10	29	21	28	21
RZ8207	9	14	19	16	18	4	21	11	15	14
RZ8904	19	10	6	3	22	19	12	8	9	7

Table 3 – Results for genotype by site interaction for all evaluated traits $\sigma^2_{G\times E}$ - genotype by site variance component

Trait	p-value of the test: $H_o : \sigma^2_{G\times E} = 0 \quad vs \quad H_1 : \sigma^2_{G\times E} > 0$
Yield (kg/plant)	0.0014
Bunch weight (kg/bunch)	< 0.001
IFPR	0.3759
IFPO	0.2919
Volume of grape must (mL)	0.0416
Weight of berries (g/60 berries)	0.0064
Soluble solids(ºBrix)	0.0443
Titratable Acidity (tartaric acid, g/L)	0.0961
pH	0.1367